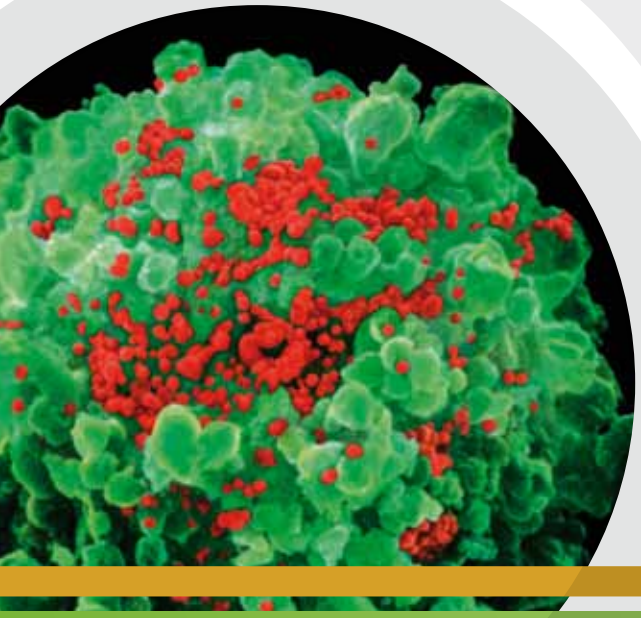

TECHNICAL REPORT

Risk assessment of HTLV-I/II transmission by tissue/cell transplantation

Part 2: Risks by tissue type, Impact of processing and effectiveness of prevention measures

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Part 2: Risks by tissue type, impact of processing and effectiveness of prevention measures

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Contents

Abbreviations

AATB	American Association of Tissue Banks
ATL/ATLL	Adult T-cell leukaemia/lymphoma
CLIA	Chemiluminescence Immunoassay
CoE	Council of Europe
ECDC	European Centre for Disease Prevention and Control
EIA	Enzyme Immune Assay (= ELISA)
ELISA	Enzyme Linked Immune Sorbent Assay (= EIA)
EUTCD	European Union Tissues and Cells Directive
FDA	Food and Drug Administration (United States)
HAM/TSP	HTLV-I Associated Myelopathy/Tropical Spastic Paraparesis
HCT/Ps	Human Cells, Tissues, and cellular and tissue-based Products (US regulation)
HTLV	Human T-lymphotropic Virus (used to describe all types)
HTLV-I	Human T-lymphotropic Virus, type I
HTLV-II	Human T-lymphotropic Virus, type II
HTLV-Ab	Antibodies specific for HTLV
NAT	Nucleic Acid Test
PBMC	Peripheral Blood Mononuclear Cells
PTLV	Primate T-lymphotropic Virus(es)
PVL	Pro-viral Load

1 Executive summary (Part 2)

Introduction

An important part of ECDC's function is to provide its stakeholders with scientific advice upon their request. On 2 August 2010, ECDC received such a request from the European Commission's Directorate-General for Health and Consumers (DG SANCO) – Directorate C Public Health and Risk Assessment.

ECDC was asked to assess the epidemiological history of Human T-lymphotropic Virus (HTLV), possible risks of HTLV transmission through transplantation of human tissues and cells, and the possible measures to prevent such transmission, in particular with regard to tissues and cells imported from the United States. Testing for HTLV-I/II infection among most tissue and cell donors was recently discontinued in the US.

In the European Union, testing for HTLV-I is required for tissues and cells donors living in, or originating from high-incidence areas, or with sexual partners originating from those areas, or where the donor's parents originate from those areas.

Methodology

In accordance with its internal procedures for providing scientific advice, ECDC addressed the question by setting up an ad hoc expert group of internal and external experts in the field. The expert group was set up in cooperation with the EU Commission's DG SANCO (Directorate C Public Health and Risk Assessment)and the EU Competent Authorities for Tissues and Cells. However, in accordance with its internal procedures, ECDC selected experts based on their individual scientific and professional qualifications.

After receiving clarifications on specific questions from the Commission, ECDC performed a literature review to capture all evidence available. The expert group then reviewed the scientific evidence, assessing its validity and generalisability in relation to the question asked.

ECDC is presenting the results of its assessment in two separate technical reports (Parts 1 and 2), of which this is the second part. This report addresses the potential risk of HTLV transmission through transplantation of human tissues and cells and possible measures to prevent such transmission, in particular in relation to tissues and cells imported from the United States.

Results and conclusions

The predominant modes of transmission for HTLV-I are blood components, infected mother's breast milk, sexual intercourse and the sharing of injection equipment among injecting drug users. One major difference in transmission risk compared to many other blood-borne infections is the exclusive association between transmission and the presence of infected cells of lymphocyte origin in blood and tissues. As almost no free virus is produced in vivo, cell-free blood components do not transmit infection. Refrigerator storage for a period of over two weeks seems to remove the risk of transmission in cell-containing blood components due to loss of viability of the lymphocytes. This is one reason for performing leukocyte reduction in blood service facilities. Transmission by infected cells only in the case of blood transfusion was found to be well supported by scientific evidence.

Transplanted tissues and cells as well as organs have been shown to transmit HTLV-I infection. According to both the AATB Standards and the FDA requirements[1], only those donors whose donations of cell and tissue are 'rich in viable leukocytes' need to be tested for HTLV-I/II infection. This is derived from experience with blood transfusion. A threshold of a total 10^8 cells of viable leukocytes is considered by the AATB as indicative of transmission risk.

Assessment of the evidence could not conclude whether the findings for blood and blood components can be generalised to tissue and cell preparations of non-blood origin. Evidence for a generic threshold of a total 10^8 cells of viable leukocyte class was found to be insufficient. The use of the current threshold of 10^8 cells of viable leukocyte class to determine tissue safety in relation to HTLV-I/II transmission may still place individuals receiving cell and tissue transplants at risk of infection with the viruses.

The US FDA gives some guidance as to which cell and tissue types should be considered 'rich in viable leukocytes'. ECDC's ad hoc expert panel considered the US classification insufficient to remove the risk of HTLV transmission. Similarly, claims that tissue and cell processing remove the risk were considered difficult to evaluate without

[1] FDA Final Rule, Eligibility Determination for Donors of Human Cells, Tissues, and Cellular and Tissue-Based Products, dated 25 May 2004 (effective 25 May 2005).
http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Tissue/ucm073964.htm

transparent validation of processing methods. Given the epidemiological situation in the USA, testing should be required until methods have been validated.

The ECDC ad hoc expert panel concluded that donor selection by risk factor exclusion is an inefficient strategy for reducing transmission risk, except when evaluating clinical contra-indications. The ad hoc expert panel suggests that donor examination cannot replace HTLV-I/II testing, particularly in the case of donors originating from endemic regions.

While only a single test for HTLV infection is currently FDA-approved on the US market, the review showed that the performance characteristics of the HTLV-I/II screening tests available on the EU market are good, especially when confirmatory testing is systematically applied. This is routine in the EU, but is not performed in the USA. No confirmatory tests for HTLV have been approved for diagnostic use on the US market by the US FDA.

The review shows that tests are available which, if they received FDA approval, would be technically suitable for testing tissue donations, as illustrated by the fact that they are used in Europe and other parts of the world.

Considerations

Based on examination of the evidence, ECDC suggests the following for the consideration of the EU Commission's Directorate-General for Health and Consumers (Directorate C Public Health and Risk Assessment) and the EU Member States Competent Authorities for Tissues and Cells.

- Consider discouraging use of the threshold value of 10^8 leukocytes for safe lymphocyte content. Recommend studies to assess transmission risks related to lymphocyte content and discuss the validity of the threshold with the AATB and other relevant US actors.
- Motivation: if a threshold value for lymphocyte content is desirable as a risk assessment parameter for determining the transmission risk of HTLV-I/II infection, it should be based on relevant studies with strong statistical evidence. The current evidence for the threshold is insufficient to justify safe use of the threshold value for risk assessment of HTLV-I/II transmission in relation to cell and tissue donations. Such studies may not be easy to organise, but could possibly be performed by combined look-back and tissue type assessment studies. If lymphocyte content by tissue type and the type of donation are known, it could be possible to study the issue by retrospective studies.
- If testing is implemented among blood donors, consider recommending HTLV-I/II testing of tissue and cell donors.
- Motivation: prevalence of infection among tissue and cell donors is shown to be higher than among blood donors. Therefore testing of tissue and cell donors could be cost-effective if testing is implemented for blood donors.
- Consider discussing with US regulators the availability of HTLV-I/II tests and the need to apply a confirmatory test to improve positive predictive values.
- Motivation: availability of a suitable test on the US market is a prerequisite for the application of EU testing requirements. As the withdrawal of the only suitable test from the US market made it impossible to follow existing testing requirements, arguing for the approval of another test could be justified on the grounds of patient safety. It should be noted that there are several categories of test that the FDA allows and the 'approved' category may not be the only option. Furthermore, neither the current nor the discontinued test had been approved for cadaveric sample use.
- Consider requiring validation in accordance with transparent, predetermined standards for the processing of tissue and cell materials to remove HTLV-I/II transmission risks and applying these to all untested materials imported to the EU.
- Motivation: In contrast to EU requirements, ethnic minorities among whom HTLV-I/II infection may be endemic and migrants from high-prevalence areas are not directly addressed when assessing donor eligibility in USA (despite the fact that living in Europe for over five years results in deferral due to concerns over variant Creutzfeldt-Jacob disease transmission risk). There has been a decision to abolish HTLV-I/II testing in USA, despite the fact that the epidemiology of the infection does not differ significantly from that in the European Union. It may not be feasible to reintroduce testing simply for the purpose of imports to the EU. However, the claim that processing removes the transmission risk is poorly documented and evidence for the various methods used is not readily available. This evidence needs to be made available for validation. Alternatively, donors should continue to be tested.
- Consider recommending a comprehensive evidence-based review of lymphocyte content in tissues relevant for donation
- Motivation: comprehensive, evidence-based information on lymphocyte content in tissues relevant for donation would aid risk assessment and enable better planning of guidance for donor testing. Competent authorities for tissues and cells may have the necessary expertise for such an evaluation.

2 Request from the European Commission

On 2 August 2010, ECDC's Director received the following request from the European Commission's Directorate-General for Health & Consumers (DG SANCO) – Directorate C 'Public Health and Risk Assessment' (transcript):

Dear Dr Sprenger,

Subject: Request for ECDC advice on testing requirements for Human T-lymphotropic virus (HTLV) for tissue and cell donors

For a number of years the American Association of Tissue Banks (AATB) has required systematic testing for antibodies to human T-lymphotropic virus type I and type II (HTLV-I/II testing) for donations of tissues and cells occurring on US territory. Recently the AATB board agreed to change standards for tissue banking in order to remove the requirement to test donors of processed conventional human tissues (e.g. bone, tendons, ligaments, skin, heart valves etc). However, donors of viable leukocyte-rich tissue (e.g. semen, hematopoietic stem/progenitor cells) must continue to be tested and found to be negative for anti-HTLV-I and anti-HTLV-II to be considered suitable for release for transplantation. According to AATB this decision harmonises HTLV testing with US Food and Drug Administration (FDA) regulations which require HTLV test only for donors of viable, leukocyte-rich tissues and cells.

More information is available on the AATB website:

http://archive.constantcontact.corn/fs076/110205S357439/archive/l102820896575.html

Directive 2006/17/EC requires that HTLV-I antibody testing is performed for donors living in, or originating from, high incidence areas, with sexual partners originating from those areas or where donor's parents originate from those areas. Several Member States have HTLV testing required for human tissues and cells imported from the United States.

The recent change to the AATB standards was discussed at the joint meeting of the Competent Authorities and Regulatory Committee on tissues and cells which was held in Brussels on 20–21 May 2010 and several Member States expressed concerns about the change of AATB's testing requirements for HTLV and the potential impact on the safety of human tissues imported to the EU from the United States. In addition it became clear that different EU Member States apply different testing requirements regarding HTLV. Several Competent Authorities have therefore agreed to meet later this year in order to assess this situation and the potential consequences for tissue collection in Europe.

We herewith send you a letter prepared by the Human Tissue Authority (HTA, UK) summarising the problem and listing some questions for discussion, as well as background information collected by HTA.

We would like ECDC to contribute to this discussion later this year and therefore to assess the situation and questions in the letter by HTA. These cover the epidemiological history of HTLV, the possible risks for HTLV transmission through transplantation of human tissues and cell, and the possible measures to prevent such transmission, in particular regarding tissues and cells imported from the United States. Considering the planned timing we would appreciate if ECDC could complete its assessment by 15 October 2010.

Yours sincerely,

Signed Andrzej Rys

3 Background and methods

Legal authority

According to the founding regulation of ECDC, Regulation (EC) No 851/2004[2] Art 9(2), 'the Centre may be requested by the Commission, the Member States, third countries and international organisations (in particular WHO) to provide scientific or technical assistance in any field within its mission. Scientific and technical assistance provided by the Centre shall be based on evidence-based science and technology.'

ECDC shall:

- search for, collect, collate, evaluate and disseminate scientific data (Art 3(2)(a));
- provide scientific opinions and timely information (Art 3(2)(b),(c));
- exchange information, expertise and best practices (Art 3(2)(e)); and
- facilitate the development and implementation of joint actions (Art 3(2)(e)).

Evidence-based public health

Evidence-based decision-making in a public health setting has to carefully incorporate the best available scientific evidence from research and other reliable sources with considerations of values, perceived needs and recourses in the given context. Evidence-based medicine is often defined as the integration of expertise, values, and the best available evidence into the decision-making process [1].

A public health decision may be rather complex and needs to take several determinants of health into account, such as genetic factors, lifestyle, physical environment, socio-economic conditions, biological environment and health services at different levels [2].

Only some of these factors are relevant to the prevention and control of HTLV I/II in tissue and cell donations.

Evidence-based methodologies

ECDC has tried to compile this risk assessment in accordance with the following procedure applying evidence-based methodologies:

- Formulate questions.
- Search for evidence.
- Assess the evidence.
- Formulate an answer.
- Disseminate and implement
- Evaluate.

[2] Regulation (EC) No 851/2004 of the European Parliament and of the Council of 21 April 2004 establishing a European centre for disease prevention and control. OJ L 142, 30.4.2004, p. 1.

Questions from the European Commission

After a request for clarification, the following rephrased questions were posed by DG SANCO's Directorate C – Public Health and Risk Assessment:

Question 1

What is the epidemiology of HTLV-I/II viruses, including distribution by geographic region, risk group and other factors?

Question 2

Do the risks of virus transmission differ by type of tissue/cell or processing and if yes, how do they differ?

Question 3

What is the evidence for effectiveness of measures used to prevent virus transmission through transplantation of tissues/cells?

For this part of the ECDC risk assessment (Part 2), only Questions 2 and 3 above are addressed. Question 1 was addressed in the first part of the ECDC risk assessment (Part 1).

Search strategies

This part of the risk assessment involved exploratory keyword-based searches, complemented by information retrieved from searches conducted for Question 1. As relatively few publications could be retrieved for Questions 2 and 3, ECDC relied on expert advice from the ad hoc expert panel to develop this part of the risk assessment.

To ensure that the questions posed by the Commission were searchable in electronic databases, they were split into the following subcategories:

Population: donors of cells and tissues, living and cadaveric. (In addition, donors of blood/blood components and pregnant women were identified as additional populations for which screening programmes in many countries may produced relevant comparative data).

Interventions: testing for HTLV-I/II infection, exclusion by clinical and risk factor criteria, exclusion by lymphocyte content.

Comparison: US/North American versus EU donors, donors in the rest of the world.

Outcome: infection due to transfusion, tissues and cells donation or receipt of blood components, prevalence of HTLV-I/II infection, implications for safety of human tissue- and cell-derived products imported to the EU.

Reviews and original research articles were retrieved from PubMed, Embase and the Cochrane Library bibliographic databases on 27 January 2011 for Question 2.

The concepts used in the systematic search were taken from the controlled vocabulary available in the bibliographic databases (i.e. MeSH and Emtree terms). These were complemented with multiple field search combinations by using natural vocabulary (i.e. keywords). The retrieved records were in several languages, but with a majority in English. Some more relevant studies were selected from reading reference lists.

Results from primary searches were screened by reading titles and abstracts (when available). Studies were selected for full reading according to relevance for the different questions. Selection criteria were decided by a group of reviewers. One reviewer read the articles, but questions and uncertainties were discussed by a group of reviewers. Due to time constraints it was not possible to retrieve all potentially relevant articles from reference lists. Some relevant articles without English abstracts as well as reports in the grey literature might also have been missed.

Question 3 was mainly evaluated through consultation with the expert panel, with the exception of testing, for which references were identified through a keyword search. A PubMed search using the criteria *HTLV AND screening AND evaluation* was performed on 11 September 2010. Articles dated before 1995 were excluded because of technical developments in the field. This literature search generated 195 publications. Ten articles summarising testing procedures and evaluations of commercial tests for HTLV diagnostics were selected.

Studies will be categorised according to the following study designs: reviews, trials and observational studies. The observational studies were sub-classified into the following categories: cohort studies, case series, case–control studies, case studies, cross-sectional studies, time series and 'before and after' studies.

The following sections will be included in the evidence table to be produced for the final risk assessment:

- Bibliographic citation
- Type of study
- Number of patients or size of population
- Study outcome
- Strengths of study
- Limitations of study.

Assessment of the evidence

Validity: to assess the validity of a study is to evaluate whether the results of the study are trustworthy. In this study, one of the problems was that comprehensive incidence and prevalence data on HTLV-I/II is currently not available in Europe or the US/North America since infection by this virus is not subject to systematic surveillance schemes in either region. Few studies on HTLV-I/II transmission risks are available for the population under review (donors of cells and tissues), especially from Europe. Tests used for sero-epidemiological studies may provide inflated prevalence due to low test specificity, particularly if there is no confirmatory testing in the very low prevalence populations.

Generalisability (external validity): to assess external validity or generalisability is to evaluate whether the studies are transferrable to other settings or circumstances. In this assessment the challenges were connected to uneven comparability of different studies on HTLV-I/II and lack of epidemiological studies on the exact study population.

Grading of evidence according to strength of documentation: an evidence-based approach implies trying to draw explicit conclusions and building on the best available evidence, thus giving more weight to the studies which are of the highest quality and employ the most robust methods. The problems faced in this risk assessment were connected to a lack of studies and systematic reviews for the regions concerned. For some of the questions, the reviewers had to start by examining studies assessing populations (i.e. blood donors) different to the study population. Nevertheless, such studies can be useful, even if their design indicates that little weight can be given to the evidence.

Obtaining expert advice: ECDC ad hoc expert panel

While performing initial evidence assessment based on explorative and systematic literature searches for Questions 1-3 as described above, the ECDC internal review group recognised that expertise within the Agency was insufficient to properly address certain elements of Questions 2 and 3. Preliminary results were presented to the EU Commission Competent Authorities on Tissues and Cells at a meeting in Brussels on 6–7 December 2010. At this meeting, it was agreed that the Commission (Directorate C Public Health and Risk Assessment) would request nominations for national experts from the Competent Authorities on Tissues and Cells to support the ECDC risk-assessment work.

The Commission (DG SANCO Directorate C Public Health and Risk Assessment) made a request for potential experts and nominations by the Competent Authorities on Tissues and Cells were sent directly to ECDC. On the basis of its internal procedures[3,4], ECDC selected nine experts by reviewing their scientific and professional qualifications and ruling out any potential conflicts of interest. Following their appointment to the ad hoc expert panel by the ECDC Director, the selected experts were invited to review ECDC work and attend an expert meeting in Stockholm on 4 March 2011. Seven experts attended the ECDC meeting and their advice has been incorporated into this risk assessment. A separate meeting report will also be made available.

References: Background and methods

1. Straus SE, et al. Evidence-Based Medicine. How to Practice and Teach EBM. 3rd ed. Churchill Livingstone; 2005.

2. Gray M. Evidence-based Health Care and Public Health: How to Make Decisions About Health Services and Public Health. 3rd ed. Churchill Livingstone; 2009.

3 Internal procedure (work instruction) on handling requests for scientific advice at the European Centre for Disease Prevention and Control. ECDC/SAU/001 – rev. 1. Issue date (revised): 1 February 2011

4 ECDC policy on declarations of interest and handling of potential conflicts of interest. Draft endorsed by ECDC Management Board at its 20th meeting, Stockholm, 9–10 November 2010 (Agenda item 14)

4 HTLV-I/II infection and disease

Etiological agents

Human T-lymphotropic virus types I and II (HTLV-I and II; also denoted as HTLV-1 and 2) are two closely related retroviruses belonging to the Retrovirus family and the *Deltaretrovirus* genus. They belong to the Primate T-lymphotropic viruses (PTLVs) along with a number of simian counterparts. While HTLV-I and II are retroviruses, they differ from the *Lentivirus* genus to which the more common human immunodeficiency virus types 1 and 2 (HIV-1 and 2) belong. Recently, two new genetically distinct, but closely related viruses have been described from Africa (and named HTLV-III and IV), but their epidemiology and disease-causing properties are as yet unknown [1]. Therefore, and since they seem very rare, they are not included in this risk assessment.

HTLV-I and II are RNA viruses which can reverse transcribe their genome into DNA and integrate into their host T-lymphocytes. Infection with both viruses is chronic and lifelong and only a fraction of those infected eventually develop disease (see below). No vaccine against infection by either of the viruses.

Infection can be detected in the laboratory for diagnosis and epidemiological studies using a variety of methods, most commonly by demonstrating anti-HTLV I and II antibodies through serological assays. The serological screening assays do not distinguish between HTLV I and II infection and confirmatory testing is necessary to distinguish the variants and diagnose infection. Polymerase Chain Reaction (PCR) based tests have also been developed and can be used to quantify the viral load in blood samples. Virus isolation is possible but labour intensive.

Disease presentation

Infection with HTLV-I has been linked to two major diseases: HTLV-I associated myelopathy/tropical spastic paraparesis (HAM/TSP) and adult T-cell leukaemia/lymphoma (ATL/ATLL) [2, 3] as well as several other inflammatory conditions. Clinical presentation of HAM/TSP includes muscle weakness in the legs, hyperreflexia, clonus, extensor plantar responses, sensory disturbances, various urinary manifestations, impotence, and low-back pain [3]. High titres of HTLV-specific antibodies are found in the blood and cerebrospinal fluid of those with the disease. Other uncommon inflammatory disease associations include infective dermatitis, uveitis, myositis, and HTLV-associated arthropathy [3]. Adult T-cell leukaemia/lymphoma is associated with the malignant proliferation of transformed leukocytes carrying HTLV-I provirus.

Development of HTLV-I/II associated disease is generally slow and most of those infected remain asymptomatic for life. The average age of diagnosis is 40 which is commonly preceded by adult-acquired infection. The lifetime risk of HTLV-I carriers developing HAM/TSP varies according to geographic and ethnic determinants. Lifetime risk of HAM in Japan has been estimated at 0.25% [4], in Jamaica and Trinidad at 1.8% [5] and in the UK at 3%, based on incidence /prevalence data [6]. Prevalence of HAM (including incident cases) among US blood donors during follow up for up to 10 years was 3.7% [7]. The risk of disease development is increased among transfusion and transplant recipients, who may develop the disease with a much shorter incubation time, particularly in case of immune suppression [8-10]. The age-standardised incidence rate is 2/100 000 person-years, with a higher risk in females [8].

It is estimated that 2–7.3% of those infected with HTLV-I develop ATLL during their lifetime, although the proportion varies in different parts of the world and by gender [8, 11-14]. Infection in early life appears to be important for the development of ATLL [8,11]. The incidence rate is 2–4/100 000 person-years, with a higher risk in males than females, the direct opposite to the sex-ratio of HAM/TSP [8]. In Japan average age of onset is 60 years, while in Trinidad, Jamaica and Brazil it is 40 years [8]. The difference is unexplained but has been suggested to be dependent on host factors, among other possibilities [8].

Infection with HTLV-II has not been associated with malignancies; however, it has been associated with a neurological disease resembling HAM/TSP. The estimated lifetime risk of disease development for HTLV-II-infected persons is unknown but appears to be less than that estimated for persons with HTLV-I infection. While the majority of HTLV-II-infected persons remain asymptomatic (>95%) from the virus infection per se, recent studies report an increased incidence of other infectious diseases (e.g. bronchitis, kidney or bladder infections) in HTLV-1- and HTLV-II-infected persons [15].

HAM/TSP is a progressively disabling disease which frequently can have a severe impact on the quality of life of those affected. Secondary complications may lead to death after many years. No effective treatment exists against HTLV-I and II infection exists. Symptomatic or targeted treatment is still the main approach for HAM/TSP patients. Although zidovudine (AZT) and alpha interferon (IFN-α) in combination yield some response and improve ATL prognosis, better treatments need to be developed [16]. Antiretroviral treatment does not seem to have a sustained effect on proviral loads [16]. ATLL carries a poor prognosis in Europe, with a life expectancy of five months following diagnosis [17].

Transmission mechanisms and tissue/cell tropism

Both HTLV-I and II have been shown to be transmitted by blood contact, mother-to-child transmission (breastfeeding) and sexual contact, although the role of sexual transmission for HTLV-II is less clear. Unlike HIV-1/2, transmission of HTLV-I/II is more dependent on cell-to-cell contact. In the case of HIV it is possible to infect target cells using purified virions in vitro, whereas HTLV-I/II requires the co-culture of infected cells with target cells for efficient transmission. Moreover, cell-to-cell contact appears to be more important for transmission in vivo, as free virions cannot be demonstrated in plasma or serum and cell-free blood components have been shown not to transmit the infection. This is in stark contrast to HIV and Hepatitis viruses, where plasma products are infectious and high levels of virions are frequently found in the cell-free blood components of infected individuals.

For HTLV-I/II, models of transmission rely on the presence of infected cells, of which the most prominent seem to be CD4 and CD8 positive T-lymphocytes. These cells, which are part of the white blood cell (leukocyte) family, are carriers of the virus in infected individuals. In vitro models suggest that transmission between infected and uninfected cells requires physical contact and involves specific receptor molecules that interact with viral surface proteins. There is evidence that multiple cell types, such as dendritic cells, other monocyte/macrophage lineage cells and even epithelial cells found in breast milk may be important for transmission between individuals, even if infected CD4+ T-cells are most frequently detected in vivo [18-20]. The virus expands in vivo both by transformative proliferation of the infected cells and by re-infection of new cells through direct cell-to-cell contact.

The main cellular receptor of HTLV-I has been recently described and shown to be the ubiquitous glucose transporter protein GLUT-1, which is present on a wide variety of cells [21]. In addition, other cellular molecules, such as heparan sulfate proteoglycans (HSPGs), neuropilin-1 (NRP-1) and DC-specific intercellular adhesion molecule-3 (ICAM-3)-grabbing non-integrin (DC-SIGN) may contribute to the transmission mechanisms [18, 22-26]. To be able to transfer the infection from one cell to another, a specific structure dependent on cell-to-cell direct contact, sometimes called a viral synapse, needs to form [25]. This may explain the apparent inability of free virus to confer infection in vivo.

HTLV-I is readily detected in CD4+ T-lymphocytes in vivo. However, the virus has also been shown to be present in other cells of the leukocyte lineage, such as CD8+ cells, monocytes and B-lymphocytes [24]. In addition, in vitro infection has been successful for a wide variety of cells types from non-haematopoietic lineages and even non-human cells. There are models for persistent HTLV-I infection in the rat, rabbit, mouse and squirrel monkey. In these models, the virus infects and can be detected in a wide variety of haematopoietic and non-haematopoietic cells and tissue types, including brain, lung, kidney, heart, liver, thyroid, as well as thymic and endometrium epithelia [24]. In the monkey model, the most frequently infected cells were peripheral blood mononuclear cells (PBMC), but infection of thyroid and salivary glands, lung, liver, pancreas, intestine, muscle and spinal cord were sporadically detected in some monkeys [24].

While these observations have not been verified by human studies, and their significance for HTLV-I pathogenesis is uncertain, it is likely that they are partly explained by the very common occurrence of the GLUT-1 receptor on multiple cell types. Some models of HTLV-I infection suggest that many cell types are (at least initially) infected in vivo, but that this infection is non-productive due to intra-cellular constraints on replication which can only be avoided in activated T-cells.

Manel et al. suggest that there may be a direct infection role of HTLV-I through the GLUT-1 receptor in a variety of the pathophysiological effects of the viral infection, including infection of the basal cells of the corneal epithelium and some endothelial cells [24].

Relevance for donations of cells and tissues

There is a risk that HTLV-I/II may be transmitted through the use of donated human tissues for transplantation or other human medicinal use as transmission through blood transfusion and bone transplantation [9, 27-30]. The recent discovery of the main HTLV-I/II receptors [21, 24], the potential role of dendritic cells for transmission [18, 23] and animal models in which HTLV infects a number of tissues and cells of non-leukocyte lineage [24] raise questions on the relevance of infection in other tissues/cells than those of the haematopoietic lineage which could be transmitted through donated substances of human origin. Moreover, cells of the haematopoietic lineage are known to be present in multiple tissue types in human, but their exact concentration is not known for all tissue types relevant for transplantation [21, 24].

5 EU legal requirements on HTLV testing of tissue donations

COMMISSION DIRECTIVE 2006/17/EC of 8 February 2006 implementing Directive 2004/23/EC of the European Parliament and of the Council as regards certain technical requirements for the donation, procurement and testing of human tissues and cells (EUTCD)[5] sets down requirements for technical procedures which are designed to exclude donors of tissues and cells which would constitute a risk for transmission of infectious diseases. The Directive specifies both general and specific exclusion measures, including exclusion based on clinical signs of disease, medical history examination, review of behavioural and social risk factors and screening of evidence for previously undiagnosed infections by specific tests.

The legal requirements for testing tissue donations state that 'HTLV-I antibody testing must be performed for donors living in, or originating from, high-incidence areas or with sexual partners originating from those areas or where the donor's parents originate from those areas' (2006/17/EC, Annex II, point 1.2). Therefore, the Directive does not make HTLV I/II screening mandatory for all tissue donations, but only if the donor evaluation (Directive 2006/17/EC, Annex II, point 1.2) reveals that the donor falls into the category described above.

Some Member States have more stringent national requirements for application of the HTLV-I/II test. Moreover, preliminary review of the interpretation of the 'high incidence areas' for HTLV-I/II indicates that the areas/countries considered to belong to this category vary among Member States (information provided to ECDC by the UK Human Tissue Authority – HTA).

Detailed criteria of donor evaluation relevant for controlling risks of HTLV-I/II infection are described in the extract of Directive 2006/17/EC in Annex 3.

[5] COMMISSION DIRECTIVE 2006/17/EC of 8 February 2006 implementing Directive 2004/23/EC of the European Parliament and of the Council as regards certain technical requirements for the donation, procurement and testing of human tissues and cells

6 Recent changes in tissue donor testing requirements in the US concerning HTLV-I/II infection

The American Association of Tissue Banks (AATB)

The following section is modified from the web page of the AATB (http://www.aatb.org/About-AATB):

The AATB is a voluntary, professional, scientific and educational organisation founded in 1976. The AATB's mission is public health. It is the only national tissue banking organisation in the United States, and its membership totals more than 100 accredited tissue banks and 1 000 individual members. These banks recover tissue from more than 30 000 donors and distribute in excess of two million allografts for more than one million tissue transplants performed annually in the US. The overwhelming majority of the human tissue distributed for these transplants comes from AATB-accredited tissue banks.

The AATB publishes standards and accredits tissue banks. It certifies personnel and operates a tissue network. The association also interacts with regulatory agencies and conducts educational meetings.

First published in 1984 and presently in its 12th edition, the AATB's *Standards for Tissue Banking* are recognised in both the United States and around the world as the definitive guide for tissue banking. These standards are the only private tissue-banking standards published in the United States, and they are the most comprehensive and detailed tissue-banking standards in the world. As such, the AATB's *Standards* have served as the model for federal and state regulations as well as several international directives and standards. While the AATB's *Standards*, have no direct legal regulatory force by themselves, the statutes and/or regulations of more than 20 [US] states reference AATB's *Standards*, institutional accreditation, or individual certification. At least six states require AATB accreditation for any tissue bank operating in their state.

In 1986, the AATB initiated a mandatory accreditation programme for its institutional members to ensure that tissue-banking activities are performed in a professional manner in compliance with its standards. Today, the AATB Accreditation Program remains the only private accreditation programme for tissue banks in the USA.

Removal of requirement for HTLV I/II testing for tissue donations

On 11 November 2009, the American Association of Tissue Banks (AATB) issued a news release stating that its Board of Governors had approved changes to AATB's *Standards for Tissue Banking* removing the requirement to test all tissue donors for antibodies to human T-lymphotropic virus type I and type II (anti-HTLV-I and anti-HTLV-II)[6] (Annex 4). The changes were recommended by the AATB Physicians' Council and Standards Committee. On 20 October 2009, the Board of Governors voted to approve these changes[7]. This represented a reversal of a requirement for HTLV I/II testing introduced on 18 March 1993 by the AATB's Board of Governors.

6 http://archive.constantcontact.com/fs076/1102056357439/archive/1102820896575.html

7 HTLV TESTING Recommendation from the Physicians' Council & Regulatory Review Document. Compiled by Scott Brubaker/AATB August 18, 2009

According to the news release on its website, the AATB evaluated relevant, scientific literature as well as current state, federal, and international requirements for the screening and testing of donor tissue for HTLV-I and HTLV-II. The press release also states that due to administrative and operational considerations, there is no requirement to implement these changes by a specific date, but they can be made at any time at the discretion of the tissue bank. The press release refers to the paragraphs of the AATB's *Standards for Tissue Banking* for the exact content of the change in the standards. The lifting of the requirement is not total and testing must still be performed for some tissue and cell components that are 'viable, leukocyte-rich cells/tissues'.

Since the standards were amended, Florida and California have lifted their state requirement for HTLV testing of tissue donations (news releases posted on the AATB website).

Reasons for the change

There are several reasons for the AATB decision to change its standards and remove the requirement for HTLV-I/II testing. The rationale behind the changes has been described in an AATB document[8] provided to ECDC by the AATB on 4 August 2010.

US FDA requirements

The US Food and Drug Administration (FDA) regulations for human cells, tissues, and cellular and tissue-based products (HCT/Ps) described HTLV infection and risk as relevant only for donors of 'viable, leukocyte-rich cells and tissues', and has only required testing for donations falling into this category. The AATB considers that the change in their standards reflects a fallback to the FDA requirements.

Test withdrawal

In the US, only tests approved for human diagnostic use by the FDA may be marketed and used for purposes other than research. At present, three test kits are licensed by the FDA, but only one remains in production[9].

The table of FDA licensed assays for Human T-Lymphotropic Virus Types I & II lists three test kits with these trade names and testing formats:

- Abbott HTLV-I/HTLV-II EIA (EIA);
- Abbott PRISM HTLV-I/HTLV-II Assay (ChLIA); and
- Vironostika* HTLV-I/II Microelisa System (EIA). (*Licensed for donor screening, but may not be available).

At present, the Vironostika HTLV Ab kit is no longer commercially available. Similarly, in February 2009 Abbott gave notification that the last shipment date of the Abbott HTLV-I/HTLV-II EIA kit was 31 December 2009. The expiration date of this kit was 18 April 2010. In the past, this test was widely used for tissue and donor testing as a result of its reasonable performance characteristics and applicability for relatively small sample series.

Poor performance of the only available test on cadaveric materials

In the US, only one test kit manufactured by one company is currently available for HTLV screening of tissue donors. This test kit, the Abbott PRISM HTLV-I/HTLV-II Assay (ChLIA), is only licensed for testing blood samples from 'living' donors (see the FDA website link cited above.) The kit has not been approved for testing blood specimens from cadaveric donors and use of this sample type is not recommended. It is a high-throughput assay system which is only suitable for the running large numbers of samples.

Poor availability of alternative test

The performance of a CE-marked test kit when using cadaveric blood specimens is still being evaluated by a testing laboratory in the US. This test kit has not been evaluated by the FDA and it is not licensed in the US.

[8] Rationale for Changing the Requirement to Test Tissue Donors for HTLV, AATB document, May 10, 2010

[9] http://www.fda.gov/BiologicsBloodVaccines/SafetyAvailability/TissueSafety/ucm095440.htm#approved

Risk assessment – transmission of HTLV and the need to screen certain tissues

AATB considers, in line with the US FDA, that as HTLV I and II are retroviruses infecting CD4 and CD8 positive lymphocytes, and transmission has been linked exclusively to exposure to viable lymphocytes/leukocytes and not to non-cellular blood components such as plasma, transmission risks will only be relevant for those tissue products containing viable leukocytes. Studies of HTLV virus transmission following blood transfusion have found that a sufficient number of viable leukocytes must be present for transmission to occur, hence the term, 'rich in viable leukocytes', used by FDA, to describe a tissue type relevant for HTLV risk. The AATB refers to a threshold of $1x10^8$ viable leukocyte cells as the minimum number of cells that can result in transmission of the virus.

Therefore, the AATB concludes that HTLV testing is only relevant for those products that are 'rich in viable leukocytes'. Tissue types not considered 'rich in viable leucocytes' and for which HTLV-I/II testing is not required are processed as conventional human tissue (i.e. bone, tendons, ligaments, skin, fascia, nerves, cartilage, heart valves, cardiac conduits, and vessels/vascular tissue).

The AATB change of recommendations does not cover certain other tissue/cell types regarded as falling into the category 'rich in viable leucocytes', for which testing is still required by the AATB and the US FDA. These include hematopoietic stem/progenitor cells and semen.

HTLV-I/II infection in US donor populations

According to the AATB, the prevalence of HTLV infection is low in the United States, and the country is not considered an endemic area. In certain population groups infection is more common, but these individuals are not eligible for cell or tissue donation (e.g. injecting drug users and sex workers).

However, US guidelines for tissue and cell donor selection contain no reference to other US population groups for which there is evidence of a higher prevalence than in the general population (see Section 7 in Part 1 of this risk assessment for a review of epidemiology).

7 Implications of changes in US HTLV-I/II testing requirements on tissue and cell donors for the EU

In its list of questions the Commission asked ECDC to determine whether the changed testing requirements for HTLV-I/II in relation to tissue and cell donors in the US represent an obstacle to importation and use of tissue and cell products of US origin in terms of compliance with the requirements of the EUTCD. This is particularly relevant for the risk of HTLV-I/II transmission.

Directive 2006/17/EC, Annex II, point 1.2 stipulates that 'HTLV-I antibody testing must be performed for donors living in, or originating from high-incidence areas or with sexual partners originating from those areas or where the donor's parents originate from those areas'. The Commission asked ECDC to examine the epidemiology of HTLV I/II, to evaluate in which geographical areas of the world HTLV I/II infections are most common and to establish the status of North America in this regard, and how it compares with the EU/European region.

In US documentation on the change of requirements for HTLV-I/II testing, factors affecting the transmission of HTLV-I/II form part of the motivation for testing fewer categories of materials with human tissue cell origin. In particular, the restriction of HTLV-I/II transmission risk, and therefore the need for testing, to materials that are 'rich in viable leukocytes' and for a threshold value of 10^8 leukocytes is applied in the US for the classification into this category.

In the initial question and in subsequent clarifications, ECDC was asked to examine the evidence for classification of materials into risk categories based on their leukocyte content and especially the threshold mentioned in the US rationale documents for the change of HTLV-I/II testing requirements. It was agreed that ECDC would not try to examine this through a comprehensive review of tissue types, as this would require extensive tissue-specific expertise which was not readily available to ECDC. Instead, ECDC will concentrate on evaluating the evidence as to whether transmission is possible only by exposure to sufficient numbers infected leukocytes and whether the threshold for transmission risk is 10^8 leukocytes.

ECDC was also asked to examine whether measures used to prevent transmission of HTLV-I/II during transplantation of tissues/cells are effective even after the change in US requirements for testing.

8 Results of ECDC Review

Transmission mechanisms for HTLV-I/II

To a certain extent, the HTLV-I/II viruses have similar transmission mechanisms to other blood-borne and sexually transmitted infections. The predominant modes of transmission for HTLV-I are blood and blood components, infected mother's breast milk, sexual intercourse and the sharing of injection equipment among injecting drug users [8]. The same transmission modes exist for HTLV-II, except for sexual transmission which has not been convincingly demonstrated [31]. However, one major difference in transmission risk compared to many other blood-borne infections is the exclusive association between transmission and the presence of infected cells of lymphocyte origin in blood and tissues. Many other blood-borne infections can be transmitted by exposure to plasma or other a-cellular blood components.

Evidence for transmission linked to the presence of infected lymphocytes

Early studies of HTLV-I epidemiology after the identification of the causative agent [32] identified receipt of infected blood components as a major risk factor for HTLV-sero-conversion and adult T-cell leukaemia/lymphoma [28, 33, 34]. Large-scale look-back studies among blood donors both in Japan and USA confirmed the findings and also suggested that there is a dependence on cellular components in the blood for transmission to occur [30, 33, 35]. A comparative study of HTLV-I, HTLV-II and HIV-infection demonstrated with a high degree of confidence that both HTLV-I and II are exclusively transmitted by blood components containing cells, and that loss of ability to proliferate or become activated due to refrigerator storage over a period of >14 days also prevented transmission by blood components containing cells [27, 35]. A highly significant inverse time-trend for the effect of the length of storage on risk of transmission prior to transfusion was discovered [27, 35]. In contrast, no transmission occurred among recipients of a-cellular blood components from HTLV-I or II positive donors [27].

At the time of the studies establishing the strong link between cellular blood components and HTLV transmission, quantitative methods for estimating virus load in cells were not widely available. Virus load (i.e. the number of provirus copies per cell unit) has since been shown to vary tremendously among asymptomatic individuals [16, 36, 37].However, it has not been possible to determine a minimum infectious dose for human infection [16].

Evidence for infectious dose threshold of 10^8 leukocytes

A key element in establishing whether donors of cells or tissues need to be tested for HTLV-I/II infection according to the requirements of the AATB *Standards* is being able to determine whether the leukocytes in donated cells or tissues are viable and present in large quantity. Both in the AATB *Standards*, and in the FDA requirements[10], only those donors whose cell and tissue donations are 'rich in viable leukocytes' need to be tested for HTLV-I/II infection.

In the AATB documentation[11], there is additional guidance on how to determine whether a donation should be considered 'rich in viable leucocytes'. This identifies a threshold value 'in the range of 10^8 cells' which represents a transmission risk. Products containing fewer than this number of cells are deemed not to constitute a transmission risk and the donor will not need to be tested.

The threshold value of 10^8 viable leukocytes originates from a look-back blood donation study carried out in Japan during the 1980s by Okochi et al. In the study 103 sero-conversions were found among 1 153 recipients of blood and blood components only partly screened for HTLV-I/II antibodies [33]. Risk factors associated with sero-conversion were examined among the recipients and sero-conversion was found to be strongly linked to the receipt of blood components containing cells. In fact, none of the 52 individuals who had received only frozen plasma from anti-HTLV-I/II positive donors sero-converted during the study observation time (from one to more than four years). Identified risk factors included short storage of packed red cell preparations and one method for preparation of platelet concentrates.

Within the same study, the authors found that seroconversion occurred among all six patients receiving HTLV-I positive platelet concentrates prepared by platelet apheresis while none of the four patients who had received HTLV-I positive platelet concentrates prepared from a single donation of whole blood sero-converted. The authors

[10] FDA Final Rule, Eligibility Determination for Donors of Human Cells, Tissues, and Cellular and Tissue-Based Products, dated May 25, 2004 (effective May 25, 2005).
http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Tissue/ucm073964.htm

[11] Rationale for Changing the Requirement to Test Tissue Donors for HTLV, AATB document, May 10, 2010

concluded that employing Fisher's exact method there was a significant difference between the groups in terms of sero-conversion and they quoted a p-value of 0.004.

The authors compared the properties of platelet preparations and concluded that the number of lymphocytes in the apheresis platelet concentrates was 2–4 x 10^9 lymphocytes, whereas those taken from single blood collections contained 1 x 10^7 lymphocytes.

The authors suggested that the number of lymphocytes required for transmission of HTLV-I transmission is 10^8 since many of the recipients of infected cells containing 2 x 10^8 lymphocytes sero-converted, but none of the patients receiving infected concentrates containing only 10^7 lymphocytes did so.

No other studies supporting the threshold number of 10^8 lymphocytes were identified by ECDC's literature search, although many studies support the strict linkage to transmission by cell and not via a-cellular blood components for HTLV-I and II infection.

Evidence of infectivity or non-infectivity of tissue types relevant for donations

Tissue and cell transplantation as well as organ transplantation has been shown to transmit HTLV-I infection. One of the earliest cases described occurred in a recipient of a deep-frozen bone allograft [9]. This case resulted in development of disease, despite long-term storage of the bone allograph in a frozen state. Several studies have described transmission and development of HTLV related disease in multiple recipients of tissues or organs from a single donor [10, 38-42]. In these cases, use of immunosuppressive drugs in the recipients appeared to speed up the clinical symptoms.

In the US, FDA guidance classifies cells and tissues into categories requiring donor screening and those not requiring donor screening for HTLV-I/II infection as per Box 1 below (quote from FDA guidance for industry[12]):

Box 1 – FDA guidance for industry

FDA classification of tissues into categories requiring donor screening for HTLV-I/II infection:

Examples of viable, leukocyte-rich cells or tissue include, but are not limited to:

- Hematopoietic stem/progenitor cells
- Semen.

You should consider cells and tissues to be viable and leukocyte-rich based on their status at the time of recovery, even if later processing might remove leukocytes.

FDA's classification of tissues into categories not requiring donor screening for HTLV-I/II infection:

Examples of cells or tissue that are not considered viable, leukocyte-rich cells or tissues include, but are not limited to:

- Corneas
- Sclera
- Skin
- Heart valves
- Dura mater
- Bone
- Tendons
- Ligaments
- Cartilage
- Oocytes.

Literature searches performed by ECDC did not identify any relevant literature that linked to the direct or indirect risk of HTLV-I/II transmission in relation to the above division, except the documented transmission by bone marrow (presumably containing haematopoietic cells) and bone (containing bone marrow) [9, 39, 43]. These data, although based on case studies only, do not support the classification as described above by the FDA.

12 FDA. Guidance for Industry "Eligibility Determination for Donors of Human cells, Tissues, and Cellular and Tissue-Based Products (HCT/Ps) "; August 2007. Note: the FDA document is a guidance document aiming to help establishments to comply with Code of Federal Requirements (21 CFR, part 1271, subpart C);

As little evidence to assess the infectivity of various tissue and cell types could be identified through literature searches, ECDC asked the ad hoc expert panel to address the question of tissue types constituting a risk and those which may be used safely without HTLV-I/II testing. The experts were also asked to address the question of whether certain methods of processing harvested cells and tissues prior to transplantation can remove transmission risk.

After a thorough discussion, the ad hoc expert panel advised as follows:

With regard to the classification of tissues not requiring HTLV-I/II testing due to absence of leukocytes (as presented in the FDA list), the panel did not support the use of this list and provided the following justifications:

- the link between viable leukocyte content and risk of transmission has not been demonstrated for tissues and cells other than blood and their presence alone may be a poor marker of infectivity
- the infectious dose of HTLV-I/II in terms of cell-associated viral load is unknown and the threshold used by the AATB for leukocyte content is not supported by rigorous and valid studies
- many of the tissue categories (skin, oocytes, heart valves) listed as not requiring testing may naturally contain or be contaminated with cells that have been indicated as being involved in transmission of HTLV-I/II in in vitro settings and animal models
- certain tissue types giving rise to concern for potential transmission are missing from the list, i.e. blood-vessels, pancreatic islets, etc.
- many of the a-cellular materials (dura mater, tendons, ligaments, cartilage) classified as not requiring testing may in practice contain cellular tissue parts attached (bone, muscle tissue)
- fresh and frozen bone has been shown to be capable of transmitting infection but bone is listed in the category not requiring testing.

With regard to the effectiveness of various processing methods the ad hoc expert panel considered that there may be processing methods resulting in the removal of all risk of transmission, i.e. methods that completely remove cellular live materials from the tissue. However, not all processing methods can be easily evaluated without proper evidence validating the removal of risk. Methods developed for removal of bacterial contamination cannot usually be assumed to sufficiently remove HTLV-I/II transmission risks. Similarly, methods developed to remove viral particle associated risks may not be sufficient for HTLV-I/II risk removal as the risk is likely to be linked to cell-bound viruses. Moreover, many tissue and cell types cannot be processed in a manner which would sterilise them without destroying their donor usefulness.

A further complication is that many processing methods developed in the US which claim to remove the risk of transmission are proprietary or patented. Insufficient information is available on these methods to be able to objectively evaluate their potential for risk reduction.

The ad hoc expert panel therefore considered the current lack of HTLV-I/II testing for tissue and cell donors in the US to introduce a higher risk of HTLV-I/II transmission than exists in the EU. The experts suggested that the change in US testing policy constitutes a de facto trial of the consequences, which can only be evaluated after many years of follow-up.

Effectiveness of exclusion methods for transmission prevention

Risk factor review

The major documents in the EU and the US that establish requirements for examining contra-indications in relation to donor eligibility are, on the one hand, regulatory and on the other professional guidance. In the EU, the main regulatory document establishing minimum criteria to be implemented throughout the Member States is the EUTCD[13] (relevant sections included in Annex 3). In addition, the Council of Europe has issued detailed professional guidance for stakeholders: 'Council of Europe Guide to safety and quality assurance for the transplantation of organs, tissues and cells 3rd edition, 2009'.

In the US, the Food and Drug Administration (FDA) has issued a guidance document for industry 'Eligibility Determination for Donors of Human cells, Tissues, and Cellular and Tissue-Based Products (HCT/Ps)' which is intended to help establishments comply with the 'Code of Federal Requirements (21 CFR, part 1271, subpart C)'. In the US, the American Association of Tissue Banks (AATB) has also issued professional guidance in their 'AATB Standards for Tissue Banking, 12th edition, 2008'.

Annex 6 presents a comparison of the various documents, together with an analysis of some additional supporting references. In many regards the evaluation of donor eligibility is based on similar principles, but there are some

13 COMMISSION DIRECTIVE 2006/17/EC of 8 February 2006 implementing Directive 2004/23/EC of the European Parliament and of the Council as regards certain technical requirements for the donation, procurement and testing of human tissues and cells

clear differences for which ECDC sought the advice of external experts. In particular, the question of indications for HTLV-I/II testing differs between the regions and the effect on the comparative level of safety for the end products needed to be addressed.

As with tissue types, no relevant studies on the effectiveness of donor risk factor evaluation were found by the ECDC literature searches, and the question was therefore referred to the ad hoc expert panel. The panel was unanimous in its assessment that risk factor evaluation, particularly in the case of cadaveric donors, is likely to ineffective in revealing past risks of HTLV-I/II exposure. The strong role of mother-to-child transmission on the epidemiology and maintenance of increased prevalence, the potential for transmission through regular sexual activity and the dominance of asymptomatic infection in the case of HTLV-I/II makes identification of risks by risk-factor review close to impossible with deceased donors and very difficult even in the case of live donors.

A major difference between the US and EU criteria for donor selection identified by ECDC and the ad hoc expert panel concerns the criteria for HTLV-I/II testing. In the US FDA guidance, testing based on origin from areas of HTLV-I/II endemicity defined by high prevalence or incidence is not included.

Considering the clinical evaluation criteria for contra-indications with the donation of tissues and cells, the US and EU criteria were considered fairly similar.

As a conclusion, the ad hoc expert panel suggested that risk factor evaluation was insufficiently effective to replace testing for HTLV-I/II in situations where individuals originate from endemic areas with a high prevalence of infection.

Microbiological screening

The scope of this section is to clarify the HTLV I/II laboratory testing procedures and summarise the results of published evaluation studies for the available commercial tests.

Blood donor screening for HTLV was introduced in Japan in the mid-1980s, followed by USA and Canada in 1988 and France in 1991 [44]. Numerous other countries subsequently introduced various screening programmes for HTLV in blood and tissue donors. ranging from full scale testing of all donors to the testing of new donors from selected risk groups. Despite the rapid evolution of clinical diagnostics over the last 30 years, there is still no golden standard for laboratory testing of HTLV-I/II infection [45]. Laboratory diagnostics are dominated by commercial serological methods for detection of HTLV-I/II antibodies in sera, such as ELISAs, western blots and agglutination assays.

Assays for the detection of HTLV nucleic acids by PCR have been published, but are not superior to serological methods in terms of specificity, sensitivity or economy. Therefore, the use of nucleic acid testing for laboratory screening of HTLV-I/II infection is still limited. Currently, there are no nucleic acid tests approved by FDA for diagnostic purposes.

The number of commercially available antibody screening assays is limited and the developments and releases of new assays have been relatively rare [46]. However, in recent decades there have been some important improvements in the serological tools for detection of HTLV-I/II antibodies. The first generation of serological assays, introduced in the middle of the 1980s, utilised whole HTLV-I lysate proteins as an antigen source. Since HTLV-I and HTLV-II share approximately 60% homology at the genetic level, some HTLV-II infections were not identified using these assays [45]. In the second generation of assays, HTLV-II antigens were also included, either in the form of crude lysate or as recombinant proteins [45]. The third generation assays use a technically improved detection methodology where recombinant proteins and synthetic peptides are used both to capture and detect anti-HTLV antibodies [47]. The conclusion of most of the evaluations of HTLV-I/II diagnostic tools is that these developments have increased specificity and sensitivity for detection of HTLV-I and HTLV-II, although some studies indicate the opposite [48-50].

Table 1 summarises information and evaluation results of the most commonly used assays for laboratory detection of HTLV I/II information.

Most assays evaluated have 100% sensitivity for detection of HTLV-I/II infection in clinical samples. However, no assay has shown 100% specificity in repeated investigations (Table 1). The Positive Predictive Value (PPV) of a diagnostic test can be defined as:

$$\frac{\text{Number of true positives}}{(\text{number of true positives} + \text{number of false positives})}$$

or

$$\text{PPV} = \frac{(\text{sensitivity})(\text{prevalence})}{(\text{sensitivity})(\text{prevalence}) + (1 - \text{specificity})(1 - \text{prevalence})}$$

When using a test with less than 100% specificity to screen a low prevalence population, as is the case when screening for HTLV-I/II infection in blood and tissue donors in Europe and US, the PPV is low (i.e. the frequency of false positive results is high). If tissue donors are deferred based on false positive results, the mortality of patients on organ waiting lists may outweigh the benefits of testing. To overcome the problem with low PPVs in HTLV screening, several studies have focused on testing algorithms – i.e. how to best combine different screening assays and confirmatory tests in order to improve the accuracy of results. Confirmatory testing is traditionally performed in reference laboratories and methods used here are western blots (Diagnostic Biotechnology HTLV WB 2.3/2.4, Genelabs Diagnostics, Singapore), line immunoassay (INNO-LIA HTLV-I/HTLV-II, Innogenetics N.V. Belgium) and in-house PCR methods. Evaluation studies of confirmation assays have shown that the line immunoassay is the most efficient method to confirm and resolve indeterminate testing results [51].

Assay evaluation studies and reaching agreement on a unified testing algorithm for HTLVI/II testing is problematic for a number of reasons. The lack of a golden standard for testing and confirmation introduces a bias into the definitions of positive and negative serum panels used by research groups for assay evaluations. Results are difficult to compare since studies are based on different serologic assays and testing algorithms in combination with different interpretation criteria [44]. Moreover, specificities and sensitivities of screening and confirmation assays have been shown to change depending on the geographical origin of the serum panels or incidence of disease [45, 49]. In addition, the best testing algorithm does not only include the best possible performance of the tests under review, but also practical and economical parameters related to the purpose of testing.

Thorstensson et al. proposed a diagnostic algorithm including testing repeatedly reactive samples from ELISAs in the primary laboratory with third generation ELISA assays from Murex or Ortho, followed by INNO-LIA and finally western blot. [44]. To reduce the number of false positive results and the money and time spent in sending samples for confirmatory testing to reference laboratories, Berini et al recommended primary testing using a combination of Serodia HTLV-I particle agglutination and ELISA or a dual ELISA testing with Biokit and Murex [45].

In a study evaluating confirmatory assays for HTLV-I/II diagnostics, 292 out of 18.169 samples showed repeated reactivity in second generation ELISA used for primary testing. In the confirmatory testing over 75% of the positive samples were shown to be negative, underlining the importance of confirmatory testing to validate screening data [51]. In a Swedish study evaluating the accuracy of blood donor screening, eight out of 21.189 screening tests were reactive to HTLV-I/II. Confirmatory testing showed that all eight samples were false reactive, but given the relatively low overall false reactivity (0.04%) this was not considered a problem from a donor-deferral point of view [52]. Compared to earlier Swedish studies on HTLV-I/II testing this result indicated a highly improved accuracy of the primary HTLV-I/II screening. In a study from the US, 29% of the repeatedly reactive specimens from HTLV screening were further investigated using commercial western blots [53]. In other similar studies, line immunoassay has been used for confirmation and this has shown higher rates of false reactivity, indicating that this result may be a low estimate of the true number of false reactive samples.

Since 31 December 2009, the only FDA-licensed HTLV assay available for use in the US has been the Abbott PRISM HTLV-I/II. This assay is part of an automated testing system for high throughput screening. Most organ procurement organisations in the US do not have access to the instrument needed to execute the testing with this assay in a cost-efficient and quality-controlled manner [47].

Table 1. Commercial HTLV assays and their evaluation results

Manufacturer	Assay name	Principle	Reference	Sensitivity HTLV-I (%)	Sensitivity HTLV-II (%)	Specificity HTLV-I/II (%)[1]	Positive predictive value (PPV) in low prevalence situation (%)	Approved by FDA	Additional information
Abbott	HTLV-I 2.0 EIA	Indirect ELISA	Andersson et al.	100	85.5	98.4/99.3	0.33	Yes	No longer available
Abbott	HTLV-I/II	Indirect ELISA	Andersson et al.	100	96.8	90.2/99.8	1.14		
Abbott	Prism HTLV-I/HTLV-II	Sandwich ChLIA[2]	Andersson et al.	100	100	NT/100	100	Yes	
Abbott	rHTLV-I/II	Sandwich ChLIA[1] (3rd generation assay)	Malm et al.	100	100	99.8			For high throughput screening
			Kapprell et al.	100	100	99.9			
			Qui et al.	100	100	99.9			
Abbott	Murex HTLV -I+2	ELISA (3rd generation assay)	Berini et al.	98.8	94.6	99.7			
			Kapprell et al.	100	100	99.9			
Biokit	HIV 1&2 +HTLV-I/II	Indirect ELISA	Andersson et al.	98.9		94.2/99.5	0.45		
			Berini et al.	100	96.4	97.0			
Genelabs	HTLV-I/II ELISA	Indirect ELISA	Andersson et al.	100	91.9	96.8/98.2	0.13		
Genelabs	HTLV-I/II ELISA 3.0	Indirect ELISA	Andersson et al.	100	96.8	90.8/99.8	1.14		
Fujirebio	Serodia HTLV-I	Particle agglutin-ation	Andersson et al.	100	100	97.6/100	100		
			Berini et al.	98.8	98.2	95.8			
Murex Diagnostics	Cambridge Anti-HTLV-I/II EIA (VK 80/81)	Indirect ELISA	Andersson et al.	100	90.3	89.6/96.0	0.06		
Murex Diagnostics	HTLV-I/II (GE 80/81)		Andersson et al.	100	100	99.7/98.4	0.14		
Organon Vironostika	HTLV-I	Indirect ELISA	Andersson et al.	100	87.1	96.5/97.1	0.07		
Organon Vironostika	HTLV-I/II	Indirect ELISA	Andersson et al.	100	91.5	100/100	100		
BioMerieux-Vironostika	HTLV-I/II microelisa system			100[9]		93.6%[9]		Yes	No longer available
			Berini et al.	97.7	94.6	92.9			
Ortho	Cambridge HTLV-I/II ELISA	Indirect ELISA	Andersson et al.	100		91.9/93.6	0.04		
Ortho	HTLV-I/HTLV-II Ab Capture	Sandwich ELISA (3rd generation assay)	Andersson et al.	100	100	95.5/99.4	0.38		
Hoffman-La Roche	Cobas Core Anti HTLV-I/II EIA	Indirect ELISA	Andersson et al.	100	100	99.7/99.8	1.14		

9 Discussion and conclusions

Transmission in the context of tissue and cell donations (Question 2)

The evidence review assessed the validity, generalisability and strength of documentation concerning some of the factors influencing transmission of HTLV-I/II infection in the context of cell and tissue donations. The review supported the conclusion that HTLV-I/II transmission in the context of blood and blood component donation is dependent on the presence of lymphocyte cells in the transfused component. This conclusion was found to be confirmed by a number of well-designed studies. Furthermore, the idea that lymphocytes associated with transmission in blood and blood components lose the ability to transmit infection, if appropriately stored for a period of more than 14 days, was also found to be valid and well supported by several studies. In addition, the finding that a-cellular blood components, such as plasma and other cell-free fractions, do not represent a transmission risk was found to be valid and confirmed by multiple studies. All these findings could also be generalised to apply to products derived from blood donations and plasma donations (provided that plasma donations efficiently avoid the collection of cells of lymphocyte origin).

However, the evidence assessment could not conclude whether the findings for blood donations can be generalised to apply to tissue and cell preparations not of blood origin. Although this may be the case the evidence available is currently insufficient to be able to draw this conclusion. Transmission of HTLV via deep frozen bone after prolonged storage suggests that conservation alone may not be sufficient to remove the transmission risk for tissues.

Evidence to support a generic threshold of a total number of 10^8 cells of viable leukocyte class was found to be insufficient. This threshold is based on a single observation among a group of 10 patients receiving concentrated blood components which were estimated to contain different concentrations of lymphocytes. Six patients estimated to have each received a concentrated platelet blood component containing 10^9 lymphocytes from HTLV-I positive donors became infected. On the other hand, another set of four patients estimated to have each received a platelet blood component, prepared in a different manner from a single HTLV-I positive donor containing less cells (10^7), did not become infected. Based on this evidence, the authors concluded that at least 10^8 cells are needed for transmission to occur.

The evidence for the threshold value in relation to leukocyte cell content is clearly neither sufficient nor generalisable. A conclusion based on 10 events with two different outcomes contains too much uncertainty to be generally applicable. Even though the study claims that the difference was statistically significant, this cannot be considered reliable given the low number of events observed.

In addition, the two outcomes were based on products from different patients with unknown proviral load and prepared in different ways. It cannot be excluded that during preparation factors other than the final number of cells (which was not measured in the actual product lots used) affected the transmission. Finally, as the derived threshold was obtained entirely from a blood component study, there is no guarantee that it is generally applicable to other tissue and cell products. It cannot be concluded that other factors, dependent on the tissue and cell type, would not have an impact on the number of cells required for efficient transmission. It is possible that in certain tissues additional factors exist which may lower the threshold of cells required for infection.

The conclusion is that the evidence for the threshold of 10^8 cells is far too weak to be used as a general criterion for determining whether a particular cell or tissue type is devoid of HTLV-I/II transmission risk and safe for use. Further studies with greater scope and understanding of lymphocyte content are needed to address this question. It is possible that the use of the current threshold (10^8 cells of viable leukocyte class) to determine tissue safety in relation to HTLV-I/II transmission is placing individuals in receiving cell and tissue transplants at risk of infection.

With regard to the relevance of different tissue and cell types for HTLV-I/II transmission risk, the assessment is mainly based on professional advice from ECDC's ad hoc expert group. The panel considered the US classification of tissues into two categories ('require testing' and 'do not require testing') as insufficient for risk removal.

Furthermore, the panel considered that details of the processing methods for certain tissue and cell types could not be evaluated for their potential to remove infectivity due to non-transparency of proprietary processing methods. Instead, the panel concluded that validation of tissue and cell processing methods to remove transmission risk should be mandatory if testing for HTLV-I/II is not performed. Where tests are performed and positive cases excluded, this validation would not be necessary.

Effectiveness of measures to prevent transmission (Question 3)

The ECDC ad hoc expert panel concluded that donor selection by risk factor exclusion is an inefficient strategy for transmission risk reduction, except for evaluation of clinical contra-indications. The ad hoc expert panel suggests that donor review cannot compensate for lack of HTLV-I/II testing, especially in the case of donors originating from endemic regions.

The review of HTLV-I/II tests in the EU and in the US revealed that tests are available on the European market, but only one test approved for diagnostic use by the FDA is available in the US. This test is only suitable for use in high-throughput settings where sample numbers are high enough to achieve a cost-effective operation, which prevents its use in cell and donor services. The other test that was widely used by tissue and cell banking facilities in the US was discontinued by the manufacturer in 2009. As this was the only other FDA-approved test for HTLV-I/II testing, the AATB may not have had any other option than to change their testing requirements for the US tissue donor services.

The review showed that the performance characteristics of the HTLV-I/II screening tests available on the EU market are comparatively good, but due to the low prevalence of HTLV-I/II infection in both the US and the EU, their positive predictive value may still remain low. In principle, this can lead to some donated materials being discarded unnecessarily, especially if there is no confirmatory testing as the majority of positive results may be false positives. However, the ad hoc expert panel was unanimous in its assessment that false positive rates can be significantly reduced by applying confirmatory testing and cooperating with test providers that have sufficient experience and high throughput. The ad hoc expert panel suggested that this issue is frequently addressed through cooperation with blood donation services.

A low positive predictive value in low prevalence settings is an inevitable consequence of a highly sensitive screening test in a two-step diagnostic algorithm. Nevertheless, the review shows that tests are available which, if they were to receive FDA approval, would be technically suitable for testing tissue donations, as illustrated by the fact that they are being used in Europe and other parts of the world.

The decision to require screening for a specific infection to prevent its transmission has to be based on a comprehensive evaluation of the benefits for the individual and society. In the case of cell and tissue donations, infection transmission risks have to be balanced against the consequences of morbidity, illness, disability or even death due to the loss of tissues and cells that may be discarded due to the false positive reaction of a screening test. However, as cells and tissues from a single donor may be used for 20 or more recipients, the potential exposure from one untested and HTLV-infected individual is large.

The ECDC ad hoc expert panel suggests that if HTLV-I/II screening is implemented in a Member State or region for blood donations, it should also be implemented for tissue and cell donations. Since several studies indicate that HTLV prevalence is up to ten times higher in tissue donors than blood donors, such an approach would be justified.

10 Considerations

These considerations are based on a review of the findings of a literature search, as described in Section 2, (Search strategies), on the advice of ECDC's ad hoc expert panel given during its meeting (4 March 2011), and on a further review of the conclusions drafted by ECDC following the meeting.

Based on an examination of the evidence, ECDC suggests the following for the consideration of the European Commission's Directorate-General for Health and Consumers (DG SANCO) – Directorate C Public Health and Risk Assessment and the EU Competent Authorities for Tissues and Cells:

- Consider discouraging the use of the threshold value of 10^8 leukocytes for safe lymphocyte content, recommending studies to assess transmission risks related to lymphocyte content and discussing the validity of the threshold with the AATB and other relevant US actors.
  Motivation: if a threshold value for lymphocyte content is desirable as a risk assessment parameter for determining HTLV-I/II infection transmission risks, it should be based on relevant studies with sufficient statistical evidence . The current evidence for the threshold is not sufficient to justify safe use of the threshold value for risk assessment of HTLV-I/II transmission in relation to cell and tissue donations. Such studies may not be easy to organise, but one possibility could be combined look-back and tissue type assessment studies. If lymphocyte content by tissue type and the type of donation are known, it could be possible to study the issue via retrospective studies.
- Consider recommending HTLV-I/II testing of tissue and cell donors if testing is implemented among blood donors.
  Motivation: prevalence of infection among tissue and cell donors is shown to be higher than among blood donors. Therefore testing of tissue and cell donors could be cost-effective.
- Consider discussing with US regulators the availability of HTLV-I/II tests and the need to apply a confirmatory test to improve positive predictive values.
  Motivation: availability of a suitable test on the US market is a pre-requisite for the application of EU testing requirements. As the withdrawal of the only suitable test from the US market has resulted in an inability to follow existing testing requirements, it could be justifiable to argue for the approval of another test on patient safety grounds. It should be noted there are several categories of tests allowed by the FDA and the approved category may not be the only option. A second note is that neither the current nor the discontinued test had been approved for use on cadaveric samples.
- Consider a requirement for all untested materials imported into the EU to be validated in accordance with transparent, pre-determined standards for the processing of tissue and cell materials in order to remove HTLV-I/II transmission risks.
  Motivation: in contrast to EU requirements, ethnic minorities in which HTLV-I/II infection may be endemic and migrants from high-prevalence areas are not directly addressed when assessing donor eligibility within the US (however, living in Europe for over five years results in deferral due to concerns regarding variant Creutzfeldt-Jacob disease transmission risk). There has been a decision to abolish HTLV-I/II testing in the US, despite the fact that the epidemiology of the infection does not appear to differ significantly from that in the EU. The reintroduction of testing may not be feasible for the purpose of imports to the EU only. However, the claim that processing removes the transmission risk is poorly documented and evidence of the various methods used is not readily available for assessment. Until such evidence is presented for validation, donors should continue to be tested.
- Consider recommending a comprehensive evidence-based review of lymphocyte content in tissues relevant for donation.
  Motivation: comprehensive, evidence-based information on the lymphocyte content in tissues relevant for donation would facilitate risk assessment and enable better planning of guidance for donor testing. Competent authorities for tissues and cells may have the necessary expertise for such an evaluation.

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Annex 1. Systematic literature search strategy

PUBMED: Transmission (indirect search)

Concept 1	Boolean operator	Concept 2
OR		OR
"HTLV-I Infections/transmission"[Mesh] "HTLV-II Infections/transmission"[Mesh] "Leukemia-Lymphoma, Adult T-Cell/transmission"[Mesh]	AND	"Leukocytes"[Mesh] "white blood cell"[Title/Abstract] "white blood cells"[Title/Abstract] "lymphoid cell"[Title/Abstract] "lymphoid cells"[Title/Abstract] "thymus dependent lymphocyte"[Title/Abstract] "thymus dependent lymphocytes"[Title/Abstract] "leukocytes"[Title/Abstract] "leukocyte"[Title/Abstract] "Dendritic Cells"[Mesh] "Macrophages"[Mesh] "dendritic cell"[Title/Abstract] "dendritic cells"[Title/Abstract] "monocyte"[Title/Abstract] "monocytes"[Title/Abstract] "macrophage"[Title/Abstract] "macrophages"[Title/Abstract] "cd4 positive lymphocyte"[Title/Abstract] "cd4 positive lymphocytes"[Title/Abstract] "t4 cell"[Title/Abstract] "t4 cells"[Title/Abstract]

Concept 1	Boolean operator	Concept 2	Boolean operator	Concept 3
OR		OR		OR
"htlv i"[Title/Abstract] "htlv ii"[Title/Abstract] "htlv 1"[Title/Abstract] "htlv 2"[Title/Abstract] "human t lymphotropic virus 1"[Title/Abstract] "human t lymphotropic virus htlv i"[Title/Abstract] "human t lymphotropic virus 2"[Title/Abstract] "human t lymphotropic virus type 1"[Title/Abstract] "human t lymphotropic virus type 2"[Title/Abstract] "atlv"[Title/Abstract]	AND	"disease transmission, infectious"[MeSH Terms] "disease transmission"[Title/Abstract] "Infection Transmission"[Title/Abstract] "pathogen transmission"[Title/Abstract]	AND	"Leukocytes"[Mesh] "white blood cell"[Title/Abstract] "white blood cells"[Title/Abstract] "lymphoid cell"[Title/Abstract] "lymphoid cells"[Title/Abstract] "thymus dependent lymphocyte"[Title/Abstract] "thymus dependent lymphocytes"[Title/Abstract] "leukocytes"[Title/Abstract] "leukocyte"[Title/Abstract] "Dendritic Cells"[Mesh] "Macrophages"[Mesh] "dendritic cell"[Title/Abstract] "dendritic cells"[Title/Abstract] "monocyte"[Title/Abstract] "monocytes"[Title/Abstract] "macrophage"[Title/Abstract] "macrophages"[Title/Abstract] "cd4 positive lymphocyte"[Title/Abstract] "cd4 positive lymphocytes"[Title/Abstract] "t4 cell"[Title/Abstract] "t4 cells"[Title/Abstract]

Limits: English, French, German, Spanish, Finnish, Swedish, Romanian

PUBMED: Transplantation (indirect search)

Concept 1	Boolean operator	Concept 2	Boolean operator	Concept 3
OR		OR		OR
"Organ Transplantation"[Mesh] "Tissue Transplantation"[Mesh] "organ graft"[Title/Abstract] "organ grafting"[Title/Abstract] "tissue graft"[Title/Abstract] "tissue grafting"[Title/Abstract]	AND	"HTLV-I Infections"[Mesh] "HTLV-II Infections"[Mesh] "Human T-lymphotropic virus 1"[Mesh] "Human T-lymphotropic virus 2"[Mesh] "Leukemia-Lymphoma, Adult T-Cell"[Mesh] "htlv i"[Title/Abstract] "htlv ii"[Title/Abstract] "htlv 1"[Title/Abstract] "htlv 2"[Title/Abstract] "human t lymphotropic virus 1"[Title/Abstract] "human t lymphotropic virus htlv i"[Title/Abstract] "human t lymphotropic virus 2"[Title/Abstract] "human t lymphotropic virus type 1"[Title/Abstract] "human t lymphotropic virus type 2"[Title/Abstract] "atlv"[Title/Abstract]	AND	"Meta-Analysis "[Publication Type] "Meta-Analysis as Topic"[Mesh] "Guideline "[Publication Type] "Guidelines as Topic"[Mesh] "Practice Guideline "[Publication Type] "Evidence-Based Practice"[Mesh] "Consensus Development Conference "[Publication Type] "Consensus Development Conferences as Topic"[Mesh] "Review Literature as Topic"[Mesh] "Review "[Publication Type] "Epidemiology"[Mesh] "Cross-Sectional Studies"[Mesh] "Risk"[Mesh] OR "Prevalence"[Mesh] "Incidence"[Mesh] "Prospective Studies"[Mesh] "Mass Screening"[Mesh] "Epidemiologic Studies"[Mesh] "systematic review"[Title/Abstract] "cross sectional"[Title/Abstract] "meta analysis"[Title/Abstract] "screening"[Title] "screen"[Title] "risk"[Title] "prevalence"[Title/Abstract] "incidence"[Title/Abstract] "cohort study"[Title/Abstract] "cohort studies"[Title/Abstract] "guideline"[Title] "guidelines"[Title] "epidemiology"[Title] "prospective study"[Title/Abstract] "prospective studies"[Title/Abstract] "case report"[Title/Abstract] "case reports"[Title/Abstract] "Case Reports "[Publication Type]

EMBASE: Transmission (indirect search)

Concept 1	Boolean operator	Concept 2	Boolean operator	Concept 3
OR		OR		OR
'human t cell leukemia virus 1'/exp 'human t cell leukemia virus 2'/exp 'human t cell leukemia virus infection'/exp 'htlv i':ab 'htlv i':ti 'htlv ii':ab 'htlv ii':ti 'htlv 1':ab 'htlv 1':ti 'htlv 2':ab 'htlv 2':ti 'human t lymphotropic virus 1':ab 'human t lymphotropic virus 1':ti 'human t lymphotropic virus htlv i':ab 'human t lymphotropic virus htlv i':ti 'human t lymphotropic virus 2':ab 'human t lymphotropic virus 2':ti 'human t lymphotropic virus type 1':ab 'human t lymphotropic virus type 1':ti 'human t lymphotropic virus type 2':ab 'human t lymphotropic virus type 2':ti atlv:ab atlv:ti	AND	'disease transmission'/exp 'disease transmission':ab 'disease transmission':ti 'infection transmission':ab 'infection transmission':ti 'pathogen transmission':ab 'pathogen transmission':ti	AND	'leukocyte'/exp 'white blood cell':ab 'white blood cell':ti 'white blood cells':ab 'white blood cells':ti 'lymphoid cell':ab 'lymphoid cell':ti 'thymus dependent lymphocyte':ab 'thymus dependent lymphocyte':ti 'thymus dependent lymphocytes':ab 'thymus dependent lymphocytes':ti leukocyte*:ab leukocyte*:ti 'dendritic cell'/exp 'macrophage'/exp 'dendritic cells':ab 'dendritic cells':ti 'dendritic cell':ab 'dendritic cell':ti macrophage:ab macrophage:ti macrophages:ab macrophages:ti monocyte:ab monocyte:ti monocytes:ab monocytes:ti 'cd4 positive lymphocyte':ab 'cd4 positive lymphocyte':ti 'cd4 positive lymphocytes':ab 'cd4 positive lymphocytes':ti 't4 cell':ab 't4 cell':ti 't4 cells':ab 't4 cells':ti

Limits: English, French, German, Spanish, Finnish, Swedish, Romanian

EMBASE: Transplantation (indirect search)

Concept 1	Boolean operator	Concept 2	Boolean operator	Concept 3
OR		OR		OR
'human t cell leukemia virus 1'/exp 'human t cell leukemia virus 2'/exp 'human t cell leukemia virus infection'/exp 'htlv i':ab 'htlv i':ti 'htlv ii':ab 'htlv ii':ti 'htlv 1':ab 'htlv 1':ti 'htlv 2':ab 'htlv 2':ti 'human t lymphotropic virus 1':ab 'human t lymphotropic virus 1':ti 'human t lymphotropic virus htlv i':ab 'human t lymphotropic virus htlv i':ti 'human t lymphotropic virus 2':ab 'human t lymphotropic virus 2':ti 'human t lymphotropic virus type 1':ab 'human t lymphotropic virus type 1':ti 'human t lymphotropic virus type 2':ab 'human t lymphotropic virus type 2':ti atlv:ab atlv:ti	AND	'organ transplantation'/exp 'tissue transplantation'/exp 'tissue transplantation':ab 'tissue transplantation':ti 'organ transplantation':ab 'organ transplantation':ti 'tissue graft':ab 'tissue graft':ti 'organ graft':ab 'organ graft':ti	AND	'cohort analysis'/exp 'cross-sectional study'/exp 'prevalence'/exp 'incidence'/exp 'screening'/exp 'risk'/exp 'evidence based practice'/exp 'systematic review'/exp 'meta analysis'/exp 'practice guideline'/exp 'systematic review':ab 'systematic review':ti 'cross sectional':ab 'cross sectional':ti 'meta analysis':ab 'meta analysis':ti 'screening':ti 'screen':ti 'risk':ti guideline:ti guidelines:ti epidemiology:ti 'cohort study':ab 'cohort study':ti 'cohort studies':ab 'cohort studies':ti 'case report'/exp 'case report'

Limits: English, French, German, Spanish, Finnish, Swedish, Romanian

Annex 2. Evidence table

Citation	Title	Type of study	Area covered	Population/ sample size	Outcome	Strengths	Limitations
WHO and IARC (1996). International Agency for Research on Cancer (IARC) Monographs on the evaluation of carcinogenic risks to humans. 67: 447.	Human Immunodeficiency Viruses and Human T-Cell Lymphotropic Viruses	Epidemiological review and expert assessment of clinical significance of HTLV-I and HTLV-II for cancer	Global	n/a	Estimate of global prevalence. Review of links to ATLL, HAM/TSP and other possible conditions. Estimates of lifetime risk of ATLL (2 %).	Comprehensive review of epidemiology and most aspects of HTLV-I/II infection. Summary of most available data at time of production. Convincing conclusions on a causative link of HTLV-I to ATLL.	Lack of population representative data. Does not present comprehensive conclusions concerning links to diseases other than HAM/TSP and ATLL. Does not present lifetime risks of HAM/TSP development among those infected with HTLV-I.
Okochi K and Sato H, 1986. AIDS Res. 2 Suppl 1; S157-61	Transmission of adult T-cell leukemia virus (HTLV-I) through blood transfusion and its prevention.	Retrospective look-back cohort study.	Japan	1 153 recipients of infected blood; 103 seroconversions 52 received plasma; no seroconversion, 4+6 cases in arm used to establish infection threshold.	Claims to establish infectious dose for cells.	Look-back population size.	Sample size too small to establish infection threshold.
Sullivan MT et al. 1991. Arch Intern Med. 151; 10: 2043-8	Transmission of human T-lymphotropic virus types I and II by blood transfusion. A retrospective study of recipients of blood components (1983 through 1988). The American Red Cross HTLV-I/II Collaborative Study Group	Retrospective look-back cohort study	US	28 centres 17/133 recipients seroconverted.	Infection, effect of storage time on infectivity.	Multicenter study	Relatively few events.
Kleinman S et al. 1993. Transfusion. 33; 1: 14-8	Transfusion transmission of human T-lymphotropic virus types I and II: serologic and polymerase chain reaction results in recipients identified through look-back investigations.	Retrospective look-back cohort study	US	54 individuals transfused with HTLV-positive cellular blood components between 1983 and 1989.	16 (30%) of 54 evaluable recipients of transfused cellular components became infected.	Relatively long timeline. PCR used for confirmation.	Relatively few events.
Sanzen L and Carlsson A 1997. Acta Orthop Scand. 68; 1: 72-4	Transmission of human T-cell lymphotrophic virus type 1 by a deep-frozen bone allograft	Case study	Sweden	1	Development of disease despite bone being deep-frozen for an extended period.	Only study on bone-transmission	One case only.
Gonzalez-Perez MP et al. 2003. Transplantation 75; 7: 1006-11	Human T-cell leukemia virus type I infection in various recipients of transplants from the same donor	Case study	Spain	One donor, five recipients.	3/5 developed infection and disease (solid organ: kidney, liver), two did not (cornea).	Same donor	Same case as Zarranz Imirizladu et al?
Zarranz Imirizaldu JJ et al. 2003. J Neurol Neurosurg Psychiatry. 74; 8: 1080-4	Post-transplantation HTLV-1 myelopathy in three recipients from a single donor	Case study	Spain	One donor (solid organs), five recipients (1 x liver, 2 x kidney)	3/5 developed infection and disease (solid organ: kidney, liver), two did not (cornea)	Same donor	Same case as Gonzales-Perez et al?

Citation	Title	Type of study	Area covered	Population/ sample size	Outcome	Strengths	Limitations
Huang RC. and Fishman JA 2011. Transplantation 91; 2: 146-9	Screening of deceased organ donors: no easy answers	Discussion paper	US	n/a	Discuss US decision to stop screening for HTLV among organ donors.	Experts in the transplantation field involved.	Conflict of interest?
Kaul DR et al. 2010. Am J Transplant. 10; 2: 207-13	Donor screening for human T-cell lymphotrophic virus 1/2: changing paradigms for changing testing capacity	Discussion paper	US	n/a	Call for development of tests suitable for organ donors.	Experts in the transplantation field involved.	Conflict of interest?
Nowicki MJ et al. 2006. Transplantation 82; 9: 1210-3	High seroprevalence of anti-HTLV-I/II antibodies among solid organ donors necessitates confirmatory testing	Discussion paper	US	n/a	Bring up need for confirmation testing to avoid loss of organs due to false positive results.	Experts in the transplantation field involved.	Conflict of interest?
Magnus P et al. 1996. Tidsskr Nor Laegeforen. 116; 10: 1229-32	[Quality adjusted life years in assessment of preventive measures. Should blood donors be tested for HTLV-I/II infections?]	Cost-effectiveness modelling study	Norway	n/a	Cost per QALY NOK 2.33 million when prevalence is 1 per 50 000 blood donors, reduced to 190 000 per QALY when the prevalence is 10 per 50 000	Use QALY	May not apply in other countries.
Stigum H et al. 2000. Int J Epidemiol. 29; 6: 1076-84	Human T-cell lymphotropic virus testing of blood donors in Norway: a cost-effect model	Cost-effectiveness modelling study	Norway	n/a	Prevalence 10 per 100 000 intervention will cost USD 0.9 million per life saved, or USD 41 000 per quality adjusted life year gained; willingness to pay to save a statistical life approximately USD 1.2 million in Norway; Fulfilled if prevalence is over 0.8/10 000 donors.	Use QALY	May not apply in other countries.

Annex 3. Current testing requirements in Europe for tissue donation

Extract from relevant sections of EU COMMISSION DIRECTIVE 2006/17/EC dated 8 February 2006 implementing Directive 2004/23/EC of the European Parliament and of the Council as regards certain technical requirements for the donation, procurement and testing of human tissues and cells.

[Preamble]

……

(2) In order to prevent the transmission of diseases by human tissues and cells for human applications and to ensure an equivalent level of quality and safety, Directive 2004/23/EC calls for the establishment of specific technical requirements for each one of the steps in the human tissue and cell application process.

(3) The use of tissues and cells for application in the human body carries a risk of disease transmission and other potential adverse effects in recipients. That risk can be reduced by careful donor selection, testing of each donation and the application of procedures to procure tissues and cells in accordance with rules and processes established and updated according to the best available scientific advice. Therefore, all tissues and cells, including those used as starting material for the manufacture of medicinal products to be used in the Community, should meet the quality and safety requirements laid down in this Directive.

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ANNEX I: SELECTION CRITERIA FOR DONORS OF TISSUES AND/OR CELLS (EXCEPT DONORS OF REPRODUCTIVE CELLS) AS REFERRED TO IN ARTICLE 3(a)

Selection criteria for donors are based on an analysis of the risks related to the application of the specific cells/tissues. Indicators of these risks must be identified by physical examination, review of the medical and behavioural history, biological testing, post-mortem examination (for deceased donors) and any other appropriate investigation. Unless justified on the basis of a documented risk assessment approved by the responsible person as defined in Article 17 of Directive 2004/23/EC, donors must be excluded from donation if any of the following criteria applies:

1. Deceased Donors

1.1. *General criteria for exclusion*

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1.1.3. Presence, or previous history, of malignant disease, except for primary basal cell carcinoma, carcinoma in situ of the uterine cervix, and some primary tumours of the central nervous system that have to be evaluated according to scientific evidence. Donors with malignant diseases can be evaluated and considered for cornea donation, except for those with retinoblastoma, *haematological neoplasm*, and malignant tumours of the anterior segment of the eye.

……

1.1.5. Systemic infection which is not controlled at the time of donation, including bacterial diseases, *systemic viral*, fungal or parasitic infections, or significant local infection in the tissues and cells to be donated. Donors with bacterial septicaemia may be evaluated and considered for eye donation but only where the corneas are to be stored by organ culture to allow detection of any bacterial contamination of the tissue.

1.1.6. History, clinical evidence, or laboratory evidence of HIV, acute or chronic hepatitis B (except in the case of persons with a proven immune status), hepatitis C and HTLV I/II, transmission risk or evidence of risk factors for these infections.

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1.1.9. Evidence of any other risk factors for transmissible diseases on the basis of a risk assessment, taking into consideration donor travel, exposure history and local infectious disease prevalence.

1.1.10. Presence on the donor's body of physical signs implying a risk of transmissible disease(s) as described in Annex IV, point 1.2.3.

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1.2. Additional exclusion criteria for deceased child donors

1.2.1. Any children born to mothers with HIV infection or that meet any of the exclusion criteria described in section 1.1 must be excluded as donors until the risk of transmission of infection can be definitely ruled out.

(a) Children aged less than 18 months born from mothers with HIV, hepatitis B, hepatitis C or HTLV infection, or at risk of such infection, and who have been breastfed by their mothers during the previous 12 months, cannot be considered as donors regardless of the results of the analytical tests.

(b) Children of mothers with HIV, hepatitis B, hepatitis C or HTLV infection, or at risk of such infection, and who have not been breastfed by their mothers during the previous 12 months and for whom analytical tests, physical examinations, and reviews of medical records do not provide evidence of HIV, hepatitis B, hepatitis C or HTLV infection, can be accepted as donors.

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2. Living donors

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2.2. Allogeneic living donors

2.2.1. Allogeneic living donors must be selected on the basis of their health and medical history, provided on a questionnaire and through an interview performed by a qualified and trained healthcare professional with the donor, in compliance with point 2.2.2. This assessment must include relevant factors that may assist in identifying and screening out persons whose donation could present a health risk to others, such as the possibility of transmitting diseases or health risks to themselves. For any donation, the collection process must not interfere with or compromise the health or care of the donor. In the case of cord blood or amniotic membrane donation, this applies to both mother and baby.

2.2.2. Selection criteria for allogeneic living donors must be established and documented by the tissue establishment (and the transplanting clinician in the case of direct distribution to the recipient), based on the specific tissue or cells to be donated, together with the donor's physical status and medical and behavioural history and the results of clinical investigations and laboratory tests establishing the donor's state of health.

2.2.3. The same exclusion criteria must be applied as for deceased donors with the exception of point 1.1.1. [ECDC comment: NOTE, para-truncated]

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ANNEX II: LABORATORY TESTS REQUIRED FOR DONORS (EXCEPT DONORS OF REPRODUCTIVE CELLS) AS REFERRED TO IN ARTICLE 4(1)

1. Biological tests required for donors

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1.2. HTLV-I antibody testing must be performed for donors living in, or originating from, high-incidence areas or with sexual partners originating from those areas or where the donor's parents originate from those areas.

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2. General requirements to be met for determining biological markers

2.1. The tests must be carried out by a qualified laboratory, authorised as a testing centre by the competent authority in the Member State, using EC-marked testing kits where appropriate. The type of test used must be validated for the purpose in accordance with current scientific knowledge.

2.2. The biological tests will be carried out on the donor's serum or plasma; they must not be performed on other fluids or secretions such as the aqueous or vitreous humour unless specifically justified clinically using a validated test for such a fluid.

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2.4. In the case of a deceased donor, blood samples must have been obtained just prior to death or, if not possible, the time of sampling must be as soon as possible after death and in any case within 24 hours after death.

2.5. (a) In the case of living donors (except allogeneic bone marrow stem-cell and peripheral blood stem-cell donors, for practical reasons), blood samples must be obtained at the time of donation or, if not possible, within seven days post donation (this is the 'donation sample').

(b) Where tissues and cells of allogeneic living donors can be stored for long periods, repeat sampling and testing is required after an interval of 180 days. In these circumstances of repeat testing, the donation sample can be taken up to 30 days prior to and 7 days post donation.

(c) Where tissues and cells of allogeneic living donors cannot be stored for long periods and repeat sampling is therefore not possible, point 2(5)(a) above applies.

2.6. If in a living donor (except bone marrow stem-cell and peripheral blood stem-cell donors) the 'donation sample', as defined in point 2(5)(a) above, is additionally tested by the nucleic acid amplification technique (NAT) for HIV, HBV and HCV, testing of a repeat blood sample is not required. Retesting is also not required if the processing includes an inactivation step that has been validated for the viruses concerned.

2.7. In the case of bone marrow and peripheral blood stem-cell collection, blood samples must be taken for testing within 30 days prior to donation.

2.8. In the case of neonatal donors, the biological tests may be carried out on the donor's mother to avoid medically unnecessary procedures upon the infant.

ANNEX IV: CELL AND/OR TISSUE DONATION AND PROCUREMENT PROCEDURES AND RECEPTION AT THE TISSUE ESTABLISHMENT, AS REFERRED TO IN ARTICLE 5

1. Donation and procurement procedures

1.2. Donor evaluation (this section does not apply to partner donation of reproductive cells or to autologous donors).

1.2.1. An authorised person must collect and record the donor's relevant medical and behavioural information according to the requirements described in section 1.4.

1.2.2. In order to acquire the appropriate information, different relevant sources must be used, including at least an interview with the donor, for living donors, and the following when appropriate:

(a) the medical records of the donor;

(b) an interview with a person who knew the donor well, for deceased donors;

(c) an interview with the treating physician;

(d) an interview with the general practitioner;

(e) the autopsy report.

1.2.3. In addition, in the case of a deceased donor, and in the case of a living donor when justified, a physical examination of the body must be performed to detect any signs that may be sufficient in themselves to exclude the donor or which must be assessed in the light of the donor's medical and personal history.

1.2.4. The complete donor records must be reviewed and assessed for suitability and signed by a qualified health professional.

……

1.4. Donor documentation

1.4.1. For each donor, there must be a record containing:

(a) the donor identification (first name, family name and date of birth — if a mother and child are involved in the donation, both the name and date of birth of the mother and the name, if known, and date of birth of the child);

(b) age, sex, medical and behavioural history (the information collected must be sufficient to allow application of the exclusion criteria, where required);

(c) outcome of body examination, where applicable;

(d) haemodilution formula, where applicable;

(e) the consent/authorisation form, where applicable;

(f) clinical data, laboratory test results, and the results of other tests carried out;

(g) if an autopsy was performed, the results must be included in the record (for tissues and cells that cannot be stored for extended periods, a preliminary verbal report of the autopsy must be recorded);

(h) for haematopoietic progenitor cell donors, the donor's suitability for the chosen recipient must be documented. For unrelated donations, when the organisation responsible for procurement has limited access to recipient data, the transplanting organisation must be provided with donor data relevant for confirming suitability.

Annex 4. ECDC comparison of US/EU/Council of Europe guidance and regulations on donor eligibility determination by risk factor review

Q2: Do the HTLV transmission risks differ by type of tissue/cells or processing of them and if yes, how do they differ?

- Classification of grafts tissues/cells
  - FDA: viable, leukocyte-rich/non-viable, leukocyte-rich
  - Council of Europe: musculoskeletal tissues, cardiovascular tissues, skin tissues, ocular tissues;
  - Retrieved from living/deceased donors
  - Can be sterilised/cannot be sterilised
- Risk of transmission by tissue type
  - Significant risk of HIV, HVB, HVC transmission by musculoskeletal tissues because of their high blood content and high donor-recipient ratio (Pruss A.). Nucleic-acid amplification testing or quarantine is recommended for HIV, HVB and HVC.
- Processing or inactivation methods and viral reduction processes:
  - Exposure to antibiotics, disinfection, thermo-disinfection, sterilisation by ethanol oxide and gamma irradiation;
- Reduction of risk after processing
  - Validation of pathogen inactivation: infectivity reduced to less than 104 viruses.

US		Europe	
FDA. Guidance for Industry 'Eligibility Determination for Donors of Human cells, Tissues, and Cellular and Tissue-Based Products (HCT/Ps)'; August 2007 *Note: the FDA document is a guidance document aiming to help establishments to comply with the Code of Federal Requirements (21 CFR, part 1271, subpart C);*	References to HTLV testing of donors identify two types of HCT/Ps: • Viable, leukocyte-rich cells or tissues, including but not limited to: - Hematopoietic stem/progenitor cells - Semen. Mandatory tests: HTLV I/II, CMV. • Non-viable, leukocyte rich cells or tissues, includes but are not limited to: - Cornea - Sclera - Skin - Heart valves - Dura mater - Bone - Tendons - Ligaments - Cartilage - Oocytes.	**Council of Europe** Guide to safety and quality assurance for the transplantation of organs, tissues and cells. 3rd edition, 2009	Chapter 3: Selection of donors by tissue-specific criteria**.** Classification: - musculoskeletal tissues - cardiovascular tissues - skin tissue - ocular tissue *No mention of HTLV!* Tissue-specific selection criteria: - Age restrictions for some tissues - Trauma or chronic pathology of the tissue - Tumour or neoplasia of the tissue - Microbiological infection of the tissue - Specific warm ischemia time - Appropriate tissue function - Poisoning. Musculoskeletal tissue-specific selection criteria. Ocular tissue-specific selection criteria (no references to HTLV).
Eastlund T. 1995. Cell Transplantation 'Infectious disease transmission through cell, tissue, and organ transplantation: reducing the risk through donor screening' . Original contribution	Table 3: Allograft characteristics affecting ability to transmit disease • Nonviable allografts, acellular, connective tissue, can be processed, sterilised: - bone - dura mater - pericardium - tendon - costal cartilage - fascia - ear ossicles. • Viable allografts, contain cells, may be antibiotic-treated, cannot be sterilised: - cornea - heart valves - vessels, skin - marrow, blood stem cells - vascularised organs - semen and oocytes - fetal tissue.	**Germany:** **Pruss A.** Tissue donation and virus safety: more nucleic acid amplification testing is needed. Transplant Infectious Dis. 2010. Oct; 12(5):375-85 Institute of Transfusion Medicine, Berlin.	In Germany, donor selection is based on: medical history, behaviour risk factors, physical examination, lab tests, and careful management of the tissue bank. Additional measures: Quarantine storage of the donation allows retesting of living donors after a period of time. Nucleic-acid amplification testing (NAT) makes it possible to avoid the quarantine period. For deceased donors, the first recipient of a transplant should be tested after an appropriate period. NAT used to reduce the residual risk. NAT is recommended for HIV, HVB, HVC for musculoskeletal donors when virus inactivation procedures are not applied, due to high blood content and high donor-recipient ratio;

US		Europe	
	• Processing techniques that reduce the risk of transmission: Bone: - blood and marrow cell purge, - ethanol soaks, - sterilisation (gamma irradiation, ethylene oxide) Dura mater: - sterilisation (gamma irradiation, ethylene oxide) Tendon: - gamma irradiation Cartilage: - disinfection, sterilisation Ear ossicles: - disinfection, sterilisation.		- NAT as an additional safety measure also for cardiovascular tissue donors; - NAT for HVC advised for corneal donors; HTLV screening is not required in Germany for epidemiological reasons. Grafts from cadaveric donors might pose a risk as the medical history might be difficult to obtain, frequently only post-mortem blood samples are available for testing. The donor-recipient ratio is high for cadaveric donors. Virus inactivation methods - majority are for non-vital musculoskeletal tissues: peracetic acid-ethanol treatment, thermo-disinfection, gamma irradiation, combined methods - the inactivation method has to be validated, less than 4 log10 reduction of infectivity (TCID50) for relevant viruses - freeze-drying hardly reduces infectivity of HVB, HVC, HIV
Yao F. 2007 Australia. The risk of HIV, HVB, HCV and HTLV among musculoskeletal donors in Australia.	The prevalence of HIV, HVB, HVC and HTLV, although low, are higher among musculoskeletal tissue donors than first-time blood donors ($p<0.05$)! HTLV prevalence: 121.88/100 000 msk tissue donors HTLV probability of viraemia (residual risk): 1: 118 000, can be reduced by NAT. Musculoskeletal allografts are the most frequently transplanted human tissue after blood.		Bio-burden reduction with gamma irradiation
Levin M. Blood 1997. Extensive latent retroviral infection in bone marrow of patients with HTLV1-associated neurological disease.	Bone marrow (BM) may be an important reservoir of HTLV-I in patients with HTLV-1 associated neurological disease. It is still unknown whether BM from asymptomatic carriers or patients with other HTLV-1 associated diseases also contains high levels of latent retrovirus.		
Yoshio Koyanagi. 1993. Virology. In Vivo Infection of Human T-Cell Leukemia Virus Type I in Non-T Cells	HTLV-I has a broad host range in vivo and monocytes or cells of monocyte lineage such as tissue macrophages might comprise a virus reservoir in vivo. The HTLV infected macrophages/monocytes may contribute to the persistent infection in tissues, including CNS and joints.		

Q3. Effectiveness of measures to prevent HTLV I/II transmission

<table>
<tr><th colspan="2">US</th><th colspan="2">Europe</th></tr>
<tr>
<td>FDA. Guidance for Industry

'Eligibility Determination for Donors of Human cells, Tissues, and Cellular and Tissue-Based Products (HCT/Ps)'; August 2007

Note: the FDA document is a guidance document aiming to help establishments to comply with Code of Federal Requirements (21 CFR, part 1271, subpart C);</td>
<td>III. The donor eligibility determination (1271.50) is based on the results of donor screening (1271.75) and testing (1271.80, 1271.85). Some exceptions.

A donor is eligible only if:
• Screening shows that the donor is free of risk factors for, and clinical evidence of, infection due to relevant CD agents and diseases (RCDADs) and free of CD risk associated with xenotransplantation

AND
• Test results for RCDADs are negative or non-reactive (exception for non-treponemal screening tests for syphilis).

Eligibility determination is made by a 'responsible person', authorised, trained and qualified (medical training, adequate knowledge of Federal reg., guidance).
RCDADs are:
• Relevant for ALL types of HCT/Ps:
– HIV 1, 2
– HBV
– HCV
– TSE, CJD
– TP (syphilis)
• Relevant for viable, leukocyte-rich cells and tissues, including reproductive cells or tissues if they are considered to be viable leukocyte-rich (section VI.B.2: hematopoietic stem/progenitor cells, semen)
– HTLV types I and II
• Relevant for reproductive cells or tissues:
– Chlamydia t.
– Neisseria gonorrhoea
– Other RCDADs (transmissible, life-threatening, available and appropriate screening methods and tests):
– West Nile Virus,
– Sepsis,
– Vaccinia (smallpox vaccination)

Quarantine: all HCT/Ps must be quarantined until completion of donor-eligibility determination.

IV. Donor screening

Sources of information for:
• risk factors: medical history interview
• physical examination: a current report of the physical assessment of a cadaveric donor or the physical examination of a living donor; results of a limited autopsy, a recent ante-mortem or post-mortem physical examination ;
• laboratory tests results (other than above);
• other (police reports

Are ineligible to donate/ exclusion criteria:

A. Those who exhibit one or more of the following risk factors (conditions or behaviours):
• MSM sex, in the preceding five years
• IDU, in the preceding five years
• Haemophiliacs receiving human derived clotting factor concentrates, in the preceding five years
• Sex in exchange for money or drugs, in the preceding five years.
• Persons who had sex with someone in four preceding categories above, or with HIV+, HVB, active hep C; in the preceding 12 months
• Persons exposed to known or suspected HIV, HVB, HCV infected blood [...],in the preceding 12 months
• Children born to mothers with HIV infection or at risk for HIV, if:
• aged 18 months or younger or if
• breastfed within the preceding 12 months</td>
<td>Commission Directive 2006/17/EC: technical requirements for the donation, procurement and testing of human tissues and cells</td>
<td>(3) The risk of disease transmission and other potential adverse effects can be reduced by careful donor selection, testing of each donation and the application of procedures to procure tissues and cells in accordance with rules and established and updated according to the best available scientific advice.

Article 2: requirements for the procurement of HTC; 2-12

Selection criteria for donors: Annex I

Laboratory tests required for donors: Annex II
Selection criteria, Annex I:

Selection of donors is based on a risk analysis.
Indicators of risk must be identified by:
• physical examination,
• review of medical and behavioural history,
• biological testing,
• post-mortem examination,
• any other when appropriate.

Donors must be excluded if/Exclusion criteria:

Deceased donors
• Cause of death unknown, autopsy not relevant;
• History of disease of unknown aetiology
• Malignant disease (present or history of). Some exceptions. Can be considered for cornea donation unless retinoblastoma, haematologic neoplasm, malignant tumours of the anterior eye.
• Risk of prion transmission:
– history of CJD, CJDv, family history of non-iatrogenic CJD,
– history of rapid progressive dementia, degenerative neurological disease, or neurological disease of unknown origin;
– recipients of hormones derived from human pituitary gland (growth hormones),
– recipients of grafts of cornea, sclera, dura mater,
– people who undergone undocumented neurosurgery (where dura mater might have been used);
• Systemic infection, not controlled at the time of donation (bacterial, viral, fungal, parasitic), or significant local infection in the tissues to be donated;
• History, clinical evidence, or lab evidence of HIV, acute or chronic HVB, HVC, and HTLV1/ 2, or transmission risk or evidence of risk factors for these infections;
• History of chronic, systemic autoimmune disease that could affect the quality of tissue;
• Invalid blood tests in case of: haemodilution (Annex II) , treatment with immunosuppressive agents;
• Travel-related risk factors according to exposure history and local infectious disease prevalence;
• Physical signs implying a risk of transmissible diseases (Annex IV, 1.2.3.)
• Ingestion of or exposure to substances (cyanide, lead, mercury, gold) that may be transmitted to recipients;
• Recent history of vaccination with live attenuated viruses, where there is a risk
• Transplantations with xenografts ;
• Deceased child donors are excluded:
– until tests are available if their mothers were HIV-infected
– definitely excluded: children less than 18 months old whose mothers are infected with or at risk of HIV, HVB, HVC, HTLV , and who have been breastfed in the previous 12 months;</td>
</tr>
</table>

US		Europe	
	• Juvenile detention, lock up, jail or prison for more than 72 consecutive hours in the preceding 12 months • Household contacts of hepatitis B cases or clinically active hepatitis C in the preceding 12 months • Tattooing, body-piercing in the preceding 12 months, with unsterilised instruments • History of symptomatic viral hepatitis after the 11th birthday (except if HAV, EBV, CMV). • A deceased person diagnosed with sepsis • Smallpox vaccination in the preceding eight weeks, or if developed complications • Vaccinia virus clinic infection through contact with smallpox vaccine recipient • Diagnosed with WNV infection (to be deferred for 120 days) • A positive test for WNV in the preceding 120 days • Syphilis in the preceding 12 months • *Chlamydia* or *Neisseria gonorrhoea* infection in the preceding 12 months, if donors of reproductive HCT/Ps • Diagnosed with CJD, CJDv • Diagnosed with dementia or any degenerative or demyelinating disease of CNS, or with other neurologic disease of unknown etiology; • Recipients of dura mater transplants, growth hormones (human pituitary-derived), or persons who have relatives diagnosed with CJD • Persons who have a history of CJD in a blood relative; • Persons who spent three months or more cumulatively in the UK, 1980-1996 • US military staff who resided in Germany, Belgium, and the Netherlands for more than six months cumulatively 1980–1990, or in Greece, Turkey, Spain, Portugal and Italy for six months, 1980–1996 • Persons who spent five years or more cumulatively in Europe from 1980-present; • Persons who received transfusion of blood or blood components in the UK or France, 1980–present • Persons or their sexual contacts born or having lived in Africa (Cameroon, Central African Republic, Chad, Congo, Equatorial Guinea, Gabon, Niger, Nigeria) after 1977; • Persons who received a blood transfusion or any medical treatment involving blood in the countries listed above; • Recipients or sexual contacts or xenotransplants. B. Those who exhibit clinical evidence of one or more RCD: • HIV infection • Hepatitis infection • Syphilis, *Chlamydia trachomatis*, *Neisseria gonorrhoea* (latter two for donors of reproductive HCT/Ps only), • Vaccinia infection • WNV infection • Sepsis (list of conditions) • HTLV infection (only for donors of viable, leukocyte rich HCT/P) : – A prior positive or reactive screening test for HTLV – Unexplained paraparesis and/or – Adult T-cell leukaemia. C. Those who exhibit one or more of the following physical evidence: • Signs of risk of STI (genital ulcers, herpes simplex, chancroid) • Syphilis • Evidence of anal intercourse/perianal condyloma • Needle tracks (IDU) • Recent tatooing, piercing • Disseminated lymphadenopathy		Living donors (allogeneic) must be selected on the basis of their state of health (clinical investigation) and medical and behavioural history (questionnaire, interview), results of lab tests. • Same exclusion criteria as for the deceased donors!! • Supplementary criteria: pregnancy, breastfeeding, potential of transmission of inherited conditions. Laboratory tests required for donors, Annex II: • only by qualified, authorised labs • using EC-marked testing kits where appropriate • on donor's serum or plasma • apply algorithms for testing in haemodilution circumstances • deceased donors to be tested just prior to death or within the 24 hours following death; • sampling at the time of donation for living donors, or within seven days post donation • repeat sampling and testing of living donor after 180 days is required when tissues and cells can be stored for a longer period. Retesting not required if NAT performed for HIV, HVB, HVC and virus inactivation methods were applied. Biological tests required for ALL donors: • Anti-HIV1-2, • HBsAg, Anti HBc, • Anti HCV-Ab, • Syphilis NAT is encouraged where appropriate and available. Tests required for donors living in or originating from high incidence areas, or with sexual partners /parents originating from high incidence areas: • HTLV-1 antibody Additional tests: RhD, HLA, malaria, CMV, toxoplasma, EBV, *Trypanosoma cruzi*;

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	• Unexplained oral thrush • Blue or purple spots (Kaposi's sarcoma) • Unexplained jaundice, hepatomegaly, icterus • Rush, fever (sepsis) • Large scab (smallpox vaccination) • Eczema vaccinatum • Generalised vesicular rash • Severely necrotic lesions • Corneal scaring V. Donor testing • only FDA licensed lab and tests, • tests for cadaveric specimens, • collect donor specimen at the same time as HCT/Ps are recovered, or within 7 days before or after HCT/Ps; some exceptions apply (30 days before); • recipients of transfusion or infusion might be ineligible Tests required for ALL donor of HCT/Ps: • HIV, type 1 (anti HIV-1, or combination anti HIV1-2 and NAT test for HIV-1 or combination NAT) • HIV, type 2 (anti HIV-2 or combined) • HBV (HBsAg, antiHBc) • HVC (anti HVC, NAT) • Treponema palidum Tests required for donors of viable, leukocyte rich cells or tissues: • HTLV, type I and II • CMV Examples of viable leukocyte rich cells or tissues: – Hematopoetic stem/progenitor cells – Semen Examples of tissues and cells not considered viable, leukocyte rich: – Cornea, sclera, skin, heart valves, dura mater, bone, tendons, ligaments, cartilage, oocytes.		
		Council of Europe Guide to safety and quality assurance for the transplantation of organs, tissues and cells 3rd edition, 2009	Chapter 3: Selection of donors 3.2.3. Evaluation of the deceased potential donors • medical history; • behavioural history, travel history; • clinical examination (to look for evidence of high risk behaviour, unexplained jaundice, hepatomegaly, neoplastic disease, trauma at site; • lab tests: HIV1/2, AgHbs, HBcAb, HCV-Ab, syphilis, CMV, EBV, toxoplasma, HTLV I, malaria for donors living in or coming from high prevalence areas (ref. WHO website www.who.int/transplantation/en) Exclusion criteria: • Transmissible diseases (HIV, active malignant neoplasia, severe systemic infections, prion risk, viral hepatitis, • Behavioural risks – MSM sex, in the preceding 12 months – IDU ,in the preceding 12 months – History of chronic haemodialysis – Sex in exchange for money or drugs, in the preceding 12 months – Persons with haemophilia receiving human derived concentrates – Sexual partners of persons with HIV, HVB, HVC history, or infection, or risk factors, in the preceding 12 months – Percutaneous exposure to blood thought to be at risk of HIV or hepatitis, in the preceding 12 months – Inmates in the preceding 12 months – Diagnosed or treated for syphilis or gonorrhoea in the preceding 12 months – If blood transfused in the preceding 12months,

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			can be accepted only after individual approval by a responsible person; – Any previously deferred donor – Unsafe tattoo, piercing in the preceding 12 months – Children less than 18 months from HIV positive mothers, breastfed in the preceding 12 months – History of travel to endemic areas (malaria, rabies, WNV, etc) • Autoimmune diseases: collagen diseases, systemic vasculitis; may require additional investigations. 3.3.3 Evaluation of potential living donors: • medical history, • physical examination, • testing.
Cell Transplantation Infectious disease Transmission through cell, tissue, and organ transplantation: reducing the risk through donor screening 1995, Eastlund Ted -original contribution-	Measures to prevent transmission: • Medical history review- exclusion of those with infections, malignancies, recipients of human pituitary gland hormones • Behavioural risk factors for HIV-1 and hepatitis • Blood tests: HTLV • Physical examination, autopsy examination Additional measures: • Aseptic procurement and processing technique • Exposure to antibiotics, disinfectants, sterilisation (ethylene oxide, gamma irradiation) may further reduce the risk of transmission.		