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Challenges in the testing of non-heart-beating cadavers for viral markers: implications for the safety of tissue donors

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Abstract

Natural changes that occur in blood and tissue after death may result in false positive results in antigen and antibody detection tests performed to identify markers of viral infection in potential tissue donors. Such tissue, which might otherwise be acceptable for therapeutic purposes, would not meet current standards for safe tissue banking. This is especially important in the context of insufficiency in the tissue supply. In this study, a series of blood samples collected during routine post-mortem examination was assayed using a range of commercially available kits for the detection of HBsAg, anti-HCV and anti-HIV 1 + 2 antibody/antigen. Results of tests on 104 samples collected from 97 individuals indicate that some kits result in a higher number of initial reactive samples than others. Approximately 40% of samples were reactive in one or more HBsAg assay, less than 10% in at least one anti-HIV kit and only 1 sample at low level on an anti-HCV kit. Liver or lymph node samples from individuals whose serum sample gave reactive results in antigen/antibody assays were tested for viral nucleic acid in the corresponding nucleic acid amplification test. Only one individual's sample was confirmed to test positive for HBsAg in a confirmatory neutralisation test and by nucleic acid amplification technology, and a second individual whose serum was scored reactive for anti-HCV, but negative for HBsAg, had a liver sample which was HBV DNA positive and HCV RNA negative. The results of the study indicate that antibody/antigen assays are not as specific as NAT using state of the art DNA extraction techniques. Both types of assay complement each other and used together will help assure the safety of tissues for transplantation.

Abbreviations: AIDS – Acquired immuno-deficiency syndrome; GZ – Grey zone; HBsAg – Hepatitis B surface antigen; HBV – Hepatitis B virus; HCV – Hepatitis C virus; HIV – Human immunodeficiency virus; NAT – Nucleic acid amplification techniques; RNA – Ribonucleic acid; RT-PCR – Reverse transcriptase polymerase chain reaction; DNA – Deoxy-ribonucleic acid

Introduction

Cadavers are a principal source of tissues for transplantation. It is, however, more difficult to ensure the microbiological safety of tissue donation from such donors compared to living donors as comprehensive medical and behavioural histories can only be obtained from third parties. Viruses can survive in tissues preserved under laboratory conditions for many hours after death (Ball et al. 1991) and viral infections transmitted by tissues and organs have been documented (Conrad et al. 1995; Eastlund 1995; Simonds et al. 1992; Cieslak et al. 2003).

Cadaveric tissue donors may be multi-organ heart-beating donors who also donate tissues. There are also tissue only donors whose samples are taken after cessation of the circulation i.e. they are non heart-beating when the samples are acquired. In the former situation, blood sample quality is similar to that taken from patients or blood donors and microbiological tests are validated for this type of analyte. In the latter case, the analyte differs significantly as these samples may be haemolysed or autolysed. Ante-mortem samples from cadaver donors may be suitable for testing but may not be available.

The testing of cadaver tissue donors for viral markers in Europe is, in principle, the same as that applied to living tissue and blood donors (Council of Europe 2004). However, commercial test kits for the detection of HBsAg, anti-HCV and anti-HIV 1 + 2 which are used for testing samples from cadavers have generally not been evaluated for this purpose. Two kits are currently licensed for use with cadaver blood specimens by the US FDA (US DHHS, FDA 2000) but these are not available in Europe as they have been superseded by more recent and more sensitive versions of the test kit. Repeat reactive results in assays for which testing is mandated, namely hepatitis B and hepatitis C viruses and HIV 1 + 2, are found more frequently with cadaver samples than with serum samples taken from patients for diagnostic purposes or from blood donors (Heim et al. 1999). Natural post mortem changes affect the quality of blood and tissue samples (due to haemolysis, autolysis etc) and reduce the reliability of the results (LeFor et al. 1996). The false negative rate is not known.

A survey undertaken in 1998 (Stanworth et al. 2000) indicated wide variation in the test procedures undertaken in UK laboratories testing samples for cadaver donors. In 2002, an informal telephone survey indicated continuing wide variation in the choice of kits used to test non-heart beating blood analytes, none of which were formally validated for this purpose. Discussions with laboratories with extensive experience of testing cadaver samples indicated that they had identified problems with the performance of these kits and undertook additional measures to improve their performance. This included centrifugation or filtration of the samples from non-heart beating cadaveric tissue donors in an attempt to reduce the incidence of reactive results (M Ferguson, unpublished observations). The choice of kit is also influenced by the laboratory's experience with such samples. The observations from both surveys clearly indicated a need to investigate the sensitivity and specificity of the various kits to identify those with the best performance for use on samples from cadaveric tissue donors.

Assays for antibodies to HCV and HIV 1 + 2 cannot exclude these viral infections as not all infected individuals are antibody positive and may be in the 'window period', i.e. the time between initial infection and detection of an antibody response (Busch et al. 1995; Kleinman et al. 1997) and an HCV window period transmission from an organ tissue donor has been documented (Cieslak et al. 2003). Following transmissions of HCV by blood products in the early 1990s, the Committee for Proprietary Medicinal Products (CPMP) recommended that plasma for fractionation into blood products such as albumin and factor VIII be screened for HCV RNA (CPMP 1997). Blood donors in the US, UK (www.transfusionguidelines.org.uk) and many European countries are now also screened for HCV RNA by nucleic acid amplification technology (NAT). Similarly, HBV may be transmitted through blood donation in the absence of HBsAg (Meisel et al. 2003)

The post mortem degradation of the viral genome and production of 'inhibitors' which interfere with the detection of viral nucleic acid, and presence of bacterial DNase and RNase may be dependent on the time of sampling after death (Stanworth et al. 2000). Previous studies have indicated that when samples from known AIDS patients taken before and after death were tested by PCR for HIV, specificity was high but sensitivity low (Burtonboye and Delloye 1996). In

another study, a significant number of samples gave indeterminate results (failure of the internal control) and this was probably related to the quality of the blood (Miedouge et al. 2002).

We undertook to study non-heart beating individuals' blood analytes in two types of microbiological analyses. The first related to NAT and this has been published (Padley et al. 2003). It demonstrated that commercially produced reagents for nucleic acid extraction are available for use in sensitive and specific NAT tests in which the problem of NAT inhibitors present in the sample has been overcome.

The second part of the study considered antigen and antibody detection tests on non-heart beating individuals' blood analytes and this paper deals with these findings. This part of the study examined the effectiveness and reliability of several different manufacturers' kits in routine use at the time of the survey (Stanworth et al. 2000). These kits demonstrated a number of challenges relating to lack of specificity. The NAT tests validated in the first study were used to further investigate the samples which were reactive in antibody and antigen assays in the second study. In addition, we extended the application of this method to tissue samples from individuals of known HCV status (one was HCV reactive and one was HCV negative).

The limitations of testing non-heart beating donor samples for antigen and antibody of the blood borne viruses will be discussed. It is emphasised that the study subjects were deliberately not tissue donors and were not subject to the donor selection criteria stipulated in the National Blood Service (NBS) and UK Blood Service Guidelines (www.transfusionguidelines.org.uk). It is therefore not surprising that a number of coincidental positive results were observed in some of the study subjects.

Materials and methods

Samples

Approximately 10ml blood sample was taken by milking the common iliac vein from 97 individuals who were undergoing routine post-mortem examination. The separated serum was stored at −20 °C or below. Liver samples were taken from all patients and lymph nodes from 82 patients. These were frozen directly at −20 °C in anticipation that individuals who gave reactive results for antigen and antibody detection markers in their serum samples could be tested by NAT on their liver or lymph node samples to confirm or refute the antigen and antibody detection results from the serum sample. In addition, in order to investigate whether sample quality deteriorated when samples from non-heart beating donors were taken at increasing periods after death, a second blood sample was taken approximately 24 h after the initial sample from 7 of the original individuals.

The time of death, along with the interval between death and placing the cadaver in a refrigerator and the length of time the cadaver was refrigerated before the initial blood sample was taken, were all known for 69 individuals. Detailed information about the timing of death and refrigeration was not available for the other samples but it was estimated by the pathologists to be >24 h. This was the minimum time required by the patient's next of kin to participate in the consent process and for sample retrieval. Local Research Ethical Committee approval was obtained at Peterborough and at St Thomas's Hospitals for the collection of the samples with family consent.

Antigen and antibody detection assays

A range of assay kits, most of which are currently used by laboratories who routinely test samples from cadavers, were included in these studies. The HIV, 1 + 2 antibody ± antigen, anti-HCV and HBsAg kits are listed in Tables 1, 2 and 3 respectively. Although the target volume of blood to be collected was 10 ml, in reality varying volumes of blood sample were available from each individual in the study. This is often the case with cadaver donors, particularly those who have suffered severe trauma. This limited the number of assays in which the samples could be tested. Initial reactive samples were not repeat tested due to restrictions associated with limited sample volume. Some manufacturer's instructions for use include the use of a 'grey zone' (GZ) to highlight samples giving reactivity within 10–20% below the calculated cut-off value in the assays. Such samples would normally be considered reactive and re-tested.

Table 1. Results of assays on HIV 1 + 2 Antibody or Antibody/Antigen detection kits.

Test kit	Number of samples tested	Number scored reactive	Percentage scored reactive	Percentage scored reactive or in GZ
Abbott Murex HIV 1.2.0 GE 94/95	74	0	0	0
Biorad Genscreen p24 Aq/Ab Hiv 1/2 72375/72376	74	2	2.7	2.7
Enzygnost HIV Integral	74	0	0	0
Organon/Ortho Vironostika HIV UniForm Ag/Ab 84138/39140	74	2	2.7	2.7
BioRad Genscreen Plus HIV Ag-Ab Access 34020	80	0	0	0
Abbott AxSym HIV 1/2 g0 3D41-22	91	5 + 2 GZ	5.5	7.7

Table 2. Results of assays on anti-HCV detection kits.

Test kit	Number of samples tested	Number scored reactive	Percentage scored reactive	Percentage scored reactive or in GZ
Biorad Monolisa Plus Anti-HCV 72318	81	1	1.25	1.25
Biorad Access EIA 34330	80	0	0	0
Abbott AxSym HCV Version 3.0 EIA 3B44-20	90	0	0	0

Table 3. Results of assays on HBsAg detection kits.

Test kit	Number of samples tested	Number scored reactive	Percentage scored reactive	Percentage scored reactive or in GZ
Abbott /Murex HBsAg GE34	82	6 + 1 GZ	7.3	8.5
Biokit Launch HBsAg colour 3000-1131	71	2	2.8	2.8
Biorad Monolisa Ag HBs Plus HBsAg 72313	81	10 + 2 GZ	12.4	14.8
Beckman Coulter Access EIA 34220	78	5 + 8GZ	6.4	16.7
Abbott AxSym HBsAg MEIA 7A40-77	76*	25 + 7 GZ	33	42

* + 15 samples rejected by system and unable to be tested GZ = grey zone – would normally be retested

Confirmatory testing was undertaken on two samples which gave reactive results in all 5 HBsAg detection assays. The test performed to confirm HBsAg reactivity was the Murex HBsAg neutralisation test kit GE37, used in conjunction with the Abbott /Murex HBsAg GE36 assay kit. No other confirmatory antigen/antibody testing was undertaken on samples which were reactive/within the GZ for any other samples due to limited sample volume.

Confirmatory NAT tests used on individuals with reactive blood samples

Tissue samples from individuals, whose serum samples were reactive in screening tests, were assayed by NAT using the method documented by Padley et al. (2003). Samples of liver were used in the confirmatory tests on individuals whose blood samples were reactive for HBsAg or anti-HCV and lymph nodes were used to confirm the status of individuals whose blood gave HIV antigen/antibody reactive results, unless this tissue was not available.

DNA was extracted using the QIAamp DNA Mini Kit (Qiagen, Basle, Switzerland). Nucleic acids from serum were extracted using the blood protocol as per manufacturer's instructions. All serum samples were treated with AX-matrix (InhibitEX, Qiagen), prior to DNA extraction, to remove inhibitors (Padley et al. 2003). Nucleic acids from tissue samples were extracted following the protocol described for tissues in the kit manufacturer's instructions and these were also treated with AX-Matrix after lysing of the tissue samples according to the kit manufacturer's instructions. All samples were tested with and without the

addition of a known amount of HCV, namely plasma containing 71 IU/ml, diluted from British Working Standard for HCV RNA (Saldanha and Minor 1996). The One-Step RT-PCR kit (Qiagen, Basle, Switzerland) was used for amplification of HCV RNA.

Assays for HBV DNA utilised the same reagents for extraction and amplification as those used for the detection of HCV RNA. The method used to confirm the detection of HBV DNA in positive samples was the ARTUS RealArt, (Hamburg Germany) hepatitis B virus LightCycler PCR (Mannheim, Germany) kit. Assays for HIV RNA utilised the same reagents for extraction of nucleic acid and a nested LTR based RT-PCR was utilised for amplification and detection of HIV RNA (Berry et al. 2001).

Results

A database was set up to record the results of the study and the number of samples initially scored reactive for each marker and kit. 104 samples were available for testing but they were not necessarily tested in all assays as there were insufficient volumes of some samples. The numbers of samples tested in each anti-HIV 1 + 2 or combined HIV antibody/p24 antigen assays are listed in Table 1, in each anti-HCV assay in Table 2 and in each HBsAg assay in Table 3. The percentage of samples which were reactive or in the GZ which would result in the need for further testing in each assay are also listed in each of the tables.

Fifty five out of 104 samples were tested in all six anti-HIV 1 + 2 antibody ± antigen assay kits and five of these samples gave a reactive or GZ response in at least one of the assays. The remaining forty nine samples were assayed in at least 2 of the assays and 5 of these samples were reactive or within the GZ in at least one of the assays in which they were tested. No sample was reactive in all the assays in which it was tested. These results are summarised in Table 4.

Sixty out of 104 samples were tested in all three anti-HCV assays. Only one sample gave a response to cut-off ratio of greater or equal to 1.0 in an anti-HCV assay as summarised in Table 4. This sample was scored negative in the other two anti-HCV assays.

Fifty out of 104 samples were tested in all 5 HBsAg assays. 23 samples were reactive or in GZ in at least one assay as shown in Table 4. However, only two of the samples were reactive in all five HBsAg assay kits studied. These blood samples were from the same donor and taken at different times after death. One of these samples was subsequently confirmed HBsAg positive in a neutralisation test and a liver sample was shown to be HBV DNA positive. A further 54 samples were tested in at least two assays and 19 of these samples were reactive or in the GZ for at least one of the assays in which they were tested (Table 4). The percentage of reactives/GZ negatives in each assay varied from 2.8% to 42%. Seven samples were in the GZ on testing by Access but non-reactive in all other assays. Two samples were reactive/in GZ on the Murex test only and one only in the Biorad test.

Nineteen samples gave reactive or GZ results only in the Axsym test. The Axsym also rejected 15 samples as 'untestable' in the HBsAg assay, but not on anti-HCV or HIV tests using the same technology. This was despite centrifugation of the samples prior to testing. Twelve of the samples rejected on the HBsAg assay were non-reactive on all other assays in which they were tested. The times after death at which the rejected samples were taken ranged from 14–27 h with only 2 being taken gt 24 h after death and 6 samples taken at unknown times. For the samples reactive on

Table 4. Summary of results from 104 samples tested in anti-HIV 1 + 2, anti-HCV and HBsAg assays.

Serology tests for	Number of samples tested in all assays giving reactive or GZ responses in at least one test () = Number of samples tested in all assays	Number of samples tested in two or more assays giving reactive or grey zone responses in at least one test () = Number of samples tested in two or more assays
Anti-HIV 1 + 2	5 (55)	5 (49)
Anti-HCV	1 (60)	0 (44)
HBsAg	23 (50)	19 (54)

AxSym other than the two confirmed positives, 8 were taken < 24 h after death.

Paired samples taken approximately 24 h apart were available from seven individuals. One pair of samples from individual related to the HBsAg confirmed positive individual (see above). The first sample from another pair was non-reactive on Axsym HBsAg, whereas the second sample was reactive on Axsym but not on the other two HBsAg assays in which it was tested. For a third pair of samples, the first sample taken at 6 h after death gave reactive/equivocal results in the Murex and Access HBsAg assays whereas the second sample taken 30 h after death showed no reactivity. For a fourth individual, both samples taken 16.5 and 39 h after death were rejected by Axsym HBsAg but no other reactivity was observed for these samples in any assay. It therefore appeared that there was no increased reactivity with the second of the paired samples. The time after death at which the samples were taken was known for 25 samples which were reactive or equivocal on any HBsAg detection assay kit, other than the confirmed HBsAg positives. Sixteen were taken < 24 h after death and nine > 24 h after death.

In order to confirm the applicability of the NAT method described by Padley et al. (2003), 40 of the individual samples from this study described in this paper were tested for HCV RNA using the published method. Each sample was spiked with an HCV RNA control (71 IU/ml) which served as an internal extraction and amplification control. The presence of NAT inhibitors was not detected in any of the samples (data not shown).

The assay was then adapted and evaluated as a confirmatory test on liver samples from two cadavers of known HCV status from individuals described by Padley et al. (2003). One of these was known to be HCV positive and one known to be HCV negative according to hospital records. These samples were not tested for other microbiological specificities. Both liver samples were taken more than 24 h after death. An aliquot of each was spiked with 71 IU/ml HCV virus. The HCV positive individual tested positive for HCV RNA in the spiked and unspiked samples with no difference in signal following AX matrix treatment. The HCV negative donor tested negative for HCV RNA in the spiked and unspiked samples but the spiked sample became positive only following AX matrix pre-treatment suggesting removal of inhibitors.

Liver samples from the 41 individuals whose serum samples were reactive in at least one HBsAg assay were tested for HBV DNA as a confirmatory test only using the NAT extraction and amplification methods described above for HCV RNA (Padley et al. 2003). Liver samples from HBsAg negative donors were not tested by NAT. The liver sample from the individual, both of whose serum samples were reactive in all HBsAg assays, and whose HBsAg reactivity had subsequently been confirmed positive in a neutralisation test, was positive for HBV DNA. All of the other 40 samples were negative for HBV DNA and none demonstrated inhibition in the NAT assay. These results are summarised in Table 5.

One serum sample was reactive in an anti-HCV assay with a response to cut-off ratio of 1.0. The liver sample from this individual was negative for HCV RNA (Table 5). It was, however, also included in the HBV NAT assays along with the liver samples from individuals whose serum samples were reactive in an HBsAg detection assay and it was found to be positive for HBV DNA. This reactivity was confirmed using ARTUS RealArt, (Hamburg Germany) hepatitis B virus LightCycler PCR kit (Mannheim, Germany).

The same nucleic acid extraction procedure was used with lymph node samples from ten samples tested for HIV RNA. All were negative (Table 5).

Discussion

Samples were analysed from 97 individuals undergoing routine post-mortem examinations in two hospitals. It had been planned that the study would comprise 200 individuals, but due to difficulties in obtaining consent from bereaved families, the numbers of samples available were limited. Even so, the numbers are greater or equivalent to those included in many other studies (Novick et al. 1993 – 43 samples; Burtonboye and Delloye 1996 – 117 samples; LeFor et al. 1996 – 19 samples; Heim et al. 1999 – 33 samples). We acknowledge that the small numbers of samples limit the conclusions which may be drawn from the study.

Testing of cadaver tissue donors for evidence of blood borne viral infections raises two concerns; false negative results with the possibility of transmission of undetected viral disease and false positive results which render donated tissues

Table 5. Summary of results from confirmatory NAT tests on samples scored reactive or in GZ in at lease one test.

		Number of samples reactive in		
Serology tests for	Number of samples reactive or in GZ in at least one test	HIV-1 NAT test on lymph nodes	HCV NAT test on liver samples	HBV NAT tests on liver samples
Anti-HIV 1 + 2	10 (8 lymph node samples available)	0/8	ND	ND
Anti-HCV	1	ND	0/1	1/1
HbsAg	41[a]	ND	ND	2/41

[a]Two samples from the same individual taken at different times after death were both reactive in all HBsAg assays.

unsuitable for use by virtue of non-compliance with tissue banking standards. Two unsuspected HBV infections were detected; one sample was reactive in all HBsAg tests employed as well as by HBV NAT and a second was detected by HBV NAT. The explanation for this high rate may be that the individuals were random cases undergoing routine post mortem examination and not potential tissue donors who had undergone a rigorous donor selection process including documenting medical and behavioural histories.

The Abbott Axsym system was used for anti-HCV and anti-HIV 1 + 2 detection assays without any problem. The assay for the detection of HBsAg was included in these studies as a comparison with other assays even though this test kit is not routinely used by any of the laboratories testing cadaver samples for HBsAg in the UK because of large numbers of reactive or problematic samples previously observed with this methodology (E McMahon, personal communication). It was therefore not surprising that this assay gave the highest proportion of initial reactive results in the HBsAg assays. The high HBsAg reactive rate could represent infection not detected by other kits but is more likely to represent false positive results. This is in line with other published reports such as Novick et al. (1993) who also observed large differences in reactivity in two EIA kits for the detection of HBsAg and Lefor et al. (1996) who observed that the number of repeatedly reactive samples was dependent on the incubation protocol used with the same assay kit.

Most of the reactive samples in this study were obtained >24 h after death and some were likely to have been >48 h. This is longer than that recommended for retrieval of blood samples for testing in the Guidelines for the Blood Transfusion Services in the UK (2002). Nevertheless, the results of the seven paired post-mortem samples from the same individual, separated by approximately 24 h, did not demonstrate any systematic difference in test results. Although two samples from the same individual were HBsAg positive, no general conclusion as to retention of sensitivity and specificity can be drawn from such small numbers.

Stanworth et al. (2000) indicated increased reactivity in samples taken at longer periods, up to 72 h in some cases, after cessation of circulation. Tissue banks which permitted long periods between cessation of circulation and sampling, gave rates of reactive results as high as 40% for HBsAg. However, this may have also been associated with the assay kits used by the test laboratories, on the conditions during the transportation or storage of the samples. Degradation of samples resulting in problems in antigen and antibody detection assays may be indicative of similar degradation in tissues donated for transplantation and it is accepted best practice to obtain samples and tissues as soon as possible after cessation of circulation so that the interval between death, sampling for blood for microbiological testing and tissue collection is minimised to ensure quality of both samples for testing (Stanworth et al. 2000) and of the retrieved tissue for transplantation Martinez and Malinin 1996).

Although we have shown that the HBsAg detection assays used in this study will detect highly reactive HBsAg carriers, we have also demonstrated high rates of false reactivity for HBsAg and HIV. This confirms that cadaveric samples taken from non-heart-beating donors perform badly compared to samples from living donors in antigen and antibody assays for HBsAg, anti-HCV and anti-HIV (Dow 2000). The implications of this are loss of therapeutic tissue in the context of tissue insufficiency for recipients. In

other circumstances, heart beating blood samples may be available for cadaver tissue donors, e.g. where blood samples are from brain-stem dead multi-organ donors on life support systems, whose tissues are also subsequently donated or from samples taken for diagnostic purposes prior to death and stored in hospital laboratories. Highly sensitive assay systems which are used for screening blood donors may be used on the ante-mortem (heart beating) samples when available, as long as they conform to quality system requirements for labelling/identification and are not plasma-diluted (Eastlund 2000). However, these assay systems may reject blood samples from non-heart beating donors.

Since the transmission of HCV by tissue and organs from an infected antibody-negative donor (Cieslak et al. 2003), it has become apparent that tests for HCV RNA should validated using cadaveric blood samples taken at different times after death so that such tests can be considered for routine implementation. We have demonstrated in just 2 individuals that liver samples can also be used for the detection of HBV and HCV. These tests included the addition of a standard amount of HCV to one of two replicate samples prior to extraction and amplification of viral nucleic acid and they corroborate the more extensive studies described by Padley et al. (2003) demonstrating the validity, sensitivity and specificity of NAT and that state of the art nucleic acid extraction procedures and NAT testing could now be applied to cadaver samples, even in samples taken >48 h after death.

At some stages of the disease it is possible that individuals may be anti-HCV positive but HCV RNA negative (Dow et al. 1996). In some instances this may be because the donor has cleared the infection or be infected with a genotype which is less well detected in the NAT assay. It is therefore still not possible to dispense with all antigen and antibody detection assays to eliminate false reactivity even though most NAT assays now incorporate the use of internal controls into the extraction procedure to ensure lack of inhibitors. Although it may be considered that such donors carry only a remote risk of being infectious, the risk benefit of using tissues for transplantation means that every precaution must be taken.

The specificity and sensitivity of NAT for cadaver donors has been demonstrated (Padley et al. 2003), but the analysis of the antigen/antibody assays of kits in use in the UK has not been able to demonstrate the same comparable specificity. The use of both antigen and antibody detection combined with NAT assays may add to safety, albeit at extra cost, and would bring testing of tissue donors into line with testing of blood donations. However, it continues to be necessary to use donor selection criteria (Eastlund 1995) to assure the safety of cadaver tissues for transplantation.

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References

Ball J., Desselberger U. and Whitwell H. 1991. Long lasting viability of HIV after patient's death. The Lancet 338: 63.

Berry N., Davis C., Jenkins A., Wood D., Minor P., Schild G., Bottiger M., Holmes H. and Almond N. 2001. Vaccine safety: analysis of oral polio vaccine CHAT stocks. Nature 410(6832): 1046–1047.

Burtonboye G. and Delloye C. 1996. Polymerase chain reaction in cadaveric blood and tissues. Transplant. Proc. 28: 2927–2928.

Busch M., Lee L., Satten G., Henrard D., Farzadegan H., Nelson K., Read S., Dodd R. and Petersen L. 1995. Time course of detection of viral and antigen and antibody detection markers preceding human immunodeficiency virus type 1 seroconversion: implication for screening of blood and tissue donors. Transfusion 35: 91–97.

Cieslak P.R., Hedberg K. and Thomas A.R. 2003. Hepatitis C virus transmission from an antibody negative organ and tissue donor – United States 2000–2002. MMWR 52: 273–276.

Committee for Proprietary Medicinal Products 1997. Introduction of Genomic Amplification Technology (GAT) for the detection of hepatitis C virus RNA in plasma pools. Addendum for Guidance on Plasma Derived Products CPMP/BWP/390/97.

Conrad E., Gretch D., Obermeyer K., Moogk M., Sayer M., Wilson J. and Strong D. 1995. The transmission of hepatitis C virus by tissue transplantation. J. Bone Joint Surg. 77-A: 214–224.

Council of Europe 2004. Guide to Safety and Quality Assurance for Organs, Tissues and Cells. Council of Europe, ISBN ISBN 92–871–5518–6.

Dow B.C. 2000. 'Noise' in microbiological screening assays. Transfusion Med. 10: 97–106.

Dow B.C., Buchannan I., Munro H., Follett E.A.C., Davidson F., Prescott L.E., Yap P.L. and Simmonds P. 1996. Relevance of RIBA-3 supplementary test to HCV PCR positivity and genotypes for HCV confirmation of blood donors. J. Med. Virol. 49: 132–136.

Eastlund D.T. 1995. Infectious disease transmission through cell, tissue and organ transplantation: reducing the risk through donor selection. Cell Transplant. 4: 455–477.

Eastlund D.T. 2000. Hemodilution due to blood loss and transfusion and reliability of cadaver tissue donor infectious disease testing. Cell and Tissue Banking 1: 121–127.

Guidelines for Blood Transfusion Services in the UK. Sixth Edition 2002. The Stationary Office, London. (Available at http://www.transfusionguidelines.org.uk).

Heim A., Wagner D., Rothamel T., Hartmann U., Flik J. and Verhagen W. 1999. Evaluation of antigen and antibody detection screening of cadaveric sera for donor selection for cornea transplantation. J. Med. Virol. 58: 291–295.

Kleinman S., Busch M., Korelitz J. and Schreiber G. 1997. The incidence/window period model and its use to assess the risk of transfusion-transmitted human immunodeficiency virus and hepatitis C virus infection. Transfusion Med. Rev. 11: 155–172.

LeFor W.M., McGonigle A.F., Wright D.L. and Shires D.L.III 1996. The frequency of false positive HBsAg screening test results with cadaver tissue donors is dependent upon the assay procedure used. Tissue and Cell Report 3: 297–300.

Martinez O.V. and Malinin T.I. 1996. The effect of postmortem interval and manner of death on blood and bone marrow cultures from non-septic cadaver donors of tissues for transplantation. Abstracts of the 96th Meeting of the American Society for Microbiology. New Orleans, Louisiana.

Meisel H. 2003. Transmission of hepatitis B virus 2 months prior to hepatitis B surface antigen positivity of donor blood. Transfusion Med. Hemotherapy 30: 228–231.

Miédougé M., Chatelut M. and Mansuy J-M. 2002. Screening of blood from potential organ and cornea donors for viruses. J. Med. Virol. 66: 571–575.

Novick S.L., Schrager J.A., Nelson J.A. and Baskin B.L. 1993. A comparison of two hepatitis B surface antigen and two human immunodeficiency virus type 1 (p24) antigen enzyme immunoassay kits using hemolyzed cadaveric blood specimens. Transfusion 33: 619.

Padley D.J., Lucas S.B. and Saldanha J. 2003. Elimination of false negative HCV RNA results by removal of inhibitors in cadaver donor blood specimens. Transplantation 76(2): 432–434.

Saldanha J. and Minor P. 1996. Collaborative study to assess the suitability of an HCV RNA reference sample for detection of an HCV RNA in plasma pools by PCR. Vox Sang 70: 148–151.

Simonds R.J., Holmberg S.D. and Hurwitz R.L. 1992. Transmission of human immunodeficiency virus type 1 from a seronegative organ and tissue donor. NEJM 326: 726–732.

Stanworth S.J., Warwick R.M., Ferguson M. and Barbara J.A. 2000. A UK Survey of virological testing of cadaver tissue donors. Vox Sanguinis 79: 227–230.

US Department of Health and Human Services, Food and Drug Administation. 2000. Guidance for industry. Availability of Licensed Donor Screening Tests Labelled for use with Cadaveric Blood Specimens http://www.fda.gov/cber/gdlns/cadbld.pdf.