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Algorithms for the Testing of Tissue Donors for Human Immunodeficiency Virus, Hepatitis B Virus, and Hepatitis C Virus

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Keywords
Donor screening · Tissue donation · Tissue bank · Virus inactivation · Virus safety

Abstract
Background: Although transmission of pathogenic viruses through human tissue grafts is rare, it is still one of the most serious dreaded risks of transplantation. Therefore, in addition to the detailed medical and social history, a comprehensive serologic and molecular screening of the tissue donors for relevant viral markers for human immunodeficiency virus (HIV), hepatitis B virus (HBV), and hepatitis C virus (HCV) is necessary. In the case of reactive results in particular, clear decisions regarding follow-up testing and the criteria for tissue release must be made. ***Methods:*** Based on the clinical relevance of the specific virus markers, the sensitivity of the serological and molecular biological methods used and the application of inactivation methods, algorithms for tissue release are suggested. ***Results:*** Compliance with the preanalytical requirements and assessment of a possible hemodilution are mandatory requirements before testing the blood samples. While HIV testing follows defined algorithms, the procedures for HBV and HCV diagnostics are under discussion. Screening and decisions for HBV are often not as simple, e.g., due to cases of occult HBV infection, false-positive anti-HBc results, or early window period positive HBV NAT results. In the case of HCV diagnostics, modern therapies with direct-acting antivirals, which are often associated with successful treatment of the infection, should be included in the decision. ***Conclusion:*** In HBV and HCV testing, a high-sensitivity virus genome test should play a central role in diagnostics, especially in the case of equivocal serology, and it should be the basis for the decision to release the tissue. The proposed test algorithms and decisions are also based on current European recommendations and standards for safety and quality assurance in tissue and cell banking.

Introduction

Transmission of pathogens is the most dreaded severe adverse reaction that can occur following allogeneic tissue transplantation. Tissues, mostly obtained from deceased donors, are transplanted in several clinical specialties. The spectrum ranges from musculoskeletal tissues, such as bone, cartilage, tendons, ligaments, and fascia, to the cornea and cardiovascular allografts (aortic and pulmonary valves, pericardium, veins, and arteries). Documentation of the medical and social history of deceased donors is challenging since it is limited to the information provided by relatives, close contacts, or treating physicians [1–3]. In the case of multi-transfused patients, the serological and molecular nucleic acid test (NAT) results could be of limited value because of relevant dilution ef-

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fects (see Hemodilution). While bacteria and fungi (including spore-forming organisms) can be mostly inactivated by methods such as treatment with peracetic acid-ethanol [4], gamma irradiation [5], or combination methods [6, 7], viral inactivation poses much more difficulty. For example, human immunodeficiency virus (HIV) is highly resistant to gamma irradiation. With a D_{10} value of approximately 8 kGy, a very high irradiation dose of 32 kGy is required for a $4\log_{10}$ virus inactivation [5]. Accordingly and due to current regulations [8], laboratory diagnosis of clinically relevant viral infections is vital. Additionally, the donor-recipient ratio (i.e., the number of recipients receiving grafts donated by a single donor) and, therefore, the tissue-specific risk ratio for corneas is 1:2, for cardiovascular transplants it is 1:1 to 1:10, and for musculoskeletal tissues it is up to 1:40 [9]. For living donors (amnion, femoral head), the donor-recipient ratio ranges from 1:1 to 1:4.

According to the current state of medical science and technology, both serological and molecular genetic methods, including cost-effectiveness analyses [10, 11], have to be considered in viral testing algorithms for tissue donors.

General Requirements

The laboratory performing the viral testing must have an established quality management (QM) system. This system should ensure that the defined screening program includes relevant pathogens and appropriate screening tests. Furthermore, appropriate completion of preanalytical requirements (e.g., sample handling, labelling, and storage and the time interval between collection and testing) and the appropriate documentation should be ensured. The QM system has to include written standard operating procedures (SOP), and the technical staff carrying out the testing and reporting the results, as well as those receiving and interpreting the results, must be trained.

Independently of laboratory training, measurements have to be implemented for all personnel involved in the collection and labelling of donor samples for sample storage and transport. The following requirements should be considered in the preanalytical framework [8, 12]:

Blood samples from living donors must be obtained at the time of donation or, if that is not possible, within 7 days before or after donation. The most adapted timing for sample collection is that which reflects the quality and safety of the collected tissues.

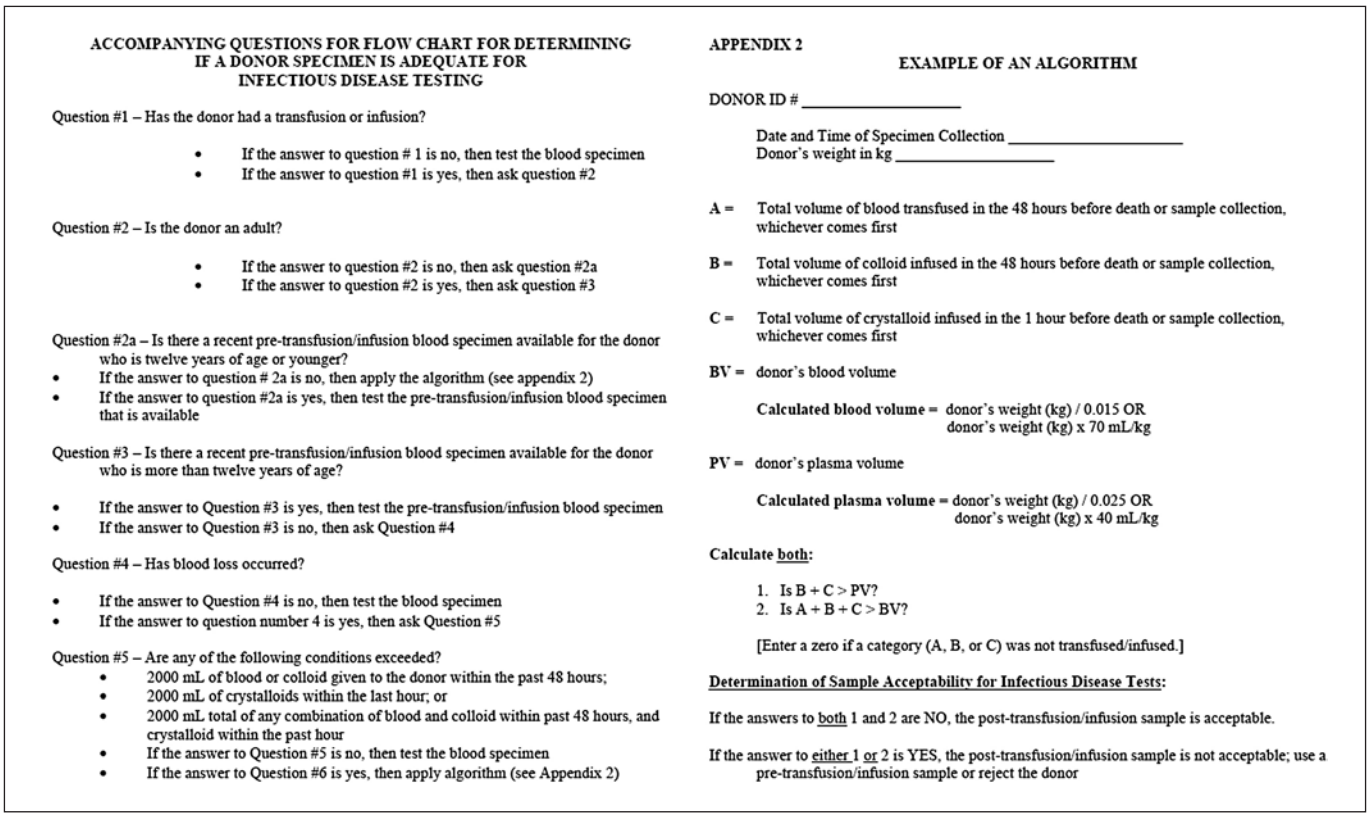
In the case of a deceased donor, blood samples must have been obtained just before death and, if that is not possible, within 7 days before death, or the time of sampling must be as soon as possible and not later than 24 h postmortem. However, several studies have demonstrated that blood samples collected more than 24 h postmortem yield valid results [13–16].

Table 1. Length of the window period for HIV, HCV, and HBV parameters

	Length of the window period, days	
	Kim et al. [60]	Kleinman et al. [61]
Anti-HIV (EIA)	22 (range 6–38)	15 (p24); 19 (anti-HIV)
HIV-NAT	11	5
Anti-HCV (EIA)	66 (range 38–94)	65
HCV-NAT	10	3
HBsAg (EIA)	59 (range 37–87)	36
HBV-NAT	not given	21

Clinical situations that could affect the quality of a test specimen must be taken into account. Infectious disease test results may be invalidated by hemolysis or by hemodilution if the extent of any dilution is such that it may cause a false-negative test result (due to a decrease in the assay detection limit). Therefore, pretransfusion/infusion samples should be obtained for testing purposes.

The screening method of testing for HCV and HIV infections is the antibody test, which becomes positive soon after the donor's immune system reacts with the virus. For HIV testing a combined assay including HIV-specific antibodies and HIV p24 antigen is mostly used (4th generation assays). Tests used for HBV detection react with a component of the viral envelope, i.e., the hepatitis B virus surface antigen (HBsAg). Freshly infected HCV and HIV donors may be infectious before the antibody test is positive (serological window period; Table 1 [60, 61]). In the case of HBV-infected donors, a negative HBsAg result is observed during the HBsAg window period (see Table 1). These gaps in routine testing can either be reduced by additional NAT tests or retesting living donors after quarantine storage. In the case of HCV and HIV, the serological window can be largely, if not completely, closed by sensitive methods detecting the virus genome by NAT. The suitability of an HBV NAT depends primarily on the sensitivity of the NAT because HBsAg testing is already sensitive. The potential late infectivity when the HBsAg test is negative is almost entirely detected by an additional antibody test, i.e., the anti-HBV core antigen (anti-HBc) test. However, the detection of anti-HBc generally indicates that the donor has had contact with HBV infection. The determination between a past or an active infection cannot be inferred from the presence of anti-HBc. Mostly (but not always) anti-HBc-positive and HBsAg-negative test results are found in cases with a past HBV infection, although the donor tissue may no longer be infectious. The concept of previous years that a donor can be accepted with anti-HBc and anti-HBs posi-



ACCOMPANYING QUESTIONS FOR FLOW CHART FOR DETERMINING IF A DONOR SPECIMEN IS ADEQUATE FOR INFECTIOUS DISEASE TESTING

Question #1 – Has the donor had a transfusion or infusion?

- If the answer to question # 1 is no, then test the blood specimen
- If the answer to question #1 is yes, then ask question #2

Question #2 – Is the donor an adult?

- If the answer to question #2 is no, then ask question #2a
- If the answer to question #2 is yes, then ask question #3

Question #2a – Is there a recent pre-transfusion/infusion blood specimen available for the donor who is twelve years of age or younger?

- If the answer to question # 2a is no, then apply the algorithm (see appendix 2)
- If the answer to question #2a is yes, then test the pre-transfusion/infusion blood specimen that is available

Question #3 – Is there a recent pre-transfusion/infusion blood specimen available for the donor who is more than twelve years of age?

- If the answer to Question #3 is yes, then test the pre-transfusion/infusion blood specimen
- If the answer to Question #3 is no, then ask Question #4

Question #4 – Has blood loss occurred?

- If the answer to Question #4 is no, then test the blood specimen
- If the answer to question number 4 is yes, then ask Question #5

Question #5 – Are any of the following conditions exceeded?

- 2000 mL of blood or colloid given to the donor within the past 48 hours;
- 2000 mL of crystalloids within the last hour; or
- 2000 mL total of any combination of blood and colloid within past 48 hours, and crystalloid within the past hour
- If the answer to Question #5 is no, then test the blood specimen
- If the answer to Question #6 is yes, then apply algorithm (see Appendix 2)

APPENDIX 2

EXAMPLE OF AN ALGORITHM

DONOR ID # _____

Date and Time of Specimen Collection _____
Donor's weight in kg _____

A = Total volume of blood transfused in the 48 hours before death or sample collection, whichever comes first

B = Total volume of colloid infused in the 48 hours before death or sample collection, whichever comes first

C = Total volume of crystalloid infused in the 1 hour before death or sample collection, whichever comes first

BV = donor's blood volume

Calculated blood volume = donor's weight (kg) / 0.015 OR
donor's weight (kg) x 70 mL/kg

PV = donor's plasma volume

Calculated plasma volume = donor's weight (kg) / 0.025 OR
donor's weight (kg) x 40 mL/kg

Calculate both:

1. Is B + C > PV?
2. Is A + B + C > BV?

[Enter a zero if a category (A, B, or C) was not transfused/infused.]

Determination of Sample Acceptability for Infectious Disease Tests:

If the answers to both 1 and 2 are NO, the post-transfusion/infusion sample is acceptable.

If the answer to either 1 or 2 is YES, the post-transfusion/infusion sample is not acceptable; use a pre-transfusion/infusion sample or reject the donor

Fig. 1. Hemodilution assessment.

tivity (titer ≥100 IU/mL) – which indicates a past infection – has to be complemented by an HBV NAT from a single sample in the case of a positive anti-HBc test [17, 18]. Furthermore, in cases of an occult hepatitis B infection (OBI), NAT shows significant advantage [19], as HBsAg is not detectable and anti-HBc may or may not be positive.

The selection of donor screening assays needs to be validated and applied in accordance with current scientific knowledge. Newer-generation tests (e.g., 4th generation) generally lead to a shortened serological window period [20, 21]. Where CE-marked assays and systems are not available for testing of postmortem blood specimens, laboratories performing this work must validate the assays for this purpose [22, 23].

Hemodilution

If a donor has recently received transfusions of blood or blood components, or infusions of colloids or crystalloids, and has lost blood, any testing of donor blood collected posttransfusion or postinfusion may not be valid due to hemodilution or plasma dilution. Assessment of the extent of any hemodilution requires the use of a formula to calculate the dilution of the donor's original blood volume (and circulating levels of antigen and/or antibody, if present) and should be done by the physician in charge or the transplant coordinator. In a number of countries, it is current practice to consider less than 50% calculated hemodilution to be the maximum safety limit with a minimal risk of a false-negative (dilution-related) test result [12]. Further possibilities for calculating hemodilution have been developed taking into account different physiological conditions [24].

A suitable example of commonly used questions and formula to assess the donor's potential hemodilution or plasma dilution is shown in Figure 1. It is based on recommendations of the US Food and Drug Administration (FDA) and the European Directorate for the Quality of Medicines & HealthCare of the Council of Europe (EDQM) [12, 25] and can be applied when the donor received fluids that may lead to hemodilution. Adaptations of the algorithms may be needed for body sizes outside the normal adult range. New calculation models should only be used after extensive studies and in compliance with the specifications of the algorithm presented here. Although it is not general practice, a tissue establishment may accept tissues and cells from a donor with plasma dilution >50 %, but only if each required test has been validated appropriately for use with a diluted test specimen. In such cases additional testing should also be per-

formed using molecular tests (i.e., NAT) for HIV, HBV, and HCV, and possibly for other viruses, depending on the donor's travel history (e.g., high-risk areas for transmission of pathogenic viruses like the Zika virus, the West Nile virus, and the Chikungunya virus), underlying disease, or other factors.

Mandatory Laboratory Tests for Viruses

Human Immunodeficiency Virus Types 1 and 2

HIV (type 1 with 3 subtypes, i.e., M(ajor), N(ew), O(utlier); subtype M with the variant AI; and type 2) belongs to the retroviruses and is a member of the genus *Lentivirus*. HIV is an approximately 100-nm, enveloped, single-stranded RNA virus and the cause of acquired immunodeficiency syndrome (AIDS) [26, 27]. The virus is transmitted primarily through sexual intercourse, intravenous drug abuse, intrauterine infection, and other transmissions via infectious blood (transfusions/organ and tissue transplants).

The risk of transmission of HIV is one of the central concerns in transplantation medicine and, accordingly, in allogeneic tissue transplantation. In the US, HIV transmission through a tissue transplant was first reported in 1988 through an allogeneic bone graft that was used in 1984 as a part of spinal surgery [28]. The graft was stored at –80 °C for 24 days and it was not subjected to sterilization. The tissue donor was not tested for HIV antibodies.

In 1985, a total of 58 tissue and organ transplants, including 28 bone grafts, were taken from a multi-organ donor with a new undetected HIV infection. Twenty-five grafts were lyophilized and treated with 30% ethanol and they did not transmit HIV after transplantation. Three bone grafts were neither disinfected nor lyophilized, with the recipients subsequently infected with HIV-1 [29]. HIV transmission through bone grafts has also been described in Germany. In 1984, sixteen bone grafts were taken from a male donor under aseptic conditions and preserved at –80 °C without a viral inactivation procedure. An HIV test was not performed because of a lack of suitable tests. Between November 1984 and May 1985, twelve patients received transplants from the donor, with 4 of them subsequently being infected with HIV [30]. Since then, no other transmission cases have been described in the international literature. It can be assumed that the risk of infection will continue to exist; however, transmission is prevented by extensive precaution measures (laboratory diagnostics, medical history, and inactivation procedures). Furthermore, it must be taken into account that tissue donors are generally first-time donors; therefore, an increased prevalence is to be expected [31, 32]. The following HIV test algorithm was elaborated by an expert group during the preparation of the 4th edition of the EDQM guide [12] (Fig. 2):

- Combination assay (4th or 5th generation), including detection of anti-HIV-1/2 antibodies plus the HIV-1 p24 antigen; both parameters should be negative for tissue release.
- If a 3rd-generation test is used, an HIV-1-RNA test (qualitative or quantitative, sensitivity limit ≤50 IU/mL) must be performed additionally; both parameters should be negative for tissue release.
- Confirmed nonnegative (i.e., weakly reactive or borderline) serological screening results can be retested in duplicate using the same assay. If the (retest) results are negative, the donated tissues can be released.
- Reactive samples can be retested using another serological assay of equal or greater sensitivity. Donations that are nonreactive in this assay and negative for HIV-1-RNA could be released for clinical use.
- It should be noted that, if an HIV-1-RNA test is performed and the result is reactive, the donations cannot be released for clinical use, independently of the serology results. The HIV-1-RNA test may yield positive results before serological tests, providing a short virological window period.

Hepatitis B Virus

Human HBV belongs to the hepadnaviruses and has a double-stranded DNA genome. The first clinical experience of the "jaundice epidemics," now understood to be caused by hepatitis B infections [33], dates back to 1885 (smallpox vaccination campaign) and 1938 (measles vaccination). The infectious virus particles are approximately 42 nm in size, are spherical, and have an outer lipid envelope. HBsAg is anchored in the viral envelope, budding at the endoplasmic reticulum membrane as a viral protein, which is also introduced as free HBsAg particles (20 nm) in serum.

The Hepatitis B Foundation (https://www.hepb.org/what-is-hepatitis-b/what-is-hepb/facts-and-figures/) reports that approximately 290 million individuals worldwide currently suffer from chronic hepatitis B, 30 million people will become newly infected each year, and around 2 billion people worldwide have had an HBV infection. A particularly high infection rate can be found in intravenous drug addicts, men who have sex with men, and commercial sex workers. Transmission is usually through sexual intercourse and contagious blood and blood products. Inadequately sterilized nonmedical instruments (e.g., tattoo needles and piercings), the contents of multiply used ampoules (e.g., heparin), as well as other bodily secretions, can be potentially infectious. The target organ of HBV is the liver (target cells: hepatocytes). The resulting immune reaction triggers the associated clinical picture of abdominal complaints, fever, joint pain, rashes,

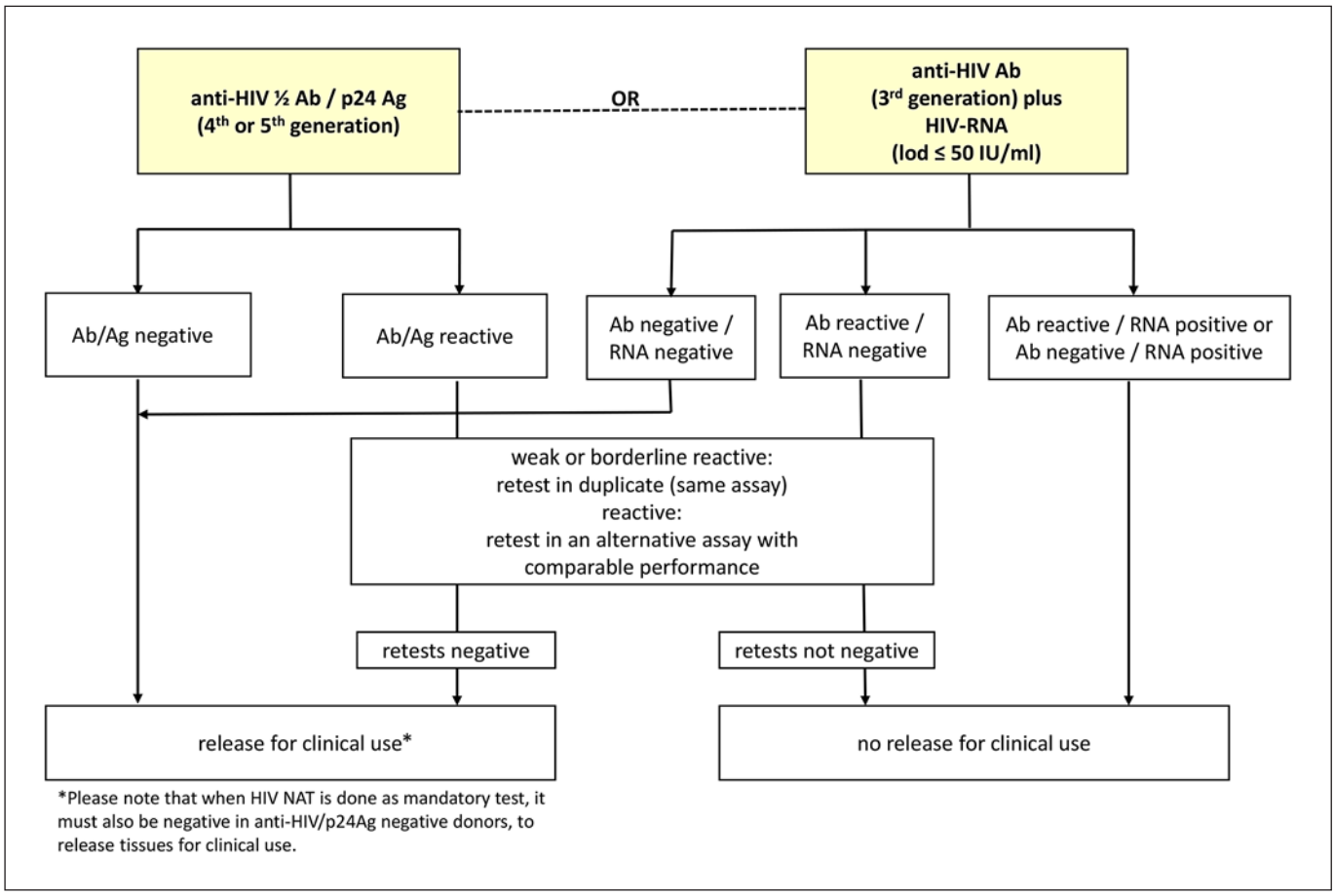


Fig. 2. Algorithm for HIV testing.

and jaundice. The disease course is fulminant with acute liver failure, resolves (in adults around 90% of cases), or becomes chronic (active or persistent). One of the most serious long-term consequences is the development of primary liver cell carcinoma.

Regarding tissues transplants, although HBV transmission has been reported only in 1 case through bone grafts [34] and in 2 cases through corneal transplants after penetrating keratoplasty [35], this virus is relevant for the risk assessment of tissue donors. A known case of HBV transmission through a red blood cell concentrate revealed the risk of blood-associated infections in the serological window phase. The donor had tested negative for HBsAg. The HBV DNA analysis of the lookback sample using NAT was positive (~2,000 copies/mL). The HBsAg test was only marginally positive 2 months after donation of the infectious erythrocyte concentrate [36].

One of the most difficult situations in HBV diagnostics is the occurrence of an occult hepatitis B infection. OBI is defined as a negative HBsAg and positive/negative anti-HBc-IgG status but HBV DNA is detectable in serum and liver tissue. The presence of anti-HBc in serum is an important key for OBI tracking, although about 20% of OBI cases are negative for anti-HBc [37]. OBI has been reported in: high-risk groups; blood donors; liver transplant recipients; patients coinfected with HCV/HIV; patients undergoing immunosuppressive therapy or hemodialysis; patients with liver cirrhosis, cryptogenic liver disease, or abnormal alanine transaminase; and patients with lymphoma or rheumatoid arthritis. Currently there is no standard assay for diagnosis of OBI in serum, and the only reliable method is the detection of HBV DNA by NAT.

The following HBV test algorithm was elaborated by an expert group during the preparation of the 4th edition of the EDQM guide [12] (Fig. 3):

- Testing of HBsAg and total antibodies to HBc, HBsAg must be negative.
- If anti-HBc is reactive, additional determination of an individual (single) sample using a highly sensitive HBV DNA method (currently ≤12 IU/mL detection limit) must be performed.
- If anti-HBc is positive and HBsAg and HBV NAT are negative, the donated tissues can be released.
- HBV DNA positivity leads, in any case, to discarding of the donated tissues.

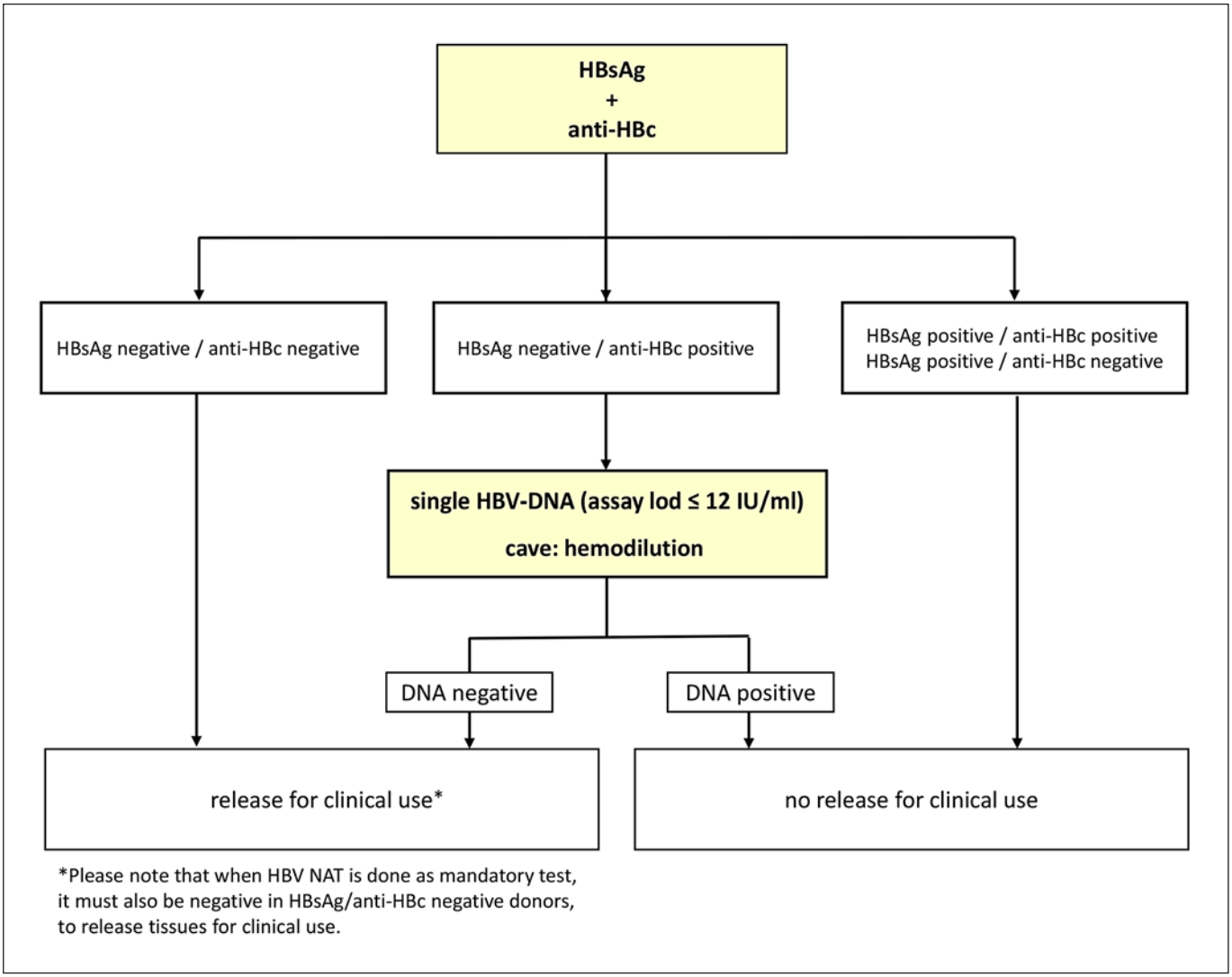


Fig. 3. Algorithm for HBV testing.

Hepatitis C Virus

After the detection of pathophysiological relationships of hepatitis A and hepatitis B and the molecular biological or serological description of the viruses underlying these diseases, a large number of parenterally transferable non-A, non-B hepatitis remained. It was not until 1989 that chimpanzee infections with subsequent DNA isolation and characterization were followed by the expression of an antigen that reacted with sera from chronically infected people with non-A, non-B hepatitis. The corresponding cDNA was sequenced, and the virus, now known as HCV, was identified as the new family of Flaviviridae [38–40]. HCV is a single-stranded RNA virus with a diameter of approximately 60–70 nm, comprising a capsid and an envelope. Like HBV, HCV is transmitted parenterally. Risk groups are intravenous drug addicts, men who have sex with men, commercial sex workers, and prison inmates. Transmission occurs primarily through infected blood and blood products. Sperm and other exudates may also contain low virus concentrations. Hepatitis C infection is usually clinically silent. Clinically visible courses are usually milder than those of hepatitis B, but a transition to cirrhosis and primary liver cell carcinoma is also possible. In 1992, HCV transmission through an allogeneic bone graft was first described. The bone graft donor became infected in 1985 following transfusion with infected fresh-frozen plasma. In 1990, a femoral head was removed from a patient during hip surgery and used as a graft after 8 weeks of cold preservation without virus inactivation. Shortly thereafter, the recipient of the femoral head graft became anti-HCV positive [41].

Another HCV transmission case was published in the USA. Ninety-one organ and tissue transplants were taken from an anti-HCV negative multiorgan donor in 2000. Of these, 40 patients were treated with transplants. HCV in-

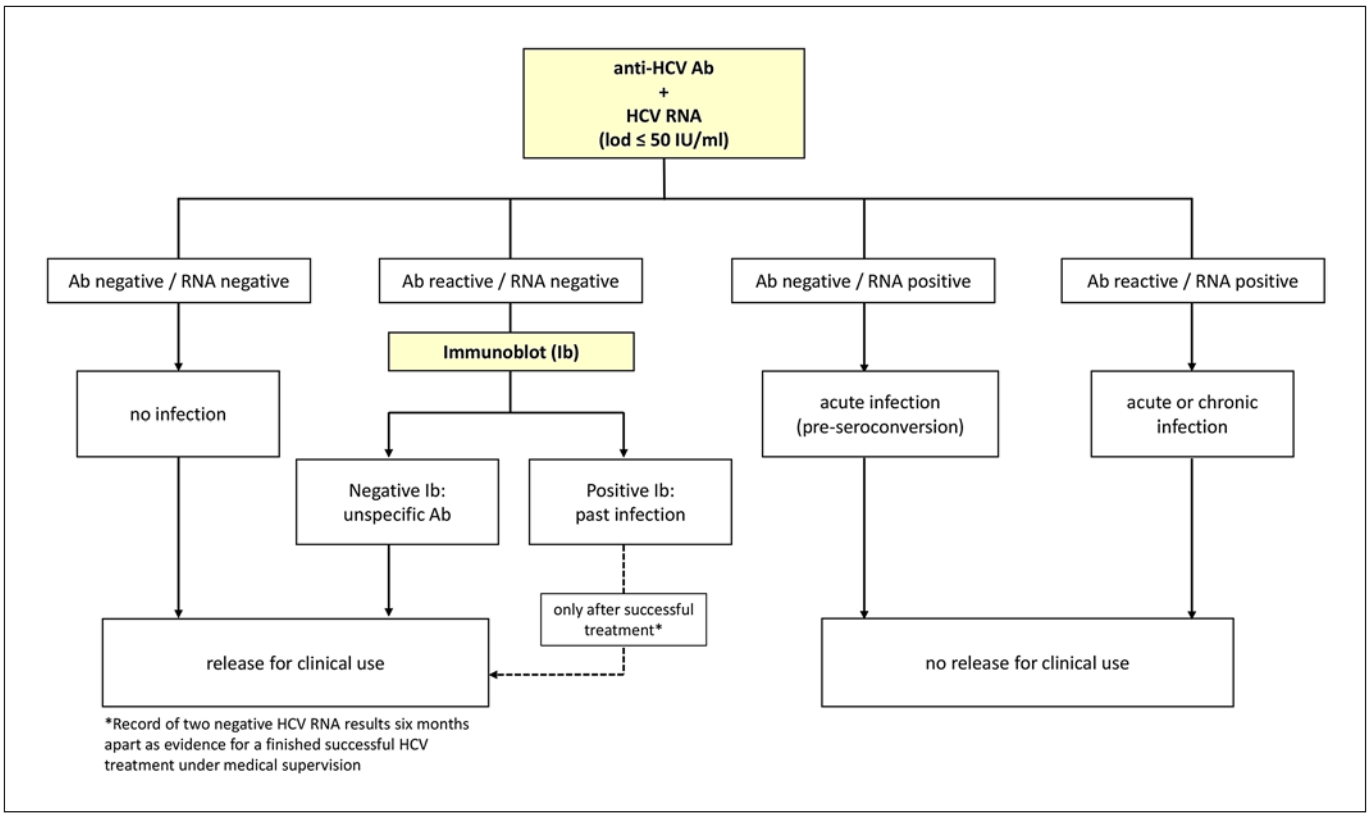


Fig. 4. Algorithm for HCV testing.

fection was detected in the organ recipients and 5 tissue recipients (1 vena saphena recipient, 1 tendon recipient, and 3 bone/tendon recipients). The donor reserve sample examined in July 2002 was positive in the HCV NAT but negative for anti-HCV [42]. No HCV infection was found in the 16 recipients who had received irradiated bone grafts (dose: 15 kGy). Other studies indicated, considering the D10 value of flaviviruses (BVDV: <3 kGy), that this dose is sufficient for depletion (by 5 $\log_{10}$ of [tissue culture infectious dose] $TCID_{50}$/ml levels) and that the irradiation, therefore, very likely prevented infection in these recipients [5].

Combined antibody-antigen detecting assays, as for HIV, are not commercially available at present, and the performance of HCV antigen assays is not sufficient to exclude an early infection. The presence of anti-HCV may indicate an acute, chronic, or past infection. Furthermore, the preseroconversion window phase takes around 2 months; therefore, an HCV RNA test is, in addition to serological testing, a mandatory part of the here recommended algorithm for HCV testing. The following HCV test algorithm was elaborated by an expert group during the preparation of the previous and the current edition of the EDQM guide [12] (Fig. 4):

- Testing of anti-HCV antibodies; anti-HCV should be negative for tissue release.
- Testing for HCV-RNA (NAT assay, sensitivity ≤ 50 IU/ml); HCV-NAT should be negative for tissue release.
- Anti-HCV-reactive and HCV-RNA-negative result, which is indicative of a past infection (medical history, type and duration of HCV treatment, and serology have to be taken into account) or a non-specific antibody test reaction. This result combination must be confirmed by immunoblot analysis. HCV immunoblot should be negative for tissue release.
- if the HCV immunoblot is positive (so called "confirmed positive anti-HCV result"), only with a record of 2 negative HCV RNA results 6 months apart as evidence of a finished successful HCV treatment under medical supervision and together with a negative HCV RNA test on the donor sample, the donated tissues can be released for clinical use.

The algorithms presented here reflect the basic requirements for testing for HIV, HBV, and HCV in tissue donors. In a number of European countries, e.g., France and the UK, mandatory NAT testing for all 3 viruses is already performed with primary serological testing.

Discussion

HIV, HCV, or HBV transmission has been described primarily in unprocessed or non-virus inactivated musculoskeletal tissues. Viral transmission through processed tissue grafts is rare. Additionally, before the year 1995 the donors were untested for these viruses or early generation kits were used. Nowadays, the safety of the tissues is improved by clinical and biological selection of the donors and, if applicable, the use of inactivation methods. In combination with other safety measures (medical and social histories, clinical notes, serology, and possibly NAT), they are suitable for ensuring a very high level of virus safety in the tissues. The suitability of validated chemical or physical methods for virus inactivation depends on the biological properties of the tissue after sterilization [43]. These procedures require validation. They usually lead to the depletion of clinically relevant viruses or model viruses (by at least 4 $\log_{10}$ levels of the $TCID_{50}$/mL) [4, 5, 44]. Finally, it should be mentioned that freeze-drying can also lead to a specific depletion (~1–2$\log_{10}$ $TCID_{50}$/mL levels) of viral infectivity [45].

The essential basis for reducing the risk of virus transmission includes a detailed medical and social history of the tissue donor and the exclusion of persons with "high-risk behavior," as well as clinical and biological testing procedures (serology with/without NAT) following the European standards [8, 12]. Testing for the HCV genome using NAT significantly reduces the virological window period in tissue donors and therefore it must be included in the routine testing. Testing or HIV genome using NAT is recommended as well and mandatory if a 3rd-generation test for anti-HIV is used. Besides serological testing for HBsAg and anti-HBc, HBV NAT may help to increase the safety of tissue preparations. Limiting the efficiency of NAT for HBV depends very much on the detection limit of the assays used. If low-level viremia is expected, for instance in occult HBV infections, the NAT must be as sensitive as possible. Tissue from a donor with suspected OBI [17, 19] and/or hemodilution should not be considered to be safe (depending on the individual constellation and the amount of hemodilution).

Other authors have described that in particular HBV NAT does not always show clear results [46, 47]. In addition to OBI, false-positive results and early window periods have been mentioned as possible causes [48–50]. However, the risk of HBV transmission through tissue preparations is significantly lower due to the inactivation methods generally used or the low amount of blood components in the processed tissue (e.g., cornea or heart valves) and therefore leads to the algorithm proposed in Figure 3. Donor samples can be pooled for NAT testing if the pooling procedure and the NAT have been validated [9], but special situations with the need for a highly sensitive NAT, e.g., OBI, should be taken in account.

In recent years, the hepatitis E virus (HEV) has assumed an important role in the transmission of viral infections in humans. HEV-1 and HEV-2 are mainly transmitted through fecally contaminated water or contaminated food. In Europe and North America, HEV-3 and HEV-4 are almost exclusively transmitted to humans from pigs and possibly also from other domestic animals (rabbits) via insufficiently heated food, especially liver. Since transmission through blood products from HEV-infected donors has also been described, many European countries have implemented HEV genome testing by NAT for blood products and stem cell preparations [51, 52]. Tissue allografts are either free of blood cells (cornea and cardiovascular tissues) or they have been processed with a virus inactivation procedure (musculoskeletal tissues) and tissue transplant recipients do not require immunosuppression. Mandatory HEV testing for tissue donors is currently not indicated since no cases of transmission have been described.

Testing of tissue donors for HIV, HBV, or HCV infection is currently performed using clinically based algorithms. Particularly in HBV diagnostics, further developments are to be expected and will primarily include the sensitivity of NAT and the relevance of surrogate markers (e.g., liver enzymes). In HCV diagnostics, the role of new therapeutics, e.g., direct-acting antivirals, must be included in the assessment of the donor [53–55]. Indeed, successful hepatitis C treatment is achievable in nearly all infected patients and it is reflected by a sustained virological response defined as the continued absence of detectable HCV RNA for 12 or more weeks after completion of therapy. This response is a marker for virological cure of HCV infection and it has been shown to be durable in large prospective studies in more than 99% of patients followed up for at least 5 years [56–59]. Therefore, it is expected that there will be more generous acceptance criteria of anti-HCV and/or HCV NAT-positive tissue donations in the near future.

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Statement of Ethics

Ethics approval was not required.

Conflict of Interest Statement

The authors have no conflict of interests to declare.

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Author Contributions

A.P. and H.F.R. developed this paper and the algorithms. A.C. contributed to evaluation of the HCV testing as well as the pre-analytical conditions. J.S.-I. made significant contributions to HBV diagnostics and OBI. U.K. wrote essential parts on virus inactivation and hemodilution. S.L.-S. assessed compliance with national and European guidelines.

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