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Prevention of transmission of HIV by organ and tissue transplantation

HIV testing protocol and a proposal for recommendations concerning donor selection

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Abstract. Human immunodeficiency virus (HIV) can be transmitted by solid organ and some forms of tissue transplantation. Although routine screening of organ and tissue donors for anti-HIV antibodies was implemented in most Western European countries and North America in 1985, several recent case reports indicate that a definite, albeit very small, risk of HIV transmission still remains. The screening tests that are currently used cannot rule out a false-negative test result occurring during the window period. Moreover, massive transfusion of the donor during the donor procedure may result in an undetectable anti-HIV antibody titer (by dilution of donor blood) that consequently leads to a false-negative test result. These risks of HIV transmission via transplantation and important issues in HIV testing are discussed in detail. Furthermore, several recommendations for the prevention of transmission and a protocol for HIV testing for both organ and tissue donation are presented. These may serve as intermediary guideliness until official ones, such as already exist for blood donation, are defined by the transplantation communities. The exclusion of donors whose behavior may place potential recipients at risk for HIV infection is essential. A thorough heteroanamnesis of the donor's next of kin during the donor procedure should provide sufficient information about donor history to enable a decision to be made in this respect. Special attention is given to the question of whether the existing donor selection criteria for blood donation should be applied in a similar way to organ donation since the strict application of selection criteria may limit the number of available donor organs. This is highly recommended in the case of tissue transplantation, which does not usually imply a sense of urgency and, thus, allows more time for donor control and selection.

Key words: HIV, organ and tissue transplantation – Donor selection, HIV – Transmission, HIV

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HIV transmission via transplantation

To date, two major variants of human immunodeficiency virus (HIV) have been described in humans: HIV-I, which causes AIDS, and HIV-II, which causes symptoms similar to AIDS and has similar modes of transmission but may be associated with a longer natural history of disease [8, 42, 57]. HIV-II is known to be the major cause of AIDS in West Africa. It is also responsible for a small number of AIDS cases in some Western European countries [30, 51, 54, 63, 67] and these can usually be traced back to endemic disease "imported" from West Africa. In Northwestern Europe and the United States, HIV-I is the major cause of AIDS. In this report, both HIV-I and HIV-II will be referred to as "HIV".

The most common and best known ways of transmitting HIV are through sexual activity, intravenous drug use, transplacental passage from mother to child, and transfusion of infected blood products [13, 22, 32, 36]. HIV infection has also been transmitted to infants via colostrum and breast milk [64] and to women by artificial insemination [60]. Several case reports indicate yet another means of HIV transmission, namely via transplanted organ and tissue allografts [4, 7, 11, 14–16, 25, 28, 40, 41, 43, 48, 49, 55, 56]. In some cases, up to 100% of the transplanted organs of a single HIV-infected donor may transmit the virus [7]. Allograft-transmitted HIV can result in overt AIDS, and this may be enhanced by the immunosuppressive therapy and the high incidence of other viral infections in the transplanted patient [52].

HIV transmission via organ transplantation has occurred in five cases since routine HIV antibody screening was implemented in 1985. One involved a donor who received multiple blood transfusions during the donor procedure, resulting in the dilution of the anti-HIV antibody titer in the control sample to below a detectable level [14]. A second case involved a living related donor who had tested negative 8 months before donation and who was not retested at the time of donation [49]. In two other cases, transplantation was carried out immediately, before the results of the HIV screening test of the donor became

available [16, 55]. In yet another case, the donor was seronegative at the time of donation; the infection was shown to have become manifest during the window period and, therefore, could not have been detected by the anti-HIV antibody test [7].

Two possible mechanisms for the transmission of HIV infection via organ transplantation can be postulated [40]. First, despite flushing with a perfusion solution, a small amount of viremic blood may remain in the donor organ and this may transmit the virus. (Not all blood cells can be removed from a donor organ, not even with the best perfusion.) The infected blood may come either from the donor himself or from infected blood products that have been transfused into the donor during treatment before brain death or during the explantation procedure. Second, the donor organ tissue itself could be infected with HIV, and cell-cell transmission or shedding may be responsible for infection in the recipient, as has been shown in the transmission of other viruses [46].

In tissue transplantation, several case reports of HIV transmission have been published. Fresh-frozen, unprocessed bone allografts were reported to have transmitted HIV to four recipients, one of whom has now contracted AIDS [7, 15]. It has been demonstrated experimentally that HIV can be harbored in bone and tendon tissue [9]. Moreover, viable HIV has been recovered from bone, marrow, and tendons of patients with AIDS, suggesting that grafting of these tissues could cause HIV transmisison [44]. One case report of HIV transmission via skin grafting has been published [16]. HIV transmission via transplantation of other types of tissue allografts has not yet been reported in the literature.

It is currently considered unlikely that HIV can be transmitted via corneal graft transplantation. Although HIV has been isolated from tears, corneal tissue, aqueous humor, and conjunctival epithelium, no HIV transmission occurred when patients received a cornea transplant from HIV-seropositive donors [7, 41, 45, 47, 56].

Reports of HIV transmission via bone marrow transplantation have not been documented. It has, however, been shown that purified bone marrow progenitor cells can be infected with HIV in vitro [31]. Therefore, it is postulated that bone marrow cells, harvested from an HIV-infected donor, may also transmit the virus when transplanted.

It is important to note that an HIV infection in a transplant recipient that is unlikely to have been transmitted by the graft may have been acquired by the recipient before transplantation. This may, for example, occur when the recipient is not routinely tested for HIV during the pretransplant screening.

Donor testing for HIV

Since 1985 all potential blood, organ, and tissue donors in Western Europe and the United States are routinely tested for anti-HIV antibodies by means of a screening assay. If the test outcome is dubious or positive, the result can be confirmed by a confirmation assay. Only the outcome of this confirmation assay is considered to be fully reliable and can give definite information on the HIV status of the donor [57]. The currently available assays for anti-HIV screening and confirmation are listed in Tables 1 and 2.

Table 1. Currently available assays for anti-HIV screening

Anti-HIV screening assays	Duration[a]
1 ELISA(enzyme-linked immuno-sorbent assay); usually HIV I/II combined	3–4 hours
2 Chemyluminescence	2 hours
3 Gelatin coated particle agglutination assay	2 hours
4 Mini-ELISA for citotesting	30 minutes

[a] From the product information inserts of various commercial test kits

Table 2. Currently available HIV confirmation assays

Anti-HIV confirmation assays	Duration[a]
Western blot	3–4 hours
Recombinant HIV I/II immunoblot	3–4 hours
PCR (polymerase chain reaction)	4–6 hours

[a] From the product information inserts of various commercial test kits

False-negative screening test result

The result of the anti-HIV screening test may be false-negative for three reasons:

1. The HIV screening test is designed to detect circulating anti-HIV antibodies. If anti-HIV antibodies have not yet developed to a detectable level in the donor serum, the test outcome will be false-negative with regard to whether the donor has been infected. The time between primary infection and detectable seroconversion is called the "window period" and is currently estimated to range from 4 to 26 weeks [37], although it has been reported that HIV provirus can be present for up to 35 months before seroconversion takes place [38].

Blood and organs that have been donated during the window period are able to transmit HIV [7, 33, 39, 66], as demonstrated by the following case report [7]: In October 1985, 4 vascular organs, 54 tissue grafts (including 2 corneas, 4 fresh-frozen, unprocessed musculoskeletal allografts, 38 treated bone allografts, and 10 treated soft-tissue allografts), and several vials of bone marrow from a male organ donor who died of a fatal head injury were harvested in a Virginia hospital. Before the man's organs and tissues were removed, his blood was screened twice and tested negative for HIV infection using FDA-licensed HIV antibody tests. The donor received no blood transfusion prior to the collection of blood specimens for serological testing.

After reports of HIV infection in recipients of these donor organs and tissues, tissue samples from the donor, including archived lymphocyte suspensions, tested positive with the sensitive polymerase chain reaction (PCR) testing technique, which detects virus nucleic acid sequences. Three recipients of vascular organs from this donor

died from AIDS-related conditions within a few years after transplantation. One of the organ recipients died of surgical complications. In addition, three recipients of unprocessed, fresh-frozen musculoskeletal allografts were reported to be infected with HIV. The remaining tissue graft recipients all tested negative for anti-HIV antibodies. The donated bone marrow vials were not transplanted.

The nature of the routinely used HIV screening test implies that it is not possible to detect an HIV infection during the window period. The test result will consequently be false-negative. New screening test techniques may detect antibodies even earlier during seroconversion, when only a few anti-HIV antibodies are present in the circulation [53, 61]. However, while in the window period, a donor infection can only be effectively discovered by a sensitive HIV antigen test, or the PCR technique [57].

It has been shown that soluble antigen (p24, gag polypeptide specificity) of HIV may be found in the circulation during the early stages of infection [1, 35]. It has therefore been suggested that blood donors be tested for this antigen to detect infection earlier than is possible with the currently used screening test. Practically speaking, however, in blood donors this does not give a substantially better detection rate [2, 3, 10]. The possible implementation of concurrent, routine screening for p24 antigen in organ donors, in addition to antibody screening, is an issue still open for discussion.

2. A large number of transfusions given to the potential organ or tissue donor prior to HIV screening may result in a false-negative test result. Massive transfusion may dilute the blood of the infected donor to below a detectable level of anti-HIV antibodies. Just how many transfusions (i.e., volume) it takes to cause such a dilution of donor blood is not known at this time.

This very phenomenon was reported in a North Carolina hospital in 1986 [14]. A cadaveric organ donor, who was admitted with a polytrauma, received 56 units of blood and blood components during the explanation procedure. A blood sample collected immediately following these transfusions was tested and found to be negative for HIV antibodies. Two days later, the organs were removed and transplanted. Blood samples drawn at explantation, however, were later found to be HIV-seropositive. A blood sample that was collected upon hospital admission, i.e., before any transfusions were administered, was later tested and also found to be seropositive. These test results were all confirmed by Western blot analysis.

To prevent this cause of a false-negative result, it is recommended that a pretransfusion blood sample always be used for the HIV screening test (e.g., the sample that has been collected upon admission to the donor hospital). This recommendation should also be applied to nonheart-beating donors, who constitute most tissue (non-organ) donors.

3. The HIV screening test result may be false-negative when the donor has received an HIV-infected blood transfusion during the donor procedure. The chance of receiving an HIV-infected blood product in Western Europe nowadays is, however, very small [21, 34]. To minimize this risk, conservative transfusion therapy and, in particular, limited use of fresh whole blood may be reasonable in donor management.

Table 3. HIV testing at the regional blood bank of Leiden, the Netherlands

Blood bank Leiden 1985–1991	Blood donors	Organ donors
Number of HIV screening tests performed	156000	4700
Intermediate or positive (first screening)	1260	67
Intermediate or positive (second screening)	246	1
Positive confirmation test	1	1

False-positive screening test result

The currently used HIV screening tests are, above all, designed for maximal sensitivity (all available tests > 99.3 %) [24]. Most HIV screening tests are relatively specific as well (> 99.8 %). However, because of the large number of screening tests performed routinely and the very low prevalence of HIV seropositivity in the donor population, a relatively large number of false-positive test results can still be expected [57]. This is illustrated by data from the regional blood bank of Leiden, The Netherlands (Table 3), which demonstrate that the number of confirmed (true) positive test results is usually very low compared to the number of initial reactive test results. It should be mentioned that the number of false-positive results varies from laboratory to laboratory, depending on the test kits in use, test sample preparation, and local factors.

A false-positive result of the HIV screening test can be caused by:

1. Hemoglobin in the test serum

2. Crossreactive antibodies:
(a) antibodies against polypeptide sequences that are encoded by the viral genome but that do not reflect actual infection with HIV [23].
(b) antibodies against HLA antigens originating from the tissue culture that was used to grow the virus for the screening test. This has been demonstrated in α-HLA DR4 sera tested in an HIV-lysate ELISA, based on "H9" spleen cell cultures.
(c) nonspecific antibodies or those with an affinity for polystyrene surfaces.

3. Anti(human)globulin enzyme conjugate that reacts nonspecifically with nonimmunoglobulin serum components and/or the ELISA carrier

4. Clots or microaggregates

It is practically impossible to prevent these causes of a false-positive outcome. To prevent the incorrect exclusion of donors, it is therefore strongly recommended that a du-

plicate repeat be performed when the screening test result is positive or intermediate [17]. When both these repeats are negative, the donor can still be used since it has been shown that a nonrepeatable reactive test finding is not associated with an increased risk of transmission [65]. To save time during a heart-beating organ donor procedure, the duplicate repeat should be performed simultaneously by using the same testing technique with, if possible, two different blood samples.

Since many of the causes of a false-positive result are sample-dependent, it is recommended that the routine testing procedures be validated with serum samples collected under identical medical and logistical conditions as expected during an organ explanation procedure.

The risk of HIV transmission via organ and tissue transplantation

The advent of routine donor screening for anti-HIV antibodies in 1985 has greatly reduced the risk of HIV transmission via organ and tissue transplantation in Western Europe and North America. However, false-negative screening test results, in particular those occurring during the window period, cannot be entirely eradicated. This has been demonstrated in several recent case reports of HIV transmission via transplanted organs and tissues [4, 7, 11, 14–16, 25, 28, 40, 41, 43, 48, 49, 55, 56]. A reliable estimation of this risk cannot be made since the prevalence of HIV infections that are still in the window period among the continuously increasing [29] number of HIV infections in the total population is unknown.

In the area covered by the Eurotransplant Foundation – the organ exchange organization for The Netherlands, Belgium, Luxemburg, Germany, and Austria – the percentage of reported donors per year with a positive HIV screening test result is 0.1%. According to Eurotransplant's current directives, these donors are immediately discarded [6]. Note that these are the cases that were actually detected by the screening test. "Missed" cases of HIV-infected donors have not been documented in the Eurotransplant area since the beginning of the routine donor screening in 1985.

Another very important instrument for identifying potentially high-risk donors is the heteroanamnesis taken from the donor's next of kin. While gathering donor history can help to preclude high-risk donation and to further minimize the risk of HIV transmission, not every donor hospital makes this effort on a routine basis. It is therefore recommended that donor referral teams always do so, and as accurately and thoroughly as possible during every donor procedure. A previous or readily available HIV donor screening test result should have no influence whatsoever on the evaluation of donor history.

It is not yet known how stringent the donor selection criteria used for blood donation [12, 20, 68] can or should be applied to organ donation. Such guidelines are not yet available for organ donation. For European blood banks, the following donor groups have been described as high risk by the Council of Europe [20] and the World Health Organization (WHO) [68]:

1. Persons with clinical or laboratory evidence of HIV infection
2. Men who have had sexual contact with other men since 1977 (or 1980, depending on the country)
3. Past or present intravenous drug users
4. All persons who have had sexual contact with individuals, male or female, who were/are inhabitants of a country with a high prevalence of AIDS cases, i.e., the African countries south of the Sahara, after 1977
5. Persons with hemophilia or related clotting disorders who have received clotting factor concentrates
6. Persons who have had sexual contact with any person meeting the above descriptions
7. Men or women who have engaged in prostitution since 1977
8. Persons transfused with blood in the past 12 months

In the United States, the following group is added [12]:

9. Persons treated for syphilis or gonorrhea in the past 12 months

One consequence of applying these selection criteria for risk behavior as strictly to organ donation as to blood donation would obviously be a reduction in the number of available organs since the slightest suspicion of high-risk behavior often results in immediate donor exclusion. The heteroanamnesis of relatives during the donor procedure may, however, be incomplete or unreliable. For ethical reasons it would be important to weigh the still minimal risk of HIV transmission, using slightly less strict donor selection criteria, against the often serious consequences of delayed organ transplantation. The current shortage of organ donors causes considerable waiting times for transplantation, with the result that a number of patients die while still on the waiting list for vital organs such as the heart and liver. On the other hand, the question could be raised of whether it is ethically and/or legally acceptable for a recipient to receive an organ transplant when not all possible measures to minimize the risk of a "missed" HIV transmission have been taken (i.e., by not using donor selection criteria as strictly as possible).

Currently, the acceptance or rejection of a donor "suspected" of risk behavior merely depends on the personal judgment of the doctor in charge of the explantation procedure at the donor hospital. It would seem reasonable for the transplantation communities to discuss and define guidelines with respect to this issue in a consensus meeting in the near future.

Until specific guidelines are defined, it is recommended that donors be excluded from donation when there are evident indications that the donor or the sexual partner belonged to a risk group. Such indications include needle puncture, evidence of homosexual activity or hemophilia from a "reliable" heteroanamnesis, etc. If there is the slightest suspicion of high-risk behavior, the decision to discard the donor can be case-dependent.

In contrast to solid organ donation, three different associations in the United States have defined recommendations for donor selection procedures to prevent HIV transmission via bone and other tissue donation [5]. In 1988 the Centers for Disease Control (CDC) recommended reviewing the medical and social history of every

donor via a direct interview with the next of kin and excluding high-risk donors. Routine testing for anti-HIV antibodies was also advised. In 1988 the American Hospital Association (AHA) advised exclusion of donors who practiced high-risk behavior, routine screening for anti-HIV antibodies on a blood sample prior to the donor's receipt of any blood transfusions, and performing a physical examination on the donor to determine obvious evidence of HIV infection. In 1989 the American Academy of Orthopedic Surgery (AAOS) made recommendations with respect to bone donation. These included: (1) taking a social and medical history of the donor and excluding high-risk donors, (2) donor testing for anti-HIV antibodies and for HIV antigen (including a PCR when available) and (3) a complete autopsy to determine whether the donor had obvious symptoms of AIDS, including a detailed histological study of lymph nodes.

In addition to these donor selection and testing procedures, tissue banks can store most tissue allografts for long periods of time, allowing for further processing or sterilization. As with viral inactivation steps for plasma products in blood donation, these tissue processing procedures may reduce, and sterilization even eliminate, the risk of transmitting HIV, as demonstrated in bone allografts [5, 50, 58]. Processing of tissue allografts may take the form of freeze-drying, ethanol treatment, or demineralization. Sterilization may be performed by gamma irradiation, ethylene oxide, or heat treatment [18, 19, 27, 59, 62]. Some tissues, however, are not able to withstand these procedures without losing their viability [26] and, therefore, are generally not subjected to sterilization. To further minimize the risk of HIV transmission via tissue transplantation, it is recommended that tissue processing or sterilization procedures be carried out on the appropriate tissue grafts in addition to routine HIV screening and the exclusion of high-risk donors by thorough heteroanamnesis. Given the general lack of urgency in tissue transplantation, it is recommended that the donor selection criteria regarding HIV risk behavior be applied as strictly to tissue donation as they are to blood donation.

HIV testing protocol for organ and tissue donation

The donor referral team may perform donor HIV testing according to the following protocol:

Blood, organ, and tissue donation is permitted when a donor has been cleared for donation on the basis of the information obtained from the donor history and when the first (i.e., routine) HIV screening test result is negative. If the screening test result is intermediate (dubious) or positive, this outcome may be false-positive. To verify this, the test should be repeated twice [17] on different donor blood samples (if available). In this way incorrect exclusion of donors can be limited, which may help to reduce the current shortage of donor organs. The donor is considered to be not infected when the outcome of both the first and the second repeat is negative and, in that case, donation of blood, organs, and tissues is permitted. If the result of one or both of the repeats is again intermediate or positive, a confirmation test can be performed to provide a definite answer as to whether the screening result was false-positive or not. However, to ensure that optimal safety measures are taken, blood donations will, in this case, be discarded without performing a confirmation test, although this would be practically possible. In organ donation a confirmation test is usually not possible since an organ donor procedure allows only very limited time for this test. In tissue donation the time schedule is very flexible, thus allowing for confirmation testing. Even when the outcome of the confirmation test is intermediate, a

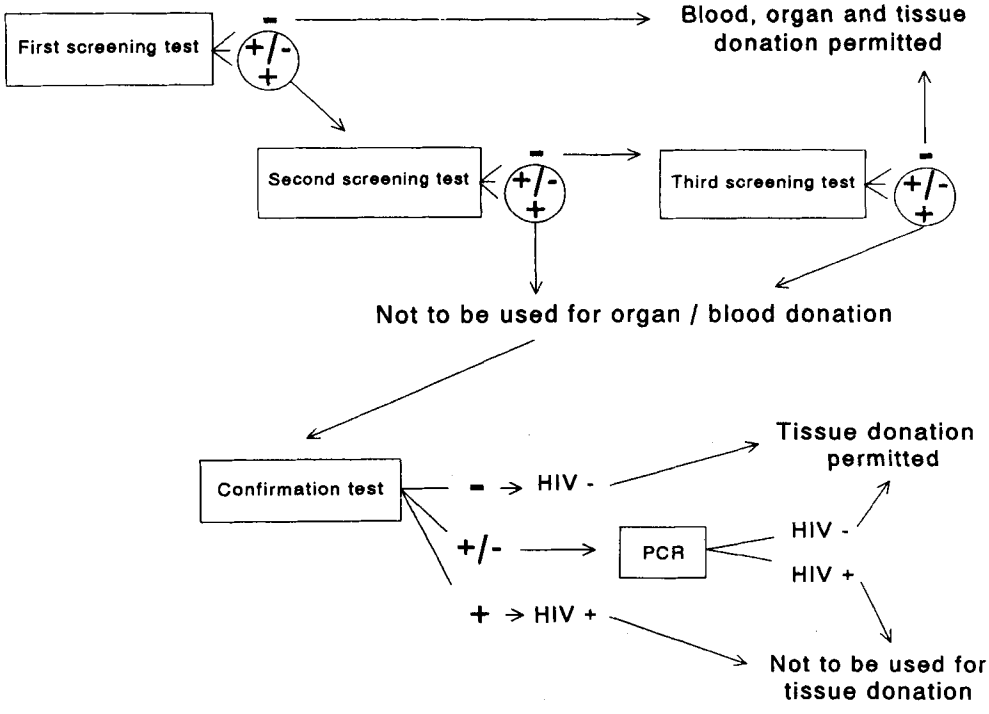

Fig. 1. HIV testing protocol for organ and tissue donation

negative result of the very sensitive PCR technique may still allow tissue donation.

Conclusion

HIV can be transmitted by solid organ and some forms of tissue transplantation. Although routine screening of organ and tissue donors for anti-HIV antibodies was implemented in 1985, several reported cases show that the risk of HIV transmission has not yet been completely eliminated. In these cases, the false-negative screening test result was caused either by HIV infection occurring during the window period (the 4–26 weeks from primary infection to seroconversion) or by dilution of the donor's blood by massive transfusion during the donor procedure, resulting in an undetectable anti-HIV antibody titer. The change of a false-negative screening test result and the consequent risk of HIV transmission is thought to be very small but definite, and will thus remain a serious concern of blood banking and transplantation communities.

The risk of a "missed" donor infection arising during the window period may only be further reduced by using very sensitive HIV antigen testing techniques (or PCR); however, this is not feasible in routine HIV screening. To avoid a false-negative test result caused by massive transfusion during the donor procedure, it is recommended that a pretransfusion blood sample always be used for the HIV screening test (also when nonheart-beating donors are concerned). The blood sample that is routinely collected upon admission to the donor hospital could serve this purpose.

In any case, it is essential that the donor referral team apply strict selection criteria prior to accepting potential donors. A donor should be excluded when, on the basis of a thorough heteroanamnesis on the donor, it is suspected, that he/she belonged to a group of persons whose behavior posed a risk for HIV infection. The question of how stringent the existing selection criteria for blood donation should be applied to organ donation remains unanswered. Very strict application of these criteria would, on the one hand, lead to a further reduction in the risk of HIV transmission. Information on donor history may, however, be incomplete or unreliable, which could easily result in false suspicion of risk behavior and the consequent exclusion of the donor, which would lead to an unnecessary loss of donor organs. The transplant communities should therefore be encouraged to address this issue in the near future and to define selection criteria specifically for organ donation. Meanwhile, it is recommended that a potential organ or tissue donor be turned away when there are evident indications from physical examination or from a "reliable" heteroanamnesis that the donor belonged to an HIV risk group. For tissue donation it is recommended that the same stringent selection criteria and high-risk group definitions be used as for blood donation since tissue transplantation does not usually imply a sense of urgency, allowing for a thorough donor selection procedure. It is also recommended that tissue graft processing and/or sterilization procedures be followed when appropriate.

It is recommended that HIV testing be carried out according to the protocol presented here, using licensed and appropiately validated assays. A reactive screening test result should be followed by a duplicate screening test repeat to prevent false-positive test results and, consequently, incorrect donor exclusion. Organ and tissue donation should be permitted when both the first and the second test result is negative. Organ donors with repeated reactive screening test results should be turned away. Tissue donation, even with a positive screening test repeat, should still be permitted, provided a subsequent confirmation or PCR test result is negative.

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