Chapter 10
Testing the Donor

Alan Kitchen and John Barbara

Introduction

The transplantation of tissues from donor to recipient is a fundamental part of medicine today. A range of tissues including bone, skin, tendons, heart valves, corneas etc. are collected from suitable donors and transplanted into those patients for whom tissue transplant would have clinical benefit.

In the same manner as blood transfusion, transplantation is not without risks, one important risk being that of the transmission of infectious disease via the transplant; transplantation transmitted infections (TTI). Although it is clear that not all tissues carry the same risk, such distinctions cannot be easily quantified and all tissues should be treated in the same way in terms of the infectious disease screening applied.

Thus, in the same way as for blood donations, all tissue donations must be screened for infectious diseases prior to release for clinical use. Although the basic principles applied to the screening of tissue donations are the same as for blood donations, or any other type of donation of biological products, there are some important differences between blood and tissue donations which result in differences in the actual screening strategies applied. Firstly, and most importantly the source of tissue donations includes both living and deceased (cadaveric) donors, and secondly, whilst the specific infectious agents screened for are broadly the same, the specific markers of infection used for the individual infectious agents may differ.

A fundamental issue when considering the screening of tissue donations for transmissible infectious agents (TIA) is the status of the donor, living or deceased. The screening of tissues collected from living donors for TIAs is effectively no different to the screening of blood donations, in that the blood samples used for screening are collected by normal venepuncture from living individuals. The

A. Kitchen (✉)
National Transfusion Microbiology Reference Laboratory, NHS Blood and Transplant, Colindale Avenue, London NW9 5BG, UK
e-mail: alan.kitchen@nhsbt.nhs.uk

G. Galea (ed.), *Essentials of Tissue Banking*, DOI 10.1007/978-90-481-9142-0_10,

characteristics of the samples are identical and the screening is performed using assays specifically designed, developed and supplied to test blood samples collected from living individuals. It is the screening of tissues from deceased donors, where the blood samples are collected post-mortem (non heart beating), that presents some specific problems that need to be overcome to ensure accurate and reliable screening results. These problems all surround the overall quality of the samples obtained and their validity as suitable substrates for the assays used; the time post-mortem of collection, the integrity of the sample, volume, level of haemolysis, biochemical changes post-mortem etc. However, as long as these problems are acknowledged and dealt with, and resolved in the right ways, the overall quality of the screening programme should be the same whether the samples collected to screen the tissue donations are from living or deceased donors.

Similarly, the screening of the donors themselves, the donor selection process, is also determined by the nature of the donor. The selection process applied to living donors is similar to that applied to blood donors, except that such donors do not generally volunteer in the same way as an "altruistic" blood donor. Living tissue donors may often have been approached directly and a direct history may therefore be taken from them. However in the case of deceased donors, although the process is the same, the history is second hand and may not be totally correct or accurate [1]. Thus the inherent risk associated with deceased tissue donations must necessarily be higher than that of living donations and the screening strategy developed accordingly.

This text looks at the screening of tissue donations for the range of infectious agents that may be present in the donor at the time of collection of the tissue(s) and which are therefore likely to be present in the donations collected and consequently likely to be transmitted through the transplantation of the donated tissues. These agents include viruses, bacteria, parasites and prions. The subject of the bacterial/fungal contamination of tissue products as a result of the collection, processing and storage processes is not covered in this particular text.

Range of Infectious Agents

The infectious agents that tissue donations need to be screened for are essentially the same as for blood donations, as are the specific markers used as screening targets for the individual infectious agents. However it may be that for some infectious agents additional markers may be included to increase the sensitivity of screening.

Table 10.1 lists the range of infectious agents that have been reported to be transmitted via blood and blood products. Although only a few of these agents have been reported to be transmitted via tissue transplantation, it is reasonable to consider that all of these agents could and would be transmitted if present in a tissue donor and in the donated tissues. Thus the same approaches in terms of identifying specific donor risk need to be followed, albeit with the obvious issues in the pre-selection of deceased donors.

Table 10.1 Infectious agents currently reported to have been transmitted via blood transfusion or tissue transplantation

Viruses	**Bacteria**
Hepatitis viruses	*Endogenous*
Hepatitis A virus	*Treponema pallidum* (syphilis)
Hepatitis B virus	*Borrelia burgdorferi* (Lyme disease)
Hepatitis C virus	*Brucella melitensis* (Brucellosis)
Hepatitis D virus (requires co-infection with HBV)	*Yersinia enterocolitica/Salmonella* spp.
Hepatitis E virus	*Exogenous*
Retroviruses	Environmental species: staphyloccocal pp./pseudomonads/*Serratia* spp.
Human immunodeficiency virus 1 and 2 (plus other subtypes)	Rickettsiae: *Rickettsia rickettsii* (Rocky Mountain spotted fever), *Coxiella burnetii* (Q fever)
Human T-cell leukaemia virus I and II	**Protozoa**
Herpes viruses	*Plasmodium* spp. (malaria)
Human cytomegalovirus	*Trypanosoma cruzi* (Chagas' disease)
Epstein–Barr virus	*Toxoplasma gondii* (toxoplasmosis)
Human herpesvirus 8	*Babesia microti/divergens* (babesiosis)
Parvoviruses	*Leishmania* spp. (leishmaniasis)
Parvovirus B19	**Prions**
Miscellaneous viruses	variant Creutzfeld Jakob Disease (vCJD)
GBV-C: previously referred to as hepatitis G virus	[classical CJD has been transmitted by corneas and dura]
TTV	
West Nile virus	
Dengue virus	
Rabies via organs	

The actual transmissible infectious agents that are of specific interest can be considered in three categories:

- those for which all tissue donations must be screened – mandatory
- those which are recognised and commonly encountered threats, but for which only specific donations need to be screened because of specific identifiable risk – discretionary (UK terminology)
- those which are rare or unknown threats and which are dealt with on a case-by-case basis – others

In addition to defining the infectious agents themselves, the specific target marker of infection for each infectious agent must also be defined. The available targets vary according to the infectious agent and theoretically there are always 3 targets for any infectious agent. However it is the suitability of each of these targets in the context of the screening of what should be low risk donors, that has to be considered, from the perspective of the biology of the agent and the host response to infection, and the sensitivity and predictiveness of the available tests. Figures 10.1, 10.2, 10.3, 10.4, 10.5 and 10.6 show the different plasma markers that can be detected following infection with HBV, HIV and HCV respectively. The figures depict the relative

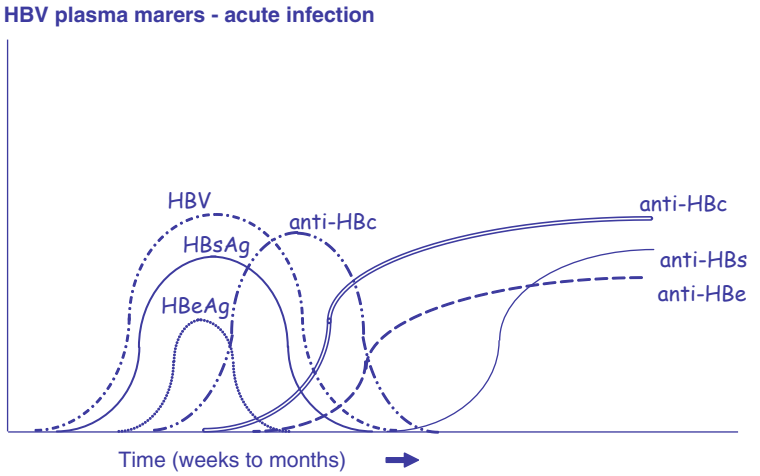

Fig. 10.1 HBV plasma markers – acute infection

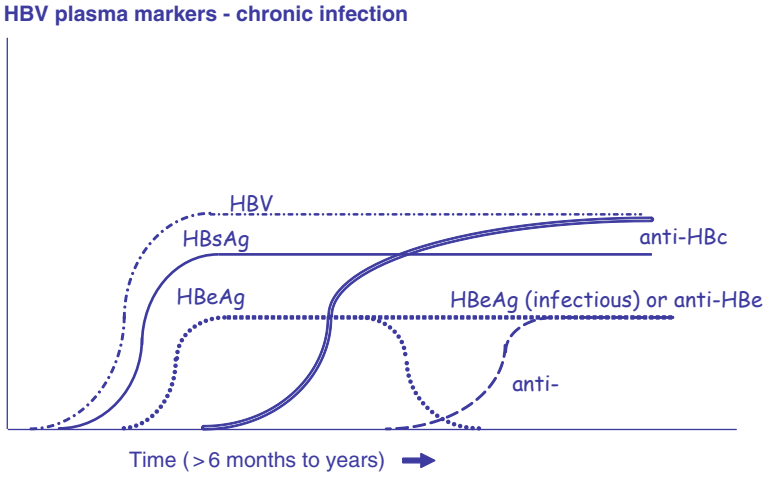

Fig. 10.2 HBV plasma markers – chronic infection

timeframes for the appearance of the different markers for each of these agents, and from these the most useful markers in terms of reliable detection of infection can be determined. A pre-requisite for any effective screening programme is always a thorough understanding of the biology of the agent and course of the infection in the host.

Even though it could be argued that some tissue products are processed in ways that remove or inactivate any infectious agent that could theoretically be present, the use of tissue products collected from a donor who may be infected is not considered to be appropriate practice, irrespective of the processing methods used. No pathogen inactivation or removal methods can be considered to be totally effective

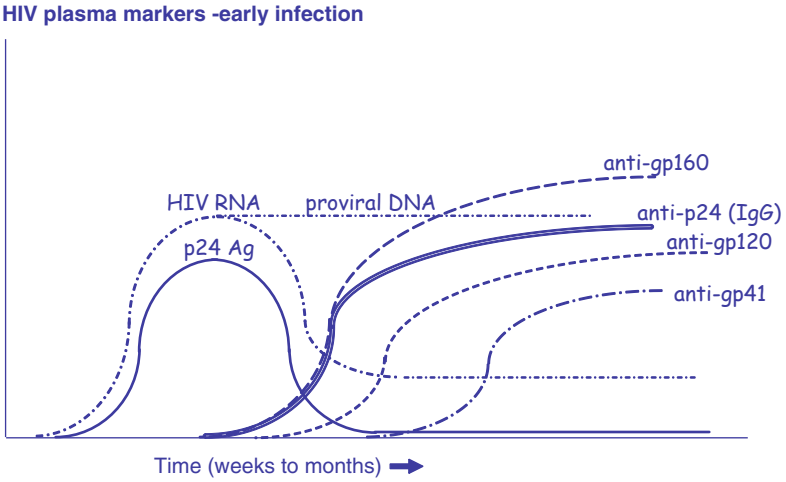

Fig. 10.3 HIV plasma markers – early infection

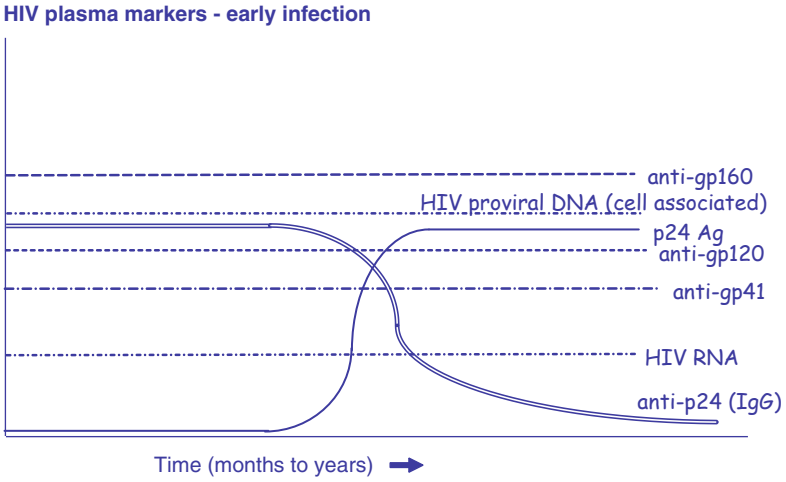

Fig. 10.4 HIV plasma markers – late infection

and therefore must be used in conjunction, not instead of, screening. If a donor is known to be infected with any of the transmissible infectious agents there is a clear risk of transmission and therefore the products should not be released for clinical use.

Mandatory Screening

The infectious agents that are currently considered to be mandatory for the screening of tissue (and blood) donations in most countries with developed healthcare systems

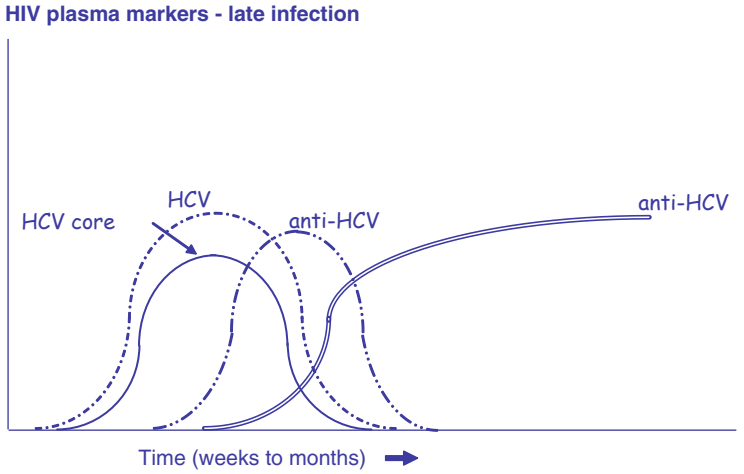

Fig. 10.5 HCV plasma markers – acute infection

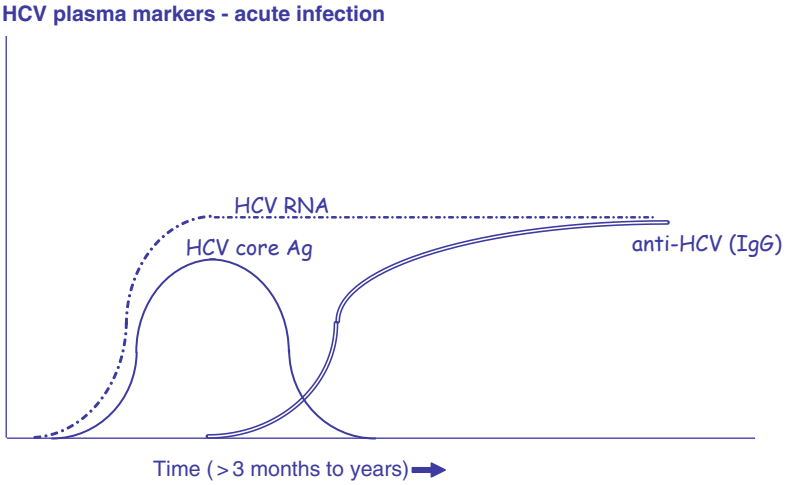

Fig. 10.6 HCV plasma markers – chronic infection

and formal regulatory authorities are hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency viruses 1+2 (HIV), human T-cell lymphotropic viruses I+II (HTLV) and *Treponema pallidum* (causative agent of syphilis). These 5 infectious agents are those for which there is published, irrefutable evidence of transmission through blood and other body fluids, and therefore the potential for transmission through residual blood in tissue products. The agents are generally present in populations worldwide, although the prevalence and incidence does vary significantly from country to country, and present such a general risk that, in theory, any donor could be infected, irrespective of absence of (known) risk or symptoms.

It is important to understand that risk is something that the pre-donation questioning and selection process seeks to identify, but donors may have been exposed to an infectious agent through a route that they were not aware of. In addition, all of these infectious agents do produce asymptomatic sub-clinical and chronic infections, and donors may appear to be fit and well at the time of donation whilst being infected and thus infectious. This is especially important in the screening of cadaveric donors as they obviously cannot be questioned directly, and the selection process is dependent upon what family and close contacts believe, which may not necessarily be the truth.

All donations are therefore theoretically at risk of being infected with one or more of these agents and so blanket screening of all donations is necessary. However, as mentioned above, as well as identifying the infectious agents, the specific screening target(s) for each infectious agent must be identified. Table 10.2 lists these specific screening targets and the mandatory screening requirement for all donations collected by the UK blood services.

Discretionary Screening

Those infectious agents for which screening can be considered to be discretionary, i.e. not all donations require screening, comprise agents for which again there is published, irrefutable evidence of transmission through blood and other body fluids and which can cause significant disease, but where risk is, and can be more easily, mitigated because the agent is not endemic or naturally present in the general population in that country, and where there are particular risk factors for infection that can be specifically identified in a donor. Specific examples of infectious agents that can be defined as discretionary are West Nile Virus (WNV), *Plasmodium* spp. (causative agents of malaria), *Trypanosoma cruzi* (causative agent of Chagas' disease). To be considered as a potential risk there is an absolute requirement for exposure of the donor to the virus (WNV) or parasite (*Plasmodium/ Trypanosoma*) by either living in, or visiting, an endemic area. Donors who are at risk can therefore be easily and specifically identified at the point of donation and only donations from such donors require this additional screening.

Other Screening

In addition to the well known, characterised and commonly screened for infectious agents, there is always the potential for the transmission of other infectious agents.

- These may be existing agents which have a generally low prevalence and incidence, often tightly geographically restricted, are rarely transmitted, but which nonetheless may be transmitted if present in the donor at the time of donation.

Table 10.2 Current mandatory screening of donations within the UK blood services

Marker/donation	HBs Ag	HIV Ag/Ab	HCV Ab	Syphilis Ab	HTLV Ab (S)	HTLV Ab (P)	HBc Ab	HCV RNA (P)	HCV RNA (S)	HIV RNA (P)	HIV RNA (S)	HBV DNA (S)	HCMV Ab	HCMV DNA
Blood	X	X	X	X		X		X						
Surgical tissue (one sample)	X	X	X	X		X	X	X		X		X		
Surgical tissue (two	X	X	X	X		X	X							
samples, 180 days apart)	X	X	X	X		X	X							
Deceased donor (adult)	X	X	X	X	X		X		X		X	X		
Deceased (I)	X	X	X	X	X		X		X		X	X		
(neonate) (M)	X	X	X	X		X	X	X		X		X		
Stem Cells	X	X	X	X	(X)[a]	(X)[a]	X							
Allogeneic cord blood	X	X	X	X	X		X		X		X	(X)[b] HBcAb+	X	(X)[c] CMV Ab+

[a]Singleton HTLV is needed in "high risk" HTLV areas.
[b]Required if the donation is found to be HBcAb+, irrespective of HBsAb status.
[c]Required if mother is CMV Ab+.

- There may be emerging agents that are transmissible, have been rarely transmitted in the past but where their incidence and prevalence are starting to increase, either through increasing incidence in endemic countries, through spread into previously non-endemic countries or through increased travel into endemic countries.
- There may be yet unidentified infectious agents that would be transmissible but which at this time have not appeared as the causes of any identifiable transfusion or transplantation associated infections.

Table 10.1 can be considered as a baseline list of infectious agents that are known to present a threat because of reported blood transfusion transmissions. Although the agents listed have been transmitted through blood, there may have been several transmissions or only a handful of cases globally especially the more "exotic" agents listed. However, to provide a definitive list of all possible current and future infectious threats is clearly impossible. In terms of potential candidate agents, meeting the following criteria would identify those particular agents that could be transmitted through tissue transplantation, if present in the donor at the time of donation. To be considered a viable threat the agent must be:

- Present in the donated tissue, in an infectious form
- Stable in the donated tissue/processed processed under the storage conditions normally required for such products
- Circulating in the donor at the point of collection of the tissue, yet without giving rise to any identifiable symptoms in the donor

This does narrow the field somewhat, and it must be made clear that not every infectious agent that afflicts man is a potential transfusion/transplantation threat. Indeed the number of potential threats is only a small proportion of those infectious agents currently known to man. Nonetheless, there are a few well characterised agents that, whilst not a major concern at present, may spread into currently non-endemic areas and therefore need to be monitored. These include viruses such as SARS, Chikungunya virus, Lymphocytic Choriomeningitis Virus (LCMV), and parasites such as *Leishmania* spp.

One major problem that may influence any screening programme for an infectious agent are the potential limitations either in the appropriate screening target(s) available and/or the corresponding screening tests being available. This is may significantly hinder impact on the ability to develop effective and appropriate screening strategies for some agents, resulting in a greater reliance on donor exclusion rather than in-vitro screening (something that is not always possible or effective with cadaveric donors/donations).

Severe Acute Respiratory Syndrome (SARS)

Severe acute respiratory syndrome (SARS) is a respiratory infection caused by a recently emergent coronavirus (SARS-CoV). The disease has severe morbidity

and mortality, but presents with non-specific signs and symptoms and there is no clear-cut diagnostic approach to prospectively identifying cases prior to the appearance of symptoms. The virus has a viraemic phase of 4–8 days, which precedes symptoms and then persists into the symptomatic phase. Viraemic individuals may transmit if blood or tissues are collected during the early asymptomatic phase of infection. Screening of individuals for SARS-CoV is possible using molecular techniques but identification and deferral of donors who may have been potentially exposed to the virus is currently the most effective way of minimizing risk of transmission.

Chikungunya

Chikungunya is an arbovirus (viruses transmitted by insects) that is transmitted primarily by mosquitoes, usually of the *Aedes* spp.. The illness is an acute symptomatic disease that characteristically begins with rapid onset of joint pains and may or may not be accompanied by muscle pain, high fever, conjunctivitis, and a rash. The severity of the illness varies and tends to be less severe in children. Most infected individual recover after a few weeks but a small proportion may have residual chronic joint pain for some years. Infection is not usually fatal, but may be a contributing factor in the death of some patients with pre-existing underlying disease. The disease occurs throughout Africa, Asia, parts of southern Europe, anywhere where the carrier mosquito species are found. More recently there have been a number of outbreaks on the islands in the Indian Ocean, one in particular on Reunion resulting in the cessation of blood collection until the outbreak had passed.

The virus has a viraemic phase prior to and during the symptomatic phase. Viraemic individuals may transmit if blood or tissues are collected during the early pre-symptomatic phase of infection. Screening of individuals for Chikungunya is possible using molecular techniques but identification and deferral of donors who may have been potentially exposed to the virus is currently the most effective way of minimizing risk of transmission. However the overall risk of the transmission of Chikungunya through tissues is very low as the disease is of acute onset and generally highly symptomatic. In 2006, 133 cases of imported Chikungunya were recorded in the UK. All of these had a clear history of risk and most had clinical symptoms.

Lymphochoriomeningitis Virus (LCMV)

Lymphocytic choriomeningitis virus (LCMV) is a virus carried by rodents that causes lymphocytic choriomeningitis (LCM). It presents as an aseptic meningitis, encephalitis or meningoencephalitis, although infection is often asymptomatic or with only mild febrile illness. The disease is rarely serious although infection during pregnancy can lead to severe disease in the infant. Infection is most commonly acquired through contact with infected rodents, often household pets, their urine, droppings bedding etc.. Transmission can also occur via bites and through open wounds where there has been contact with contaminated material. Human-to-human

transmission has not been reported except for vertical transmission from mother to foetus. However LCMV has been identified as being transmitted through organ transplantation [2], although cases of acute LCM in such donors would be very rare events. Screening for LCMV infection is possible, but molecular screening would be needed to identify viraemic donors.

Leishmania

Leishmaniasis is a parasitic disease that is found in parts of the tropics, subtropics, and southern Europe. It is caused by infection with *Leishmania* parasites, which are spread by the bite of infected sand flies. There are several different forms of leishmaniasis in humans, the most common forms being cutaneous which causes skin sores, and visceral (Kala Azar) which affects internal organs (e.g. spleen, liver, and bone marrow). The factors determining the form of disease include leishmanial species, geographic location, and immune response of the host. Although generally symptomatic, asymptomatic infections do occur and it is possible for parasitaemic individuals to present as blood or tissue donors, although there are no reported cases of transmission through blood or tissues. Screening for leishmaniasis is not straightforward as antibody responses may be low or absent in cutaneous cases, although screening is possible using molecular techniques. However identification and deferral of donors who may have been potentially exposed to the virus is currently the most effective way of minimizing risk of transmission

Screening Strategy

To be effective, in-vitro screening requires an effective screening strategy to be developed to ensure that the testing performed is targeted correctly and the outcomes are used effectively and appropriately.

Probably the first issue to be considered in the development of strategies for the screening of tissue donations is the fact that tissue donations can be divided into those from living donors (surgical tissues) and those from deceased donors (deceased tissues). The fundamental differences in the origins of the tissues, and thus the approaches available to "screen" the donors, means that the two have to be considered separately, and separate strategies developed. Whilst the core screening needs are essentially the same, the obvious restrictions in the donor selection process that can be applied to deceased donors means that there is a much greater reliance on in-vitro laboratory testing to determine the safety of the tissues collected. In the case of surgical tissues, however, the donor can be treated in virtually the same way as any blood donor, with a formal donor selection process prior to donation to identify any specific risks and determine the overall suitability of the donor as a source of a tissue donation. Although laboratory testing is still the final decider in the release of the donation for clinical use, experience from the selection of blood donors has shown the value of the donor selection process in reducing the risk of collecting a donation from a "high risk" donor, namely a donor who may have been recently

infected, and is infectious, but where the infection may not be detectable at that time with the tests in use – the window period.

There are two basic elements to a screening strategy, firstly defining the infectious agents and the individual specific screening target(s) for each infectious agent, and secondly the actual testing algorithm adopted.

As discussed above, the range of infectious agents that need to be considered to ensure tissue safety is similar to those for blood and generally well defined. Likewise the specific screening targets for each of these infectious agents are also well defined. However, these targets may differ depending upon the relative inherent risk associated with different donation types, in the case of tissues – deceased or surgical. Additional screening is added where the inherent risk is higher. Table 10.2 outlines the mandatory screening requirements for blood, tissue and stem cell donations collected and processed within the UK transfusion services. Although there is a common core, there are quite different additional requirements for different donation types. Defining the specific screening targets is therefore a fundamental part of any screening strategy, and has to reflect the specific infection risks in the donor population from which the tissues are collected.

The second key element of the screening strategy is the actual screening algorithm adopted. The algorithm essentially defines how the screening assays are used in terms of the initial and (any) repeat testing, and how the final screening result is determined. Again, this area is very similar to that for blood screening. The algorithms that are used for blood screening apply equally to tissue, and any other donation, screening. However it is essential to define the most appropriate algorithm for the particular screening performed, and it is here that blood and tissue screening may diverge. There are really only three possible algorithms that can be used for the screening of donations from low-risk donor populations to determine their fate, i.e. suitable for use or discard:

Algorithm 1 Initial screening test (Assay A) and use the results obtained to determine the donation fate. Negative – suitable for clinical use, Reactive – discard the donation

Algorithm 2 Initial screening test (Assay A) and repeat any initial reactives, in duplicate, using the same test. Use the 2 out of 3 rule (the 2 identical results out of the 3 results obtained are considered to be the true screen result) to determine the donation fate. Negative – suitable for clinical use, Reactive – discard

Algorithm 3 Initial screening test (Assay A) and repeat any initial reactives, in duplicate, using a different screening assay (Assay B). Use the 2 out of 3 rule to determine the donation fate. Negative – suitable for clinical use, Reactive – discard

The 3 algorithms are clearly quite different and have different uses, depending upon the type of donations being screened, the levels of infection in the donor population, and the complexity and effectiveness of the quality management system. Although all 3 algorithms are effective and will ensure similar levels of product

safety, it is the associated specificity that is perhaps of more interest and relevance when dealing with tissue donations. Importantly all 3 screening algorithms have the same sensitivity as all take the same approach in respect of the initial screen, and the acceptance of screen negative donations as being suitable for release for clinical use. Differences in sensitivity are solely due to the sensitivity of the screening assays used.

In the case of blood donation Algorithm 2 is the one used by the majority of transfusion services in countries with developed healthcare systems, and offers an effective approach, with an acceptable level of specificity. Although every donation is valuable, it is appropriate to point out that blood donations are more numerous than tissue donations, donors can donate regularly and it is far easier to replenish stocks. Thus the specificity of the screening and the algorithms, whilst important, is not so critical for blood donations, and the algorithm used reflects this. Indeed in those countries that use Algorithm 1, the unnecessary wastage of blood donations is even higher. However, in the case of tissue donations, and especially deceased donations where a single cadaver could provide a significant number of different tissues, the specificity of the screening algorithm is of far greater significance as repeatable screen reactivity would result in the discard of the products, even if that reactivity could be subsequently proven, through confirmatory testing, to be non-specific. Algorithm 3 is based upon the use of a second assay, Assay B that has at least equal sensitivity to Assay A, that is used to test the initial screen reactives. The algorithm is based on the fact that most non-specific reactivity is assay specific, and thus the majority of non-specific reactivity seen with Assay A, are unlikely to be seen with Assay B, whilst any true reactivity will always be seen in both assays. The algorithm increases the specificity of screening significantly, reducing the unnecessary loss of donations just to those that are reactive with both assays, generally those where infection is more likely to be confirmed. However Algorithm 3 is more challenging, both technically and from a quality management perspective, and is not an algorithm that is felt to be advantageous in a mass screening environment, screening blood donations. The major issue here is that a lot of tissue donations are collected, tested and processed by transfusion services and the screening approach used is that which was developed for blood donations, and may not be the optimal for other donation types, especially deceased donations. Thus consideration must be given to implementing the most appropriate algorithm for the product type, rather than the blanket use of algorithms that are primarily designed for blood donations, and where a lower level of specificity may be more acceptable.

This is particularly critical for deceased tissue donations where the samples are collected post-mortem and which are not the optimum substrate for the screening assays used and where overall the specificity of screening may be lower than that of blood donation screening. Although a number of authors have reported problems with the specificity of deceased screening programmes [3–7], the deceased tissue screening programme currently in place within the English National Blood Service is not encountering these levels of screen reactivity and the overall specificity of the screening programme is relatively high.

Sample Quality

The quality of the blood samples used to screen the tissue donations is clearly important. Poor quality samples produce poor quality results, with the potential resultant loss of sensitivity and/or specificity. The first issue to consider is the definition of quality as applied to blood samples collected for the purpose of screening tissue donations for release for clinical use, and here the difference between deceased and surgical tissues is very important.

Sample quality can be considered to include all of those attributes that may influence the overall reliability and accuracy of the screening results obtained. Importantly these include the condition of the sample including its age, the constituents of the sample (representativeness), and the volume. There are other, important, issues such as donor and sample ID etc., but these are outside the scope of this particular chapter.

Samples from Living Tissue Donors

The difference between deceased and surgical tissue donations is important, in relation to sample quality and in-vitro screening, as the blood samples collected from surgical donors are collected from living (“heart-beating") individuals and are thus the normal sample type that the screening assays are designed to be used for, and also should always be of sufficient volume for the screening required. Therefore the screening of surgical donations, notwithstanding the previous discussion about specificity, can be and are performed using the same systems and approaches as those used for blood donations. There should not be any differences in the nature of the blood samples collected or the reliability and accuracy of the results obtained.

Samples from Cadaveric (Non Heart-Beating) Donors

Unlike samples from living donors, blood samples from deceased tissue donors, unless the sample was collected whilst the donor was still heart-beating, cannot be expected to necessarily behave in the same way as those from heart-beating individuals, and therefore the screening performed and the results obtained have to be considered more carefully, and their validity established. A key issue, however, is not so much the fact that the blood sample is from a cadaver, but rather the representativeness of that sample, its suitability for use with the screening assays in use and therefore its ability to fully reflect the infectious disease status of the donor.

It is the nature of the blood sample collected from a cadaver that presents the major challenges. Blood samples collected post-mortem may be different from those collected ante-mortem for a number of reasons. The sample is collected from a now

static fluid that is settling into its cellular and liquid phases, in which coagulation may have occurred with the resultant biochemical changes including red cell damage, and where cell death is taking place with the release of a wide range of cell chemicals into the surrounding tissue fluids. The overall results of this may be gross haemolysis and/or other changes to the "blood" collected, including degradation of the screening targets that would be present in blood of an infected individual, and/or the release of substances that may be inhibitory to the screening assays used. However the changes that occur post-mortem obviously vary between cadavers, depending upon factors such as: age, cause of death, time between death and the cold-storage of the cadaver, time pre-mortem under direct clinical care and the interventions used, other underlying conditions (that do not de-bar tissue donation), and time post-mortem of sample collection. Thus the occurrence of post-mortem changes per se do not necessarily make any sample collected a less suitable substrate for screening. The screening of post-mortem samples from deceased tissue donors has been performed for many years in many countries with few, if any, known adverse outcomes. In addition assays can be validated for use with deceased samples, and indeed a few commercially available infectious disease screening assays for blood-borne viruses have undergone some limited validation work by the manufacturer with the resultant inclusion of claims that the assays can be used to screen deceased samples collected under certain conditions.

An important factor in the screening of deceased samples is the time, post-mortem, that the sample was collected. Clearly the sooner after death the sample is taken, the less likely it is to have been effected by any post-mortem changes. Interestingly however, currently there are few published papers that look critically at the time post-mortem for sample collection. Regulatory requirements within the EU require sample collection within 24 hours post-mortem, and in the US there is an expectation that tissues would be collected as soon as possible post-mortem, and within 24 hours, but this is currently not subject to any specific regulation. There are published studies on aspects of deceased testing that include samples collected up to 48 hours post-mortem and without any problems being encountered [4, 8], but specific studies on sample suitability related to time of collection are lacking.

Additionally there is the problem of possible haemodilution [9]. Very often the deceased donors were on life-support systems prior to their death and this may have involved either volume replacement with a range of inert volume expanders or by transfusion, in both cases potentially diluting out the constituents of the patients' own blood and therefore reducing the representativeness of the sample in terms of reflecting the true status of the donor. The immediate pre-mortem history of the donor must be known if there is any likelihood of adverse effects due to haemodilution, and at the very least the sample collection site chosen to try to minimise the risk of collection of a sample containing a significant amount of resuscitation fluid. There is a reported case of the failure of screening to identify an HIV infected tissue donor specifically because of massive fluid infusion shortly before death with the resultant dilution of HIV antibodies to below that detectable. Interestingly the antibody level returned to that normally seen, and detectable, 48 hours later as the

tissue fluids naturally re-distributed themselves [10]. However, most of the studies performed [9] have indicated that the modern screening assays used will tolerate a significant dilution of plasma before approaching the point at which a false negative result may occur. This situation is most likely to occur following significant acute blood loss and this fact will be apparent when assessing any potential deceased donor. There are FDA guidelines which seek to help clarify the situation, and these require tissue banks to develop algorithms to determine the degree of haemodilution and the action to be taken in terms of the validity of screening results [11] Within NHSBT there is a clear algorithm that can be followed in cases where haemodilution has occurred, that enables the degree of haemodilution, and thus sample suitability, to be assessed.

The actual physical process of obtaining a sample from a cadaver is very different and sample volume is often a major challenge in performing the screening required. As described above the blood settles out across the cadaver, both from the periphery into the larger vessels, and the cells settle out below the fluid component. Suitable sites for sample collection must therefore be identified, suitability depending on a number of factors. These include the presence of a suitable volume of fluid in the vessel and the accessibility of the vessel to facilitate sample collection, the probability of any resulting damage to tissues of interest, for example samples collected through cardiac stab, and the collection process itself must ensure that a suitable sample is obtained, i.e. sufficient serum/plasma to be able to perform all of the screening tests required. In addition the possibility of bacterial contamination of both the sample and the tissue itself, either due to bacteria already present in the cadaver or through the collection process itself, must always be considered. However grossly bacterially contaminated samples are usually very obvious and the suitability of the sample questioned, although bacterial contamination of the tissue itself, prior to retrieval, would not be so easily identified.

There is also a concern that if for example the sample is obtained through a cardiac stab one may damage the valve/introduce infection at the site.

Thus although a post-mortem sample from a deceased tissue donor may not be the optimum substrate for the screening assays used, if collected carefully and under controlled conditions the sample collected may resemble that obtained from any heart-beating individual with few, if any, post-mortem changes that would have any adverse effect on any screening performed. Certainly the majority of approximately 2000 samples/yr referred to the author's laboratory, the single national laboratory responsible for the screening of all deceased tissue donations collected within NHS Blood and Transplant (NHSBT) and which also provides a screening service for some non-NHSBT Tissue Banks, are suitable for screening and do not give rise to any problems beyond a slightly lower overall specificity [12].

Thus, whilst samples collected post-mortem from deceased tissue donors can be suitable substrates for many screening assays, the collection conditions must be properly controlled to ensure that they are as representative of a pre-mortem sample and the serological status of the donor as possible. In addition, the screening systems developed must be specifically designed to generate as reliable and accurate results as possible from this group of samples.

Screening

The in-vitro laboratory screening performed can be divided into serological and molecular. To be able to understand the specific role and value of the two different approaches, the basic biology of infection must be understood. Following exposure to an infectious agent there is a period during which the infectious agent either enters the body's cells and starts to replicate or is captured and eliminated by the body's passive defence mechanisms. Once the agent has entered the body's cells and started to replicate it is highly likely that a productive infection will follow. There is a period during which the infectious agent continues to replicate locally until there is sufficient to start to spread to other suitable target sites within the body. During this time levels are low and generally not detectable. However as the agent starts to spread (generally via the circulation) when levels are high enough it can then be detected. At this point nucleic acid can start to be detected, and depending upon the infectious agent, antigen may also be detectable. Subsequently as the agent spreads the immune response is initiated, symptoms appear and antibody levels start to rise and become detectable. However, again depending upon the agent, at this point nucleic acid levels in the circulation may start to fall as the immune system starts to combat the infection and a balance ensues. In the case of the majority of the blood borne infectious agents the infectious agent then sequesters itself in its preferred cells in the body and a chronic or long-term infection develops, the antibody produced simply indicating infection at some time and possibly mediating the infection, but not necessarily conferring any immunity to the agent. In the case of other infectious agents, those that give rise to acute infections only, the appearance of antibody in the circulation marks the start of the resolution of infection and the clearance of the agent from the body, and subsequent immunity, for at least a short period, to that particular infectious agent.

Serological screening can be described as the conventional approach, looking for the presence of specific serological makers of infection in blood samples collected from the donors. This approach has been in use for many years and offers sensitive, reliable, fully process-controlled and cost-effective screening which will identify the vast majority of donors who are genuinely infected (early, recent, ongoing and old infections). Serological screening is relatively cheap and although it requires dedicated, often very specific equipment, this equipment is not particularly "high-tech", nor particularly expensive when considering the work rates achieved and its overall reliability. Molecular screening is more recent in its origin and has only relatively recently been applied to mass donation screening programmes. Indeed the mass screening applications are still being developed, and currently the bespoke screening systems are primarily designed for blood rather than other donation types. Molecular screening targets the genomic material of the infectious agents and works through the cyclic amplification of low levels of target (normally undetectable) to high levels which can then be more easily detected. It offers a (theoretically) higher sensitivity than serological screening for identification of early infections, but at the same time is less effective in detecting existing/old infections. Thus molecular screening should only be considered as an addition to, not a replacement for,

serological screening. In addition it is relatively expensive when compared to serology, as it is a more "high-tech" approach utilising new and developing technologies and requiring dedicated specialist equipment for the automated mass screening of donations now required by many countries. A major question that is still to be addressed is whether cost of molecular screening is justified by the incremental benefit of molecular screening over serology alone.

Serological Screening

Serological screening is that performed to look for the presence of specific serological markers of an infectious agent that are found in the blood as a result of the infection. These markers are either specific antigens produced by the infectious agent and which generally appears on the surface of the agent, but in some cases may be expressed on infected cells, and/or specific antibodies produced by the individual's immune system in response to the infection. Table 10.2 outlines the serological markers for which tissue donations collected within the UK transfusion services are screened.

Although the immune response to any infectious agent is often associated with protective immunity, indeed the whole purpose of vaccination is to stimulate the body's immune response to produce protective antibodies, in respect of the majority of the blood borne infectious agents that are transmissible through blood and tissues, except in the case of HBsAb which is a protective neutralising antibody, the presence of antibody is not indicative of protection, rather it indicates infection and the presence of the infectious agent in that individual. A common and important feature of most of the relevant blood-borne infectious agents is that they are all persistent infections which generally do not resolve naturally. Although approximately 40% of cases of HCV do resolve, such individuals are serologically indistinguishable from persistent infections, and thus the presence of HCV antibody cannot differentiate between infectivity versus immunity.

There are a number of different types of immunoassay available, but all have broadly the same principles. However not all are suitable for screening cadaveric samples, often due to technical (sample and sampling) rather than scientific issues. In general immunoassays comprise a solid phase and a liquid phase, and the assay is performed in a number of stages, each of which has an incubation period and a wash to remove the excess materials at the end of the incubation. The solid phase carries the immobilised components of the assay, those designed to capture the specific target that may or may not be present in the liquid phase (sample). In the microwell format assays, the well is coated with either antigen (Ag) or antibody (Ab), depending on the specific target (Ag to detect Ab, Ab to detect Ag). Captured target is then detected using a conjugate which detects any Ag/Ab complex formed and has an enzyme attached to it. This enzyme triggers a colour reagent that then signals the presence of the conjugate and thus the target through the development of colour which can then be measured.

Most of the assays used today to detect both antigen and antibody, either singly or in combination, depending on the particular infectious agent, are highly

sensitive and specific immunoassays that are designed to detect specific target as early as possible in the infection process, and run on automated platforms with high levels of process control. This ensures highly accurate and reproducible results and thus the overall reliability of the screening process. However, at the same time this aspect is also a problem, certainly in the case of deceased samples, as the automated screening platforms are designed specifically for the screening of blood donations. This is primarily to do with the automated sample detection systems rather than the assays themselves, with for example, haemolysed, low volume, or slightly diluted samples all being rejected as unsuitable for processing. As identified previously, it is here that surgical tissue donations align virtually completely with blood donations as the samples for both are collected from living individuals and are thus generally good quality samples, the optimum for these systems. Whereas deceased samples are commonly rejected by such systems as they fail to be detected properly by the system and therefore not sampled and not tested. At this time there is little option but to use separate screening strategies for deceased samples, using assays and systems that can be used and have been specifically validated for the screening of deceased samples.

There are two potential performance issues that need to be considered for any screening programme, but which may be of more relevance to serological screening, and specifically to the serological screening of deceased donations; sensitivity and specificity. A past major reported issue has been the specificity of the serological screening of deceased donations. Many authors report high reactive rates with the serological screening assays in use and the consequent high losses of donations. Whilst it is true that some deceased samples do present challenges when performing serological screening, mainly those that are visibly of poor quality, our experience is that the specificity of the screening assay is not the problem that some report, rather our concern is over the overall sensitivity of the screening programme. It is important that the assays used are validated specifically for use with deceased samples to determine both any specificity issues and any sensitivity issues. The problem is that specificity issues can be seen as high screen reactive rates which are not subsequently confirmed, wasteful but not affecting safety. However any sensitivity issues would only be seen once an infected donation had been transplanted and the recipient infected. Thus an important part of assay evaluation and validation for the screening of deceased blood samples is to demonstrate that the deceased nature of the sample does not reduce the sensitivity of the assay. Unfortunately this in itself presents problems as suitable cadaveric samples are simply not available and indirect methods have to be used [13].

Molecular Screening

Molecular screening is that performed to detect the presence, in the blood, of the nucleic acids of transmissible infectious agents. The presence of viral nucleic acid specifically indicates the presence of virus, and, in most cases, infectivity. At this time the infectious agents that are targets for molecular screening are hepatitis B virus (HBV DNA), hepatitis C virus (HCV RNA) and human immunodeficiency

virus (HIV RNA), and, for one particular product human cytomegalovirus (HCMV DNA), Table 10.2 outlines the viral nucleic acids for which tissue donations collected within the UK transfusion services are screened. Essentially the molecular screening used to detect low levels of virus can be broken down into two elements, the extraction and purification of any nucleic acid in the sample and then the subsequent specific amplification and detection of any target nucleic acid present. Although viral nucleic acid may be present in the sample from the donor, and even if there are thousands of genome copies present, the overall amount of "native" nucleic acid present is still far too small to be able to detect directly with the technology currently available. Instead, the approach taken is to isolate the nucleic acid present in the sample and then amplify any specific target that may be present to a level that can then be detected either by direct visualisation using nucleic acid binding fluorescent dyes or through enzyme action with the generation of a chemiluminescent signal, both in proportion to the amount of nucleic acid present. There are currently 2 main molecular methodologies used in the molecular screening of donations for infectious agents, the polymerase chain reaction (PCR) and transcription mediated amplification (TMA). Although this chapter is not the appropriate place to provide an in-depth review of these methodologies, an overview follows.

PCR is a temperature dependent method that allows exponential amplification of short DNA sequences present within a longer double-stranded DNA molecule, in this case the genome of the infectious agents being screened for. It entails the use of a pair of primers, short sequences of nucleotides that are complementary to a defined sequence on each of the two strands of the DNA. These primers are extended by a DNA polymerase so that a copy is made of the target sequence. After making this copy, the same primers can be used again, not only to make another copy of the input sequence but also of the short copy made in the first round of synthesis. This leads to logarithmic amplification. For RNA viruses the RNA must be copied to DNA first using a reverse transcriptase (RT) step.

TMA is an isothermal method that allows exponential amplification of RNA or DNA targets in, as for PCR, the genome of the infectious agents being screened for. TMA technology uses two primers, one of which contains a promoter sequence for RNA polymerase, and two enzymes: RNA polymerase and reverse transcriptase. In the first step of amplification, the primer with the promoter sequence hybridizes to the target rRNA at a defined site. Reverse transcriptase creates a DNA copy of the target rRNA by extension from the end of the promoter primer. The RNA in the resulting RNA:DNA duplex is degraded by the RNase activity of the reverse transcriptase. Then a second primer binds to the DNA copy and a new strand of DNA is synthesised from the end of this primer by reverse transcriptase, creating a double-stranded DNA molecule. The RNA polymerase recognises the promoter sequence in the DNA template and initiates transcription. Each of the newly synthesised RNA, amplicons reenters the TMA process and serves as a template for a new round of replication. Again, as in PCR, this leads to logarithmic amplification.

However, molecular screening is a still emerging approach, not so much the underlying technology itself, which has been in use in research and development activities for over 20 years, but it is only recently that this technology has been

successfully developed into the fully automated mass screening systems that include both the sample preparation and molecular aspects and which can provide results in just 3–4 h. However, although not to the same degree, sample quality is still a factor to be considered. Although designed to work with a range of different sample types, the initial sample handling side of the automated systems may, in the same way as with serological systems, reject unsuitable samples, those that are not detected or are rejected by the sample detection systems. However, molecular techniques do have a distinct advantage over serology in that each sample has an internal control added prior to the nucleic acid extraction process that is extracted and amplified concurrently with any specific target that may be present, and which is then detected using a separate detection system. Thus every sample should score as positive with the internal control, irrespective of the presence of the specific target of interest, uniquely, validating each negative result. Failure of the internal control indicates a failure of the process at some point, whether it be due to a failure of the molecular reagents themselves or due to inhibition of the reagents by something within the sample itself, and invalidates the results for the sample.

As discussed previously the value of molecular screening is primarily to detect early infection in those donors who have been recently infected. Later in infection the serological markers present are far easier to demonstrate and the assays significantly less expensive than molecular assays. The important question is then to determine the increased value provided by including molecular screening in addition to serological screening. Although molecular screening does theoretically offer earlier detection of infection, its actual value is very much related to the biology of the individual infectious agents, the overall performance of the serological screening and the incidence of infection in the population. Where antigen assays are available, there is generally only limited additional value to be had from molecular screening, unless the incidence of infection is high enough to indicate that significant number of donors may have been exposed to the agent. However, in low-risk populations this is rarely the case. However, where only antibody assays are available and where the window period of infection is relatively long, molecular screening may have more value. However, again it is the incidence of the infection in the donor population that determines how many donors would be likely to be detected by molecular and not by serological screening. In low risk populations the actual numbers of donors who would be detectable by molecular screening and not by serological screening is extremely low. Currently, for HCV in blood donations collected within the NBS, the number of HCV RNA positive but serology negative donations is approximately 1 in 55.4 million donations, i.e. one donation per 28 years at the current donation level.

Residual Risk

The overall effectiveness of the serological screening of tissue donations, both surgical and deceased, is without question. For many years serological screening was the only approach available for the screening of tissue donations and there is no

published evidence, excepting in cases of haemodilution [10], that this approach has led to the transmission of infection as a result of serological testing failing to detect the presence of serological markers in an infected individual. The inclusion of molecular testing, at least theoretically, increases the level of safety even more, although the incremental benefit over that gained from serological screening alone is difficult, if not impossible, to quantify accurately. This is due to the relatively small numbers of tissue donations screened, the even smaller numbers of confirmed positive donors and the virtual impossibility of accurately determining the incidence of infections in tissue donors. Overall the microbiological safety of tissue donations that have been properly and effectively screened for infectious diseases is at least equal to that of blood donations.

Unfortunately however, laboratory testing is not perfect and there are occasions (for a number of reasons) when a test may not detect its target. Consequently no screening programme has absolute sensitivity and it is possible that infected donors could be undetected with the resultant entry of an infected donation into the supply chain. This is referred to as **residual risk**, i.e. the risk of infection from a screen negative donation. However, this is generally due to the lack of detectable target, rather than the assay failing to detect target.

In brief, assays fail to detect target either because the assay has missed something that is present or because there is nothing present for the assay to detect, although the donor is infected and the donation is infectious. The first scenario arises either due to poor assay performance (analytical sensitivity) or due to poor performance of the assay (operator/system error). However, if well evaluated and validated highly sensitive assays are being used, and the screening is being performed within a formal and well designed quality management system with well trained and competent staff, then this scenario should rarely, if ever, occur. The second scenario, lack of detectable target yet with infectivity present is, however, more likely to occur, no matter how good the screening programme and quality management system, as it is related to the natural history of infection in an individual and is a stage of the normal infection process.

Window Period

The major threat, in terms of residual risk is therefore from donors who are infected yet negative on screening as the normal screening target(s) are not present, or are present but below detectable levels. This situation occurs naturally very early during the course of infection, at a time when a productive infection has arisen, but before the specific screening target(s) have either been produced or their levels have risen to those detectable by the assays in use. It also occurs later in some infections when the infection is starting to resolve, the level of the screening target has declined to below that detectable, yet infectivity remains. This is referred to as the "Window period" of an infection, infectious yet undetectable with the screening assays used.

In relative terms, specific antibody appears later during the course of infection than antigen, and antigen later than nucleic acid. Therefore if the screening target is just antibody, there will be a period of time when donations from infected

donors would give negative screening results despite the presence of antigen and its associated infectivity. The same applies when comparing antigen (with or without antibody) screening with molecular screening. In this situation the relative time between the first appearance of nucleic acid and the first appearance of antigen is much shorter, and in many instances they appear so close together that there is no actual measurable incremental benefit to be obtained through the use of molecular screening in addition to antigen or antigen/antibody screening.

The overall risk of collecting a donation from a donor who may be in the window period of infection is very much related to the overall level of infection in the population, and specifically the incidence of infection in the donor population. The higher the incidence of infection, the greater the risk that a donor could have been recently infected. At the same time the ability to detect an infected donation is dependent upon having an effective and appropriate screening programme in place. A screening programme with a relatively poor sensitivity is more likely to fail to detect recently infected donors. However the overall risk is also dependent upon the actual number of donations collected, and although the incidence of infection may be the same in the blood and tissue donor populations, as the populations are so very similar, the overall numbers of tissue donations collected is many orders of magnitude lower than the number of blood donations. Thus it can be argued that the overall probability of actually encountering a donation from a tissue donor who is in the window period, assuming a screening programme with equal if not greater sensitivity, is much lower than that for a blood donation. However, when the actual number of infectious identified in the UK are looked at the incidence of confirmed infected deceased tissue donors is significantly higher than that of blood donors [14], but the denominator is around 3 orders of magnitude less and therefore the figures cannot be compared directly. Furthermore significantly more patients have transfusions, greater number of potential exposures, and it is therefore not necessarily true to argue that tissues are a higher "risk" than blood or other donations.

The window period is thus the main reason today that donations, be they tissues or blood, have an associated residual (albeit very low) risk, and screening programmes and strategies are developed to ensure that the window period is reduced to as short a period as possible.

Conclusions

Overall the current screening of tissue donations prior to release is highly effective at preventing transmitted infections. The basic principles applied to the screening of blood donations do apply as there is considerable overlap in the need to identify low risk donors, the range of transmissible infectious diseases, the screening assays used and the screening strategies developed. Certainly there is little, if any, difference between the screening of surgical tissue donations and blood donations, as the samples are all collected by standard venepuncture from living individuals. The screening of deceased tissue donations is different inasmuch as

the nature of the samples is fundamentally different, although the screening assay themselves are the same. It is the reliability of the screening process in relation to deceased samples that needs to be assured, particularly the representativeness of the samples collected in respect of the screening results obtained reflecting the true status of the donor and therefore the donation. In addition consideration should be given to the specific screening algorithms used, as those commonly used for blood donation screening may not always offer the most appropriate approach for the screening of deceased tissue donations. The issue of specificity is something that has particular relevance as the unnecessary loss of tissue donations due to poor specificity is unacceptable because tissue products are generally in shorter supply, often with many potential recipients on waiting lists. Furthermore a cadaver may provide a large number of different tissues for clinical use, and a non-specific screening result would result in the loss of a significant number of clinically valuable products.

References

1. Scardino MK, Hwang SJ, Hanna CL, Danneffel-Mandelkorn MB, Wilhelmus KR (2002) The postmortem sociomedical interview: uncertainty in confirming infectious disease risks of young tattooed donors. Cornea 21:798–802
2. Fischer SA, Graham MB, Kuehnert MJ, Kotton CN, Srinivasan A, Marty FM, Comer JA, Guarner J, Paddock CD, DeMeo DL, Shieh W-J, Erickson BR, Bandy U, DeMaria A, Davis JP, Delmonico FL, Pavlin B, Likos A, Vincent MJ, Sealy TK, Goldsmith CS, Jernigan DB, Rollin PE, Packard MM, Patel M, Rowland C, Helfand RF, Nichol ST, Fishman JA, Ksiazek T, Zaki SR, and the LCMV in Transplant Recipients Investigation Team (2006) Transmission of lymphocytic choriomeningitis virus by organ transplantation. NEJM 354:2235–2249
3. Cahane M, Barak A, Goller O, Avni I (2000) The incidence of hepatitis C virus positive serological test results among cornea donors. Cell Tissue Bank 1:81–85
4. Challine D, Roudot-Thoraval F, Sabatier P, Dubernet F, Larderie P, Rigot P, Pawlotsky JM (2006) Serological viral testing of cadaveric cornea donors. Transplantation 82:788–793
5. Heim A, Wagner D, Rothamel T, Hartmann U, Flik J, Verhagen W (1999) Evaluation of serological screening of cadaveric sera for donor selection for cornea transplantation. J Med Virol 58:291–295
6. Padley D, Ferguson M, Warwick RM, Womack C, Lucas SB, Saldanha J (2005) Challenges in the testing of non-heart-beating cadavers for viral markers: implications for the safety of tissue donors. Cell Tissue Bank 6:171–179
7. Thomas S, Klapper PE, Mutton KJ, Turner AJ, Tullo AB, Zambrano I, Carley F, Taylor A (2007) Lack of vision, loss of sight: consequences of mandatory HTLV screening in corneal transplantation. Transpl Infect Dis 9:171–172
8. Miedouge M, Chatelut M, Mansuy JM, Rostaing L, Malecaze F, Sandres-Saune K, Boudet F, Puel J, Abbal M, Izopet J (2002) Screening of blood from potential organ and cornea donors for viruses. J Med Virol 66:571–575
9. Eastland T (2000) Hemodilution due to blood loss and transfusion and the reliability of cadaver tissue donor infectious disease testing. Cell Tissue Bank 1:121–127
10. Centres for Disease Control (1987) Human immunodeficiency virus infection transmitted from an organ donor screened for HIV antibody. MMWR 36:306–308
11. http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Tissue/ucm073964.htm#THEDONOR-ELIGIBILITYDETERMINATION1271.50

12. Kitchen AD, Gillan HL (2010) The serological screening of deceased tissue donors within the English Blood Service for infectious agents – a review of current outcomes and a more effective strategy for the future. Vox Sang 98:e193–e200
13. Kitchen AD, Newham JA (2010) Qualification of serological infectious disease assays for the screening of samples from deceased tissue donors. Cell and Tissue Banking (in press)
14. NHSBT Infection Surveillance Programme Annual Report 2007. http://www.hpa.org.uk/web/HPAwebFile/HPAweb_C/1227255714122