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Effective serological and molecular screening of deceased tissue donors

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Abstract A comprehensive and effective screening programme is essential to support the banking of tissues from deceased donors. However, the overall quality of the samples obtained from deceased donors, quantity and condition, is often not ideal, and this may lead to problems in achieving accurate and reliable results. Additionally a significant percentage of referrals are still rejected upon receipt as unsuitable for screening. We are actively involved in improving the overall quality of deceased donor screening outcomes, and have specifically evaluated and validated both serological and molecular assays for this purpose, as well as developing a specific screening strategy to minimise the specificity issues associated with serological screening. Here we review the nature and effectiveness of the deceased donor screening programme implemented by National Health Service Blood and Transplant (NHSBT), the organisation with overall responsibility for the supply of tissue products within England. Deceased donor screening data, serological and molecular, from August 2007 until May 2012 have been collated and analysed. Of 10,225 samples referred for serology screening, 5.5 % were reported as reactive; of 2,862 samples referred for molecular screening, 0.1 % were reported as reactive/inhibitory. Overall 20 % of the serological and 100 % of the molecular screen reactivity was confirmed as reflecting true infection. The use of a sequential serology screening algorithm has resulted in a marked reduction of tissues lost unnecessarily due to non-specific screen reactivity. The approach taken by NHSBT has resulted in the development of an effective and specific approach to the screening of deceased tissue donors.

Keywords Deceased donor · Tissue donor · Serological screening · Molecular screening · Organ donor

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Introduction

The screening of deceased tissue donors is a critical activity to ensure the safety and availability of the wide range of tissue products required by medicine today. Prior to release for clinical use tissue donations retrieved from deceased donors require laboratory screening for any evidence of the presence of a range of infectious agents that may be transmitted via tissue transplantation. In the UK this is performed on a venous blood sample collected from the donor up to 1 week pre-mortem or, and more usually in our experience, up to 24 h post-mortem.

Screening is performed for the 'standard set' of transmissible (blood-borne) infectious agents, using serological markers as the core screen, but in some cases with the addition of molecular screening for HBV DNA and HCV & HIV RNA. Whilst the screening of samples from deceased tissue donors is performed in essentially the same way and with the same broad approach as for blood donations, the core screening requirements do vary. Additionally the screening of deceased tissue donors may be more problematical, primarily due to the nature and quality of samples from deceased individuals, often leading to increased reactive rates (Challine et al. 2006; Kitchen and Gillan 2009; Padley et al. 2005; Thomas et al. 2007; Wilkemeyer et al. 2012).

The tissue screening guidelines applied in the UK (HTA Guidelines 2010) require that donations from screen negative living tissue donors, generally patients donating their femoral head following hip replacement surgery, are quarantined until the donor has been retested for serological markers on a follow-up sample taken at least 180 days after donation. The quarantine and 180 day follow-up sample are not required if molecular screening, in addition to the standard serology screening, is applied to the sample taken at donation. However, this 180 day quarantine rule clearly cannot be applied to tissues retrieved from deceased individuals and a more comprehensive 'at donation' screening programme is the only way to maximise the safety of donations from such donors.

Within England, National Health Service Blood and Transplant (NHSBT) is the organisation that has overall responsibility for the provision of blood and tissues, although not all tissues from deceased or living donors are retrieved and processed by the organisation itself. Within NHSBT the National Transfusion Microbiology Reference Laboratory (NTMRL) then has sole responsibility for the screening of all deceased donors from whom NHSBT Tissue Services has retrieved tissues. In addition to this screening, NTMRL also screens deceased donors for a number of other NHS bodies who also retrieve and supply tissues from deceased donors; these include the Corneal Transplant Service (CTS) and a number of heart valve banks. In 2007 NHSBT determined that all deceased tissue donors would have both serological and molecular screening applied, consequently all deceased tissue donors screened by NTMRL, including some non-NHSBT donors, are subject to the same full serological and molecular screening.

The deceased donor screening programme developed by NTMRL for NHSBT includes full serological and molecular screening using assays specifically validated for the purpose (Kitchen and Newham 2011), and using a screening algorithm developed specifically for deceased donor screening; improved specificity to minimise unnecessary loss of donated products due to non-specific reactivity (Kitchen and Gillan 2009). Here we review the overall outcomes of this screening programme from 2007 to June 2012, serological and molecular, analysed from the perspective of overall effectiveness in ensuring maximum sensitivity of the screening programme together with maximum availability of screened tissue products for recipients.

Although not the specific focus of this analysis, the situation in relation to the screening of human organs prior to transplantation needs some consideration. The serological screening of tissue and organ donors, excepting for the circumstances of donation and timing of screening, is virtually identical and our screening data, serological and molecular, can be directly extrapolated to UK organ donors. The UK guidelines from the advisory committee for the Safety of blood tissues and organs (SaBTO) (SaBTO guidelines 2011) have encouraged the implementation of molecular screening of organ donations, albeit with the provision of results as soon as possible after transplantation, further highlighted by the imminent implementation of the EU Organ directive. The molecular data generated by the screening of deceased tissue donors could be extrapolated to try to predict the impact of including molecular screening in the screening of organ donors.

Materials and methods

Study population

Samples from deceased tissue donors referred to NTMRL for serology and/or molecular screening from August 2007 to May 2012 were included in this study. All screening being performed on individual donor samples only. Only those samples requiring full serology screening and/or full molecular screening (described below) were included in this analysis.

Samples

Serum/plasma samples were collected from deceased individuals within 24 h *post-mortem*. On occasions *pre-mortem* samples with sufficient volume are available and used. Where this information was available to NTMRL, these donors were excluded from this particular analysis.

Screening assays

From August 2007 NHSBT's mandatory full serological screening profile for deceased tissue donors was set at: HBsAg, anti-HCV, HIV Ag/Ab, syphilis Ab, anti-HTLV I/II and anti-HBc; and the full molecular screening profile was set at HBV DNA, HCV RNA and HIV RNA.

Serology

Serology screening of the study population was performed for the markers listed above. Initially screening was performed using a range of microplate screening assays performed on Dynex DSX automated microplate systems (Dynex Technologies, Chantilly, USA). However, these microplate assays were replaced at the beginning of 2011 with an Architect automated system—Architect *i*1000SR, (Abbott Laboratories, Maidenhead, UK). The assays used are listed in Table 1. All assays were performed according to the manufacturer's instructions. Additionally all assays were validated within NTMRL for use with samples from deceased donors (Kitchen and Newham 2011), although the Architect validation data have not been published.

Molecular

Molecular screening of the study population was performed for HBV DNA, HCV RNA and HIV RNA. Initially molecular screening was performed using 3 individual in-house RT PCR's (Liu et al. 2007; Cleland et al. 2001). All 3 included an internal control added to the sample prior to extraction. Briefly, samples were extracted using a Qiagen Biorobot. Extracts were then amplified with individual RT PCRs based on published assays. Sensitivity for HCV and HIV RNA detection was approximately 250 Geq/ml, and for HBV DNA approximately 100 Geq/ml, determined using the appropriate international standards provided by the National Institute for Biological Standards and Controls (NIBSC, HPA, Potters Bar, England).

Subsequently in 2009 the Chiron/Novartis Ultrio triplex molecular screening assay was implemented. A single triplex transcription mediated amplification (TMA) based assay for the simultaneous detection of HBV, HCV and HIV nucleic acids in human serum/plasma. In 2011 this was upgraded to the Ultrio Plus triplex assay, with claimed improved performance for the detection of individuals with very low level HBV DNA. Both the in-house and Ultrio assays were specifically validated in NTMRL for use with samples from deceased donors, and specifically with the range of deceased donor samples referred to NTMRL (data not published).

Screening algorithms

Serology

From 2007 the standard single assay donation screening algorithm was applied, with initially reactive samples being repeated in duplicate using the same assay and the 2 out of 3 rule applied to determine the overall screen result. Subsequent to more detailed analysis of the NTMRL reference outcomes of these screen reactive samples, in October 2009 the screening algorithm was changed to a 2 assay sequential algorithm (Kitchen and Gillan 2009), with all initially reactive samples being repeated in duplicate on a second assay of at least equal sensitivity; the results of the second assay determining the overall screen result.

Molecular

Any samples reactive on any of the in-house assays were repeated to confirm the reactivity. Any Ultrio triplex screen reactive sample was retested on the Ultrio assay but using the discriminatory protocol which uses the three specific probes individually, i.e. 3 separate tests per initially reactive sample. Specific repeat reactivity was then confirmed using a second assay.

Any samples initially demonstrating inhibition of the internal control, in-house or Ultrio assays, were

Table 1 Serology assays used for the screening of deceased tissue donations

Marker	Assay	Product code	Manufacturer
HBsAg	Hepanostika HBsAg Ultra	284133	bioMerieux, Basingstoke, England
	Architect HbsAg Qual II	2G22	Abbott Diagnostics, Maidenhead, England
HIV Ag/Ab	Uniform II HIV Ag/Ab	285047	bioMerieux, Basingstoke, England
	Architect HIV Ag/Ab	4J27-22	Abbott Diagnostics, Maidenhead, England
HCV Ab	anti-HCV SAVe v3	930800	Ortho Clinical Diagnostics
	Architect anti-HCV	6C37	Abbott Diagnostics, Maidenhead, England
Syphilis Ab	ICE syphilis	8E0401/02	Diasorin Ltd, Bracknell, England
	Architect syphilis Ab	8D06	Abbott Diagnostics, Maidenhead, England
HTLV Ab	anti-HTLV I/II	8E202/04	Diasorin Ltd, Bracknell, England
	Architect r-HTLV I/II	6L61	Abbott Diagnostics, Maidenhead, England
HBc Ab	Architect anti-HBc II	8L44	Abbott Diagnostics, Maidenhead, England
	anti-HBc (total)	8G21-01/02	Diasorin Ltd, Bracknell, England

diluted 1:5 and retested. Samples still demonstrating inhibition after dilution were reported as 'inhibitory'.

Blood donation screening outcomes

The routine blood donation screening outcomes for the 12 months prior to 31st May 2012 were collected as a broad comparator for the overall outcomes and effectiveness of the deceased donor screening programme. The overall screen reactive rates for HBsAg, anti-HCV, HIV Ag/Ab and syphilis antibody were compared to determine any significant difference between the deceased tissue donor and routine blood donor screening outcomes. The anti-HTLV I/II and molecular screening outcomes could not be compared directly as blood donations are screened for these particular markers in pools of 24 whilst deceased tissue donors are only ever screened individually for all screening markers. Similarly ant-HBc screening outcomes could not be compared as anti-HBc screening is not performed routinely on blood donations.

Statistical analysis

Analysis of any significance in the difference in screen repeat reactive rates between blood donations and deceased tissue donors was performed using the Chi Squared test (SPSS v20, IBM). Confirmed positives were removed from the overall numbers of screen reactive samples for both datasets prior to calculation of reactive rates.

Results

Study population

Initially samples were referred to NTMRL from NHSBT Tissue Services (NHSBT-TS) and from the Oxford Heart Valve Bank (OHVB), but during the period from August 2007 to June 2012 the number of referring sites gradually increased and additionally includes the Corneal Transplant Service (CTS)—Bristol and Manchester Eye Banks, the Brompton Hospital Heart Valve Bank (BHVB), and the Birmingham Children's Hospital Heart Valve Bank (BCHVB). Currently not all of these referrals to NTMRL are for both serological and molecular screening. NHSBT-TS and OHVB donors require full serological and molecular screening; CTS donors are referred for serological screening only; BHVB and BCHVB donors are referred for molecular screening only

A total of 10,562 deceased donor samples referred to NTMRL for screening during the period from August 2007 until June 2012 have been included. Of these 10,411 (98.57 %) were deemed as suitable for screening, with 151 (1.44 %) considered unsuitable for screening and rejected at Sample Reception (Table 2).

Of the 10,411 samples that were suitable for screening, 7,526 (72.3 %) were referred for serology screening only, 2,699 (25.9 %) for both serology and molecular screening and 186 (1.8 %) for molecular screening only.

Table 2 Referred samples rejected due to unsuitability

Categories of samples referred but not tested	No.	% of total referred (n = 10,562)	% of rejected samples (n = 151)
Haemolysed	63	0.60	41.73
Insufficient sample	76	0.72	50.33
Sample leaked in transit	1	0.01	0.66
Sample too diluted to test	2	0.02	1.32
Sample unlabelled	9	0.09	5.96
Total	151	1.44	100

Table 3 Serological screening reactivity

	HBsAg	anti-HCV	HIV Ag/Ab	Syphilis Ab	anti-HTLV	anti-HBc	Totals
Total number of donations screened	10,225	10,225	10,225	10,225	10,225	10,225	10,225
Screen initial reactive	316	41	294	64	678	114[a]	1,507
Insufficient sample to complete screening	14 (0.14)	1 (0.01)	8 (0.08)	1 (0.01)	19 (0.18)	6 (0.05)	49 (0.48)
Screen repeat reactive on the std algorithm[b]	76 (0.74)	12 (0.12)	15 (0.14)	31 (0.3)	305 (2.9)	47 (0.45)	486 (21.8)[c]
Screen repeat reactive on the sequential algorithm[b]	43 (0.42)	6 (0.06)	12 (0.12)	19 (0.18)	18 (0.17)	61 (0.6)	159 (2.0)[3]
Total screen repeat reactive[d]	119 (1.16)	18 (0.18)	27 (0.26)	50 (0.48)	323 (3.1)	108 (1.05)	566[d] (5.5)[e]
Total screen negative	10,092 (98.7)	10,206 (99.81)	10,192 (99.7)	10,174 (99.5)	9,883 (96.7)	10,111 (98.9)	

Numbers in parenthesis are % of the number of donors screened for each marker

[a] Excludes donors subsequently confirmed as being HBV infected

[b] Total no. of IR and RR results for each assay, some donors were IR/RR for more than 1 marker

[c] % of number of donors screened using the different algorithms; 2,228 screened using the std algorithm and 7,997 using the sequential

[d] Overall figure takes into account samples RR for more than one marker (see text)

[e] Overall % of total number of donors screened

Subsequent to this 49/1,507 (0.48 %) serology initially reactive samples had insufficient volume remaining to complete the serological screening, and 52/566 repeat reactive samples requiring confirmation had insufficient sample remaining to investigate fully. All of the 2,885 samples referred for molecular screening had sufficient sample to complete the screening and any other investigations required.

Deceased donor screening outcomes

A total of 10,225 samples were screened for serological markers and 2,862 for viral nucleic acids. The overall serology and molecular screening outcomes are presented in Tables 3 and 4.

Overall 566/10,225 (5.5 %) of samples were repeat reactive on serological screening and 2/2,862 (0.07 %) on molecular screening. Additionally 21 of the samples screened for viral nucleic acids were inhibitory to the assays performed and no outcome could be obtained.

49 initially serology screen reactive samples had insufficient remaining for completion of screening; all of these were reactive on 1 assay only. Although in total 645 repeat reactive results were obtained from the 10,225 samples screened, 72 of the samples were

Table 4 Molecular screening reactivity

	In-house RT PCR (%)	Novartis Ultrio (%)	Total (%)
Total screened	698	2,187	2,885
Screen negative	694 (99.4)	2,168 (99.1)	2,862 (99.2)
Initially reactive	2 (0.3)	0	2 (0.07)
Repeat reactive	2 (0.3)	0	2 (0.07)
Inhibitory	2 (0.3)[a]	19 (0.9)	21 (0.73)
Confirmed pos	2 (0.3)[b]	0	2 (0.07)

[a] One inhibitory in all 3 virus assays, 1 in the HBV DNA assay only

[b] Both HBV DNA pos, HBsAg negative, anti-HBc pos, anti-HBs pos

repeat reactive on two or more of the screening assays. 65 of the samples were reactive in 2 of the screening assays and 7 were reactive in 3 of the screening assays (detailed breakdown not presented).

The overall specificity of the serological screening programme was 94.86 % (517 non-specific reactives out of 10,064 donor samples screened), taking into account the 49 samples with insufficient to complete screening, 72 samples with multiple screen repeat reactivity and the 112 confirmed positives. However, a significant reduction in the number of screen repeat reactive donors was achieved when the sequential screening algorithm was applied (Table 3). A total of 7,997 samples were screened using the sequential assay generating 159 repeat reactive results. Of these 75 were subsequently confirmed positive, 8 had insufficient for completion of screening and 3 were dual screen reactive giving an overall specificity of 98.9 % (81/7,914).

The overall specificity of the molecular screening programme, taking into account the 2 confirmed positive donations was 100 %. If, however, the 21 inhibitory samples are considered to impact on specificity, then the overall specificity falls to 99.27 % (21/2,883 uninfected donor samples screened).

Deceased donor reference outcomes

The serology reference investigation outcomes are presented in Table 5. Although 72 of the individual deceased donor samples were screen repeat reactive for more than one serology marker, only 4 of these donor samples were subsequently confirmed positive for more than one infection, and all 4 for both hepatitis B (HBsAg+) and syphilis. Of the samples that had insufficient to complete the reference investigations, although final outcomes were not possible, none had patterns that were considered likely to reflect true infection. Similarly none of the indeterminate/inconclusive reference outcomes were considered to be likely to reflect true infection.

Both of the molecular repeat reactive donors were confirmed to be HBV infected.

Blood donation screening outcomes

During the period from the beginning of June 2011 to the end of May 2012 a total of 2,059,475 blood donations were screened. The screen reactive and confirmed positive outcomes for HBsAg, anti-HCV, HIV Ag/Ab, and syphilis Ab screening of these donations are presented in Table 6, compared directly with the corresponding overall deceased donor outcomes for the whole of the deceased donor study period. The overall specificity of serological blood donation screening, taking into account the number of confirmed positives was 99.83 % (3,578 non-specific reactives out of 2,059,246 donations screened). The overall specificity of the molecular screening programme, taking into account the 117 confirmed positive donations was 100 % (0 non-specific reactives out of 2,059,358 uninfected donations screened).

All of the screen reactive rates, after removing the confirmed positives, were significantly higher in the deceased tissue donor group ($p < 0.001$). Even the sequential algorithm with its significantly lower repeat reactive rates still had a significantly higher reactive rates than the blood donor group ($p > 0.005$). Similarly the confirmed positive rates were also significantly higher in the deceased tissue donor group ($p < 0.005$) (Table 7).

Discussion

The provision of tissues, and organs, from deceased donors may be particularly convoluted as multiple products may be obtained from one donor, but at different times and by different organisations. In the UK a single deceased individual who becomes a donor may potentially firstly donate organs at local/regional hospital level but managed through Organ Donation and Transplant (ODT)—that part of NHSBT that

Table 5 Serological confirmatory testing outcomes

	HBsAg	anti-HCV	HIV Ag/Ab	Syphilis Ab	anti-HTLV	anti-HBc
Total screen repeat reactive	119	18	27	50	323	108
Confirmed positive (std algorithm)	4 (3.4)	2 (11.1)	0	11 (22)	2 (0.6)	18 (16.7)[a]
Confirmed positive (sequential algorithm)	7 (5.9)	3 (16.7)	2 (7.4)	20 (40)	1 (0.1)	42 (38.9)[a]
Indeterminate/Inconclusive on confirmatory testing	12 (10.1)	3 (16.7)	5 (18.5)	6 (12)	2 (0.6)	6 (5.6)
Confirmed uninfected	79 (66.4)	9 (50)	19 (70.4)	12 (24)	280 (86.7)	41 (37.9)
Insufficient sample to complete confirmatory investigations[b]	15 (12.6)	1 (5.5)	1 (3.7)	1 (2)	33 (10.2)	1 (0.9)

Numbers in parenthesis are % of the number of screen reactive donations for each marker

[a] All anti-HBc present with anti-HBs >10 miu/ml, but less than 100 miu/ml

[b] Numbers corrected to account for those referred for more than one marker

manages organ donation/transplant nationally, may then donate ocular tissue under the auspices of the national Corneal Transplant Service (CTS) and finally may then donate any number of the range of other tissues that may be retrieved by NHSBT Tissue Services for general tissue banking. It is also possible that infectious disease screening of the donor may occur at each retrieval, with the potential for not only discrepant results from different laboratories/testing systems but also exhaustion of available samples with the potential loss of tissues if for some reason screening, or part of it, cannot be performed. To try to ensure that in England screening of a deceased tissue donor is performed only once, irrespective of retrievals of different tissues by different organisations, NHSBT has sought, with significant success, to centralise the testing of deceased donors in one expert laboratory.

The study period for this work includes an overall total of 10,562 samples from deceased tissue donors, of which 10,411 were considered suitable for screening; 10,225 for serology and 2,885 for molecular. Of these 566/10,225 (5.5 %) were found to be serology screen reactive and 2/2,885 (0.07 %) were found to be molecular screen reactive. The overall specificity of the deceased tissue donor screening programme over the study period was 94.861 % for serology and 99.27 % for molecular screening (based on inhibitory results rather than unconfirmed reactivity), although when using the sequential serology algorithm the serology specificity rose to 98.9 %.

Direct comparison of deceased tissue donor serology screening outcomes with the outcomes of blood donation screening shows significant differences in terms of both screen reactive rates and confirmed positives. The comparative figures for blood donors, based on the mandatory screening only for the comparative year, are 99.83 % for serology and 100 % for molecular, although not a full like-for-like comparison. As expected, blood donor screening outcomes are better overall than deceased donor ones, with higher specificity and lower prevalence of infection in the donors. The significantly higher levels of infection in the deceased donors reflecting probable differences in the donors, their demographics and risks, and the donor selection process. When analysing blood donation screening data the prevalence of confirmed infections is always, as would be expected, higher in first time donors than repeat donors. Deceased tissue donors should be considered, in terms of infection risk, as broadly equivalent to first time blood donors, although the confirmed positive rates are still around one order of magnitude greater than in first time blood donors (Table 7). However, detailed comparisons of reactive and confirmed infections in first time blood donors and deceased tissue donors could not be performed as NTMRL does not specifically record whether donors are first time or repeat donors.

One of the reasons for the significant differences in specificity relates to the difference between first time and repeat donors. The blood donor population is continually cleared of much of the non-specific reactivity through regular donation, screen negative donors continue to donate, whilst the deceased donor population is only ever an unknown (first time) population. However, there are also other fundamental differences in the testing practices and in the samples

Table 6 Comparison of blood and deceased tissue donor/donation screening/confirmatory outcomes

Donor/donation type Deceased tissue n = 10,225 Blood donation n = 2,059,475	HBsAg (%)		anti-HCV (%)		HIV Ag/Ab (%)		Syphilis Ab (%)	
	Deceased tissue	Blood donation	Deceased tissue	Blood donation	Deceased tissue	Blood donation	Deceased tissue	Blood donation
Screen repeat reactive (%)[a]	119 (1.16)	461 (0.022)	18 (0.18)	1,432 (0.07)	27 (0.26)	740 (0.036)	50 (0.48)	1,136 (0.055)
Confirmed positive (%)[b]	11 (9.2)	69[c] (15)	5 (27.8)	65 (4.6)	2 (7.4)	10 (1.3)	31 (62)	67 (5.9)
Confirmed indeterminate/inconclusive (%)[b]	12 (10.1)	10 (2.2)	3 (16.7)	102 (7.1)	5 (18.5)	20 (2.7)	6 (12)	9 (0.8)
Confirmed negative (%)[b]	79 (66.4)	379 (82.2)	9 (50)	1,259 (87.9)	19 (70.4)	708 (95.7)	12 (24)	1,060 (93.3)
Confirmatory testing not performed/completed (%)[b]	17 (14.3)	3 (0.6)	1 (5.5)	6 (0.4)	1 (3.7)	2 (0.3)	1 (2)	0

[a] % of the number of donors/donations screened within the study periods

[b] % of number of screen repeat reactives

[c] Includes 6 samples that were confirmed low level HBsAg but HBV DNA negative and subsequently identified as recent HBV vaccinees

themselves. Both molecular and anti-HTLV screening of blood donations is performed on pools of 24 donations resulting is significantly higher specificity as the majority of non-specific reactivity would be diluted out in the screening pool. Additionally anti-HBc assays commonly demonstrate higher specificity issues than other blood screening assays, and as screening is performed on all tissue donors but on only a small percentage of blood donations, the tissue donor outcomes will therefore always be significantly worse. In addition to the above there are specific sample characteristics that influence outcomes. To address the poorer specificity, for whatever reason, during the period from which the deceased donor data were collected the serological screening algorithm changed from the standard single assay approach to a 2 assay sequential algorithm. This has significantly increased the overall specificity of the serological screening (Kitchen and Gillan 2009), decreasing screening losses by approximately 90 % (21.8–2 %, Table 3), but because it was only implemented in late 2009, its effect has been diluted in this particular analysis. Nonetheless, overall the deceased donor performance figures for NHSBT compare very well with other deceased donor screening programme data (Bensoussan et al. 2010; Challine et al. 2006; Miedouge et al. 2002; Thomas et al. 2007).

The development of a deceased donor screening programme will always present challenges: sufficiency and suitability of the samples, provision of assays that are suitable for use with samples from deceased individuals, the use of an appropriate screening algorithm, and effective confirmation. However, all of these can be effectively dealt with if the underlying issues are properly understood and a systematic approach is taken.

Sample sufficiency is a particular and ongoing problem, and particularly when screening deceased donors as there is usually no possibility of obtaining further sample. Occasionally, however, additional sample can be obtained from other laboratories within the pathology department if other diagnostic investigations had been performed on the donor pre-mortem. However, we still encounter not insignificant numbers of tissue retrievals where there is insufficient sample obtained; both to perform any screening (0.72 % of all referrals) and to complete screening (0.48 % of samples screened). Additionally 3.9 % (59/1,507) of the screen reactive donor samples referred for

Table 7 Comparative confirmed serological reactivity

	Deceased tissue donors (%)	Blood donations—all (%)	Blood donations—new donors (%)
Total screened	10,225	2,059,475	172,762
HBsAg positive	11 (0.1)	59 (0.0034)	55 (0.032)
anti-HCV positive	5 (0.05)	59 (0.003)	58 (0.034)
HIV Ag/Ab positive	2 (0.02)	11 (0.0005)	7 (0.004)
Syphilis Ab positive	31 (0.3)	67 (0.0033)	51 (0.03)
anti-HTLV positive	3 (0.03)	18 (0.0009)	Not recorded[a]
anti-HBc positive[c]	60 (0.6)	48 (0.0023)	Not recorded[b]

[a] Not recorded as screening performed on pools which include new and repeat donors

[b] Not recorded as screening only performed on donors with specific risks

[c] Evidence of HBV infection but with HBsAg not present/detected

confirmation had insufficient sample to be able to perform all of the investigations required. Although individually relatively small numbers, overall 125 (1.18 %) of deceased donor samples originally referred to NTMRL could not be screened or screening completed because of lack of sample. This resulted in the loss of all tissues retrieved from these donors; these retrievals could be single- or multi-tissue.

Screening samples from deceased individuals are generally of lower quality than blood donation samples; related to the increased time taken for samples to reach the laboratory, less controlled storage conditions, the potential presence of volume expanders and other infused preparations, a higher proportion with haemolysis, and other general post-mortem biochemical and physical changes. These factors are all relatively obvious, but there is little published work looking at these issues in any real detail although there are reports of general adverse effects on screening programmes (Baleriola et al. 2012; Cahane et al. 2000; Challine et al. 2006; Strong et al. 2005; Wilkemeyer et al. 2012). Certainly we have not knowingly identified any specific problems with such samples that have led us to question assay sensitivity as Wilkemeyer et al. (2012) have, although poorer specificity can be problematical. Some studies have specifically identified haemolysis as being a major cause of poorer specificity (Challine et al. 2006; Strong et al. 2005), but this is not our experience. We have not been able to link any particular identifiable factor with the poorer specificity seen with our samples (data unpublished), although Bensoussan et al. (2010) have reported that immediate sample separation reduces non-specific reactivity, However, we have previously shown that the majority of the non-specific reactivity is assay specific and can be managed well through the screening algorithm and effective assay selection (Kitchen and Gillan 2009).

Haemodilution is another specific issue that has been considered to impact on sample quality and therefore screening outcomes (Eastland 2000), however, we feel that this can usually be dealt with without problem. Current EU and UK tissue regulations allow for calculated haemodilution up to 50 % of circulating volume (HTA Guidelines 2010), but in reality such a figure can easily be challenged, both in terms of the accuracy of any calculated dilution and in terms of the actual effect on assay sensitivity. There are continuing issues over the precise definitions surrounding haemodilution and whether a patient is defined as haemodiluted if infused but not actually bleeding out. However, when using well understood and highly sensitive serology assays dilution of samples at 1:10 (90 % haemodilution) does not generally result in loss of signal unless the target is present at an extremely low level. Similarly 1:10 dilution (1 $\log_{10}$) rarely results in loss of signal on molecular testing. In previous published serological studies looking at assay validation for use with samples from deceased individuals, reactivity at dilutions of >1:1,000 were not unusual (Kitchen and Newham 2011). Similar results were obtained subsequently with molecular assays (data not shown). Only in situations where the screening targets are at a very low level, generally early/recent infection but in some cases late resolving infection, would there be any significant risk of failure to detect screening target due to haemodilution.

Consideration has to be given to the suitability of assays for use with samples from deceased individuals.

It is our view and experience that many of the serology assays produced by the major international diagnostic companies, with the appropriate validation and controls, are suitable for this purpose. Validation of such assays can be problematical as manufacturers, as do we, struggle to access the appropriate samples to enable them to validate their assays for use with samples from deceased donors at point of development. We, and now others, have previously suggested a way in which assays can be validated for this purpose (Baleriola et al. 2012; Kalus et al. 2011; Kitchen and Newham 2011), and in the absence of anything that can generate significantly better data, continue to use this approach. The serology assays currently used in NTMRL as the primary and sequential deceased donor screening assays, Abbott Architect assays plus specific microplate assays (Table 1) have proven to be effective and highly reliable and are performing well in our hands, and with others finding similar outcomes with the Architect system (Baleriola et al. 2012).

The situation in respect of molecular screening assays is somewhat simpler as currently there are limited commercial CE marked triplex molecular donation screening assays available. The 2 main assays are the Novartis Ultrio assays run on either the Tigris or Panther platforms, or manually, and the Roche MPX assays run on the s201 system. In general validation of molecular screening assays for deceased donor screening is relatively straightforward as these assays all include an internal control, specific independent target material added to each test sample prior to the extraction/amplification process, and which effectively individually validates the result obtained for each sample tested; concerns over 'false negative' results can be therefore be allayed. In our experience non-specific reactions are rarely seen in molecular screening.

As is clear from the overall outcomes of our screening programme, the issues predominately relate to the serology. Commonly the same serology algorithm as applied to blood donor screening is used by tissue screening laboratories, but this may not be optimal. We previously identified specificity and the influence of the algorithm as the main issue when considering options to improve overall outcomes, but this was based upon a smaller dataset (Kitchen and Gillan 2009) than available for this review. The current dataset is larger and sufficient to demonstrate the importance and effectiveness of adopting a different screening algorithm. Our laboratory screening and reference data, together with lack of evidence of transmission of infections to recipients of the tissue products, confirm for us that we should consider our screening programme as being sensitive and not missing infected donors. On the basis that it is then the specificity of the screening programme that is the greatest threat, the unnecessary loss of tissue donors/donations, any strategy that improves specificity without compromising sensitivity, and assuming relative simplicity in its implementation, can only be beneficial. Data are now building up to support the use of the two assay sequential screening algorithm that we now use, indeed in Table 3 it can be seen that 21.8 % of donors were repeat reactive when screened using the standard algorithm whilst only 2 % were repeat reactive when using the sequential algorithm. Use of the appropriate serology algorithm will significantly impact on the overall effectiveness of the screening programme.

The value of confirmatory testing is another major element of any screening programme, but which may be overlooked in the context of deceased donor screening. It is clearly always important to investigate any screening reactivity to confirm the status of the screen reactive donors (Wilkemeyer et al. 2012). In the circumstances surrounding deceased donors there is a public health issue inasmuch as for any confirmed positive deceased individual, sexual and other close contacts must be still advised of any risk that they may have been exposed to. However, confirmation also serves to validate the screening programme by determining the relative proportions of true infections versus non-specific reactivity, allowing the screening programme to be appropriately modified. Continuous monitoring of the confirmed positive versus non-specific ratios is an important tool to ensure maximum availability of tissue products.

Over the last few years there has been a clear expectation that blood screening programmes will include molecular as well as serological screening, with the, often unchallenged, assumption that this will lead to an increase in blood safety. NHSBT first implemented molecular screening of blood donations in 1999 (HCV RNA), but the molecular screening of tissue donations, in addition to serology, was not implemented until 2007 (HBV, HCV and HIV nucleic acids). The implementation for tissue donors was primarily to address the EU/HTA requirement to quarantine living donor tissues for 180 days and

obtain a follow-up sample; at that time molecular screening was not performed at point of collection (HTA Guidelines 2010). Whilst being considered good practice when applied to deceased tissue donors, it was not mandated. Importantly, however, it is the overall value of molecular screening in terms of incremental pick-ups that needs to be understood. Of the study population only 2 deceased donors (0.07 %) were NAT screen positive and subsequently confirmed (both HBV DNA), but both were also serology positive. Other NAT positives were identified in the serology screen reactives during confirmation, but these donors had not had molecular screening performed. On the other hand 21 deceased donor samples (0.73 %) were found to be inhibitory on molecular screening, even after sample dilution, resulting in the loss of the donations from these donors; not an insignificant finding. Taking these results in isolation it could be argued that molecular screening was not particularly beneficial, infact within NHSBT it resulted in the unnecessary loss of products due to assay inhibitors in a number of donor samples. This view is the opposite of that of Pruss et al. (2010) who believe that molecular screening should be routine for all tissue donations. Whilst there is no doubt that the inclusion of molecular screening does theoretically improve the overall sensitivity of screening programmes by reducing the window periods for HBV, HCV and HIV infections, it is the number of NAT positive/serology negative pick-ups that demonstrates any real benefit. In many countries with developed healthcare systems, low population disease burdens, good donor selection procedures and sensitive serology assays in use, very few additional pick-ups are likely as a result of implementing molecular screening in addition to serology, and so few as to justifiably raise questions about the overall value of implementing such screening. Our data and the view of a recent consensus conference (Humar et al. 2010) certainly support this stance.

However, in certain situations the outcomes of molecular screening can provide useful information which can offer the opportunity to re-evaluate the serology screening outcomes. In the UK and other countries, the issue of implementing molecular screening of organ donors for HBV, HCV and HIV nucleic acids has been raised (Humar et al. 2010; Nett et al. 2012). It is highly unlikely that this could be achieved pre-transplant and it has therefore been seen as an adjunct to the core serology screening, but not being an absolute requirement pre-transplant, being performed as soon as possible after transplant. There is concern that this will not produce any incremental benefit and if the measurement is simply the number of additional pick-ups then these concerns are probably justified. Greater concerns are, however, increased costs and potential loss of organs due to non-specific reactivity and/or inhibition (Humar et al. 2010; Nett et al. 2012). If, however, a broader view is taken, the value of molecular screening in this situation could be that of the additional information available to better inform organ use and subsequent clinical management of transplant recipients. Indeed the question is whether the screening of potential organ donors is actually to inform decisions about the use of the organs rather than to identify and reject screen reactive donors. The pre-transplant serology screening of organ donors identifies donors who are screen reactive, whose reactivity may be specific or non-specific, but decisions have to be taken on the basis of the pre-transplant screening. The decision to use organs in this situation rests with the transplant surgeon, after the relevant microbiological advice has been provided, and is taken on the basis of risk assessment which includes the consequences to the patient of both transplanting and not transplanting. There are many confounding factors, both patient and donor related, but in general the risk of not receiving a transplant outweighs any potential infection risk associated with the transplant. Consequently the critical issue is the appropriate management of recipients in these situations, helped greatly by knowing the true infection status of the donor. It is here that molecular screening of organ donors could be of value as, even if not performed pre-transplant, the information provided would allow better patient management in many cases; for example a significant % of HCV serology positive donors are HCV RNA negative and management of recipients of organs from HCV positive donors could be more effective if the HCV RNA status of such donors were to be known. Thus in situations where evidence of infection in donated organs or tissue does not necessarily prevent use, the value of molecular screening is arguably primarily the provision of information that can be used to make more informed decisions on the use of what are often life saving donations.

Overall the data show that it is possible to develop a fully validated and effective serological and molecular

deceased tissue donor screening programme. The screening algorithm developed currently results in only 2 % of donors referred being reported as screen reactive. The majority of these are confirmed as uninfected and this further informs changing from the current position to that of allowing tissue donations from screen reactive but confirmed negative donors to be released for clinical use. The inclusion of molecular screening cannot, at this time, be considered to have had any incremental benefit on the overall screening outcomes of the deceased tissue donors handled by NHSBT, but does represent best practice and, importantly, ensures a common approach for blood and tissue donations within England.

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