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ORIGINAL ARTICLE

Significant variation in heart valve banking practice

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Abstract

The European Blood Alliance (EBA) Tissue and Cells annual benchmarking exercise identified that in 2014, the heart valve (HV) discard rate in tissue establishments (TEs) run by EBA members was between 19 and 65%. Given this significant discard rate, a decision was taken to carry out a worldwide data-gathering exercise to assess the processing methodology in different TEs. In collaboration with the Foundation of European Tissue Banks, a questionnaire asking for the details on HV processing was sent to TEs worldwide. Nineteen questionnaires were received back from 15 European TEs and 4 non-European TEs. The data provided confirmed a significant discard rate of HVs with 43–50% of aortic valves and 20–32% of pulmonary valves being discarded in 2015. The causes of HV discard varied, with microbiology contamination, anatomical and medical reasons being the main causes. This data-gathering exercise highlighted significant variations in practice in different TEs including how donor suitability is assessed, critical timings for heart retrieval and processing, heart rinsing, HV decontamination protocols and methods of microbiological testing. To reduce the discard rates, there are several aspects of HV banking that could be validated and standardised. Here, we report the findings of this data-gathering exercise. We consider this a first step that will help lead to standardising HV banking.

Keywords Heart valves · Discard · Microbiology · Transplantation

Introduction

The European Blood Alliance (EBA) Tissue and Cells Working Group (T&C WG) carries out an annual benchmarking exercise. The results of the 2014 EBA T&C WG benchmarking exercise identified that within the EBA TEs that provided data for 2014, there was a HV discard rate ranging from 19 to 65% with a median of 41% (unpublished data, Dr S Zahra).

HVs are precious resources that may be lifesaving [1] and which may be in short supply in a number of countries. It was therefore felt important to gather further information to establish the causes of HV discard in the different TEs. Furthermore, many tissue banking and processing methodologies are not based on hard evidence. It was therefore felt important to try to gather as much information as possible about HV banking practice around the world. To this end, a detailed data-gathering exercise to map the process of HV banking was carried out in May 2016, with the aim of improving practices that could be shared with all TEs.

In order to maximise the number of participating TEs, the EBA T&C WG carried out this data-gathering exercise in collaboration with the Foundation of European Tissue Banks.

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Method

A detailed questionnaire covering all aspects of HV banking was developed which included questions on types of donors (live, multi-organ and tissue-only donors), age of donors, how donor assessment is carried out, details of retrieval, donor testing, tissue transport and HV processing. Each TE was also requested to provide data on the number of donors, number of HVs discarded and reason for discard.

The questionnaire was sent out at the end of August 2016 to TEs in 15 European countries and an additional 11 countries worldwide. There was a very good response rate of 73% with 15 European and 4 non-European TEs returning results. The responses received were anonymised.

Results

HV donors

From the responses received, all 19 TEs retrieved HVs from multi-organ donors (MODs). Of these 19, 15 also retrieved HVs from tissue-only donors (TODs) and 8 TEs also retrieved HVs from live donors (LDs) (the latter are patients undergoing a domino-donor operation during a heart transplant).

The number of HV donors in each TE is shown in Fig. 1. The TEs vary significantly in size, ranging from as few as 6 donors in 2015 to a maximum of 320 donors in the same year. The majority of HV donors are MODs, followed by TODs and a smaller number of LDs.

Donor age limit for HV donation

The upper age limit for HV donation varied from as low as 40 years for both aortic valves (AVs) and pulmonary valves (PVs) in one TE, to a cutoff of 70 years for both AVs and PVs in two TEs, with the rest of the TEs having a cutoff of 60 years or 65 years for AVs and/or PVs. HVs were not collected from donors above these age limits from any of the TEs that took part in this survey.

Proportion of HVs discarded

The TEs reported a significant discard rate (for all reasons) of both AVs and PVs, with a median discard rate for AVs of 43% from TODs and 50% from MODs and a median discard rate for PVs of 32% from TODs and 20% from MODs.

Causes of HV discard

The main causes (data not shown) of HV discard included microbiological contamination (i.e. bacterial and/or fungal contamination—variable types of contaminants are identified from the skin, airway, gastrointestinal tract or the environment, including *Coagulase Negative Staphylococci*, *Streptococci*, *E. coli* and *Pseudomonas* amongst others), anatomical abnormalities and medical deferrals. Other less frequent causes of discard included damage at retrieval, damage at dissection, blood-borne virus (BBV) positivity and other miscellaneous causes.

The discard rate due to microbiological (bacterial and/or fungal) contamination of HVs from TOD and HVs from MOD in the same TE has been compared—Fig. 2. While the microbiological contamination rate is higher in TOD in TEs C, H, I, N and O, the reverse is true in TEs A, B, L, M and R with no difference in TE G; caution is however required in interpreting this data as some TEs are very small with very

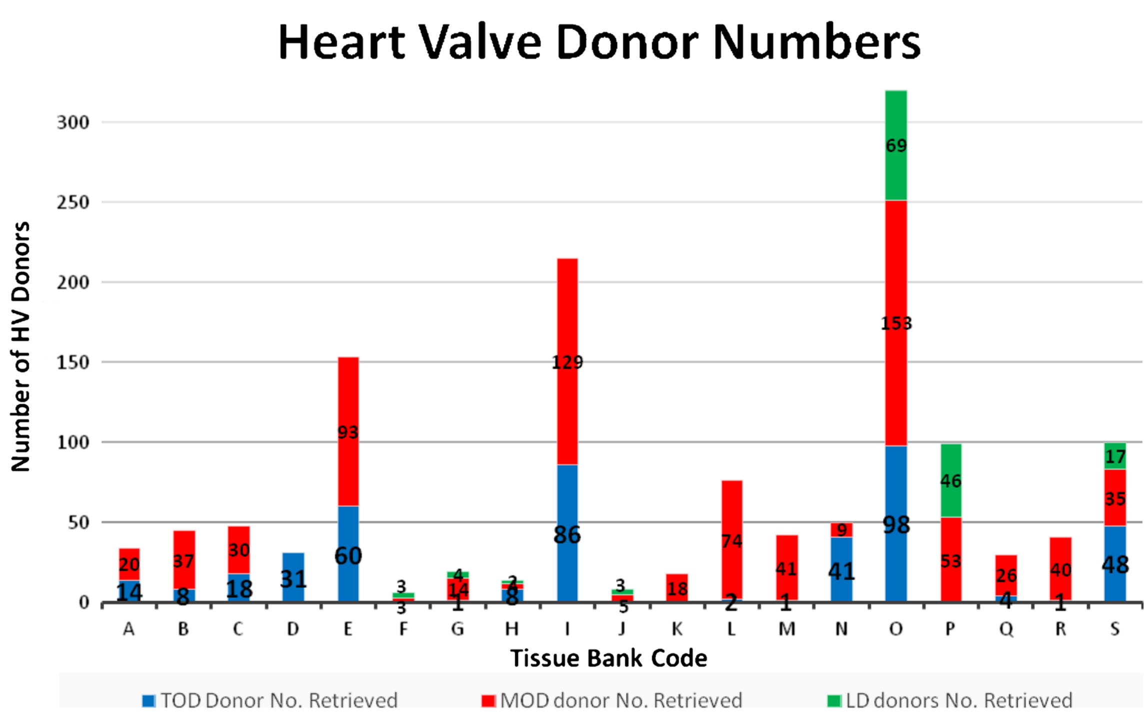

Fig. 1 Donor numbers in the different TEs in 2015

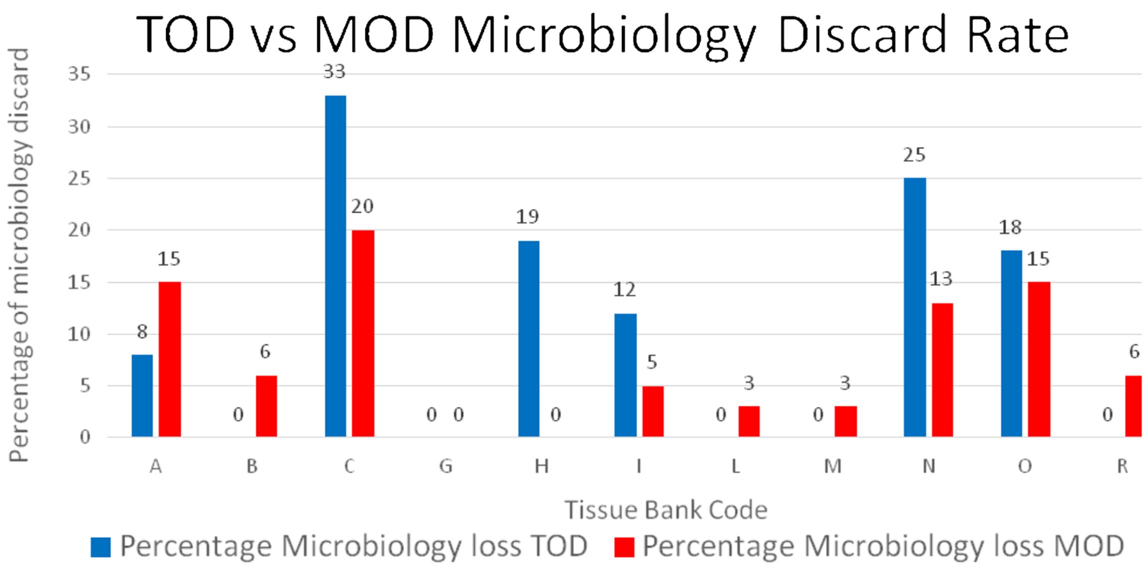

Fig. 2 Comparing the rate of microbiological contamination of HVs from MOD donors and TOD in individual TEs that bank HVs from both types of donors

few donors in a given year (refer to Fig. 1) so that results could easily vary from 1 year to another.

Donor suitability assessment

The TEs were asked to provide information as to whether potential tissue donor suitability is assessed pre- or post-donation. Not all TEs provided information for this part of the questionnaire. Of the 13 TEs that did provide information, 12 carried out some form of pre-donation assessment. One TE did not perform detailed pre-donation assessment relying instead on blood-borne virus testing and post-mortem results to confirm the suitability of the donor.

The TEs were asked whether they would routinely go through the donor medical notes, contact the donor family doctor and obtain post-mortem results as part of the donor suitability assessment. Not all the TEs provided responses for these questions, however, of the responses received, 8 TEs reviewed the medical notes while 4 did not; 7 TEs contacted the donor family doctor while 5 did not and 10 TEs reviewed the post-mortem results while 1 did not.

Blood-borne virus testing

All TEs carried out testing for BBVs, although there was variation between the different TEs—Table 1.

Heart retrieval for valve donation

The TEs indicated that heart retrieval for HV donation from MODs was always carried out (or supervised) by a medical doctor in a hospital theatre, in almost all cases at the end of other organ retrieval.

On the other hand, heart retrieval from TODs was carried out by varying groups of staff including doctors, nurses and/or laboratory staff; a doctor was involved in the retrieval process in the majority of TEs, although in one TE, the heart was retrieved by a team of trained nursing and laboratory staff and in another TE heart retrieval was carried out by trained laboratory staff only. In both organisations, a responsible medical doctor was on call and could be consulted 24/7. When the donor was a TOD, the heart tended to be retrieved as the first or one of the first tissues during the tissue retrieval process by almost all TEs.

The venue for tissue retrieval from TODs varied and included mortuaries, hospital theatres, undertakers and also dedicated tissue retrieval suites. In this data-gathering exercise, there was no correlation between the venue used for tissue

Table 1 Details of blood-borne virus testing carried out

Testing		Number of TEs
Hepatitis B	HBsAg	19/19
	Anti-HBc	18/19*
	Anti-HBs	6/19
	Anti-HBe/HBeAg	1/19
	NAT	16/19
Hepatitis C	Anti-HCV	19/19
	NAT	16/19
HIV	Ag/Ab test	16/19
	Anti-HIV	3/19
	NAT	16/19
Syphilis	Antibody assay	18/19
HTLV	Anti-HTLV I/II	13/19

*1/19 not carrying out anti-HBc testing is a non-European TE retrieval and the discard rate secondary to microbiological contamination (data not shown).

Heart retrieval from MODs took place as part of the organ donation process and as such was done very soon after the time of cardiac arrest/cross-clamp. This was different to heart retrieval from TODs. In the case of TODs, retrieval tended to take place several hours after the time of cardiac arrest with different TEs having different criteria for acceptable time limits—Table 2. From the information provided, there was no clear correlation between the retrieval timings and the rate of microbiological discard rate.

Ten TEs rinsed the heart at retrieval; six did not, while one TE had a variable practice, rinsing the heart from LDs but not from MODs. The rate of discard secondary to microbiological contamination was similar between TEs that rinsed the heart at retrieval (microbiological discard rate 2–23%) and those that did not (microbiological discard rate 0–20%), although there was a trend for a lower microbiological discard rate for TEs that did not rinse the heart at retrieval.

HV processing

Practice varied as regards how quickly after retrieval the HVs were processed: six TEs processed the HVs immediately, seven TEs had a 24 h cutoff and the rest had a variable longer cutoff for HV processing up to a maximum of 72 h. All TEs indicated that the time cutoff indicated for HV processing was the maximum acceptable, with the HVs being processed as quickly as possible. The microbiological discard rate for the three groups was broadly similar with no significant difference: 0–25% (TEs processing the HVs immediately), 0–20% (TEs processing the HVs within 24 h) and 3–23% (TEs processing HVs beyond 24 h).

HV decontamination

All the TEs decontaminated HVs; however, the practice was very variable. The antibiotic cocktails used varied significantly; combinations of 2, 3, 4 or even 5 antibiotics were used and most but not all included antifungals (data not shown).

The protocol followed during HV decontamination also varied significantly. The majority of TEs carried out decontamination at 4 °C, five TEs decontaminated at room temperature and only two TEs carried out decontamination at 37 °C—Table 3.

The duration of antibiotic decontamination was also very variable, from as short as 6 h in some TEs to a maximum of 48 h in other TEs.

Fourteen of the TEs rinsed the HVs after decontamination while five did not. This aspect of HV decontamination was subject to a detailed separate analysis.

Microbiological testing during HV processing

The samples taken and the culture methods used to detect microbiological contamination of the HVs varied significantly between different TEs—Table 4.

All TEs took a tissue sample for culture, eleven cultured the transport fluid, less than half cultured a sample of the rinse fluid, and fewer still took swabs, or cultured a sample of the cryoprotectant or the antibiotic solution.

The culture techniques used were very variable, varying from culturing in TSB (Tryptic Soy Broth, a nutritious medium for detecting aerobic bacteria) and thioglycolate (a broth suitable to detect anaerobic/facultative anaerobic bacteria) for 2 weeks at room temperature or 32 °C, to culture in medium 199 (a medium that is considered unsuitable to identify the

Table 2 Retrieval timings for HV donation from tissue-only donors

TE code	Retrieval timings		TOD microbiology discard rate: (total no. retrieved) % discarded
	Donor refrigerated	Donor not refrigerated	
B	< 6 h	< 6 h	(7) 0%
G	< 4 h up to 24 h	n/a	(1) 0%
C	< 6 h up to 24 h	12 h	(36) 33%
R	< 6 h up to 24 h	15 h	(2) 0%
O	< 6 h up to 24 h	n/a	(196) 18%
A, I	< 6 h up to 48 h	12 h	(25) 8%, (132) 12%
Q	< 6 h up to 48 h	24 h	(1) 0%
D,M	< 6 h up to 48 h	n/a	(53) 0%, (2) 0%
L	< 10 h up to 24 h	13 h	(4) 0%
N	< 12 h up to 24 h	15 h	(73) 25%
H	up to 24 h	15 h	(16) 19%

Table 3 Temperature of antibiotic decontamination

TE code	Temperature of decontamination	Percentage HV microbiology loss TOD + MOD + LD
B, C, D, F, H, I, J, K, L, O, P, S	4 °C	5, 25, 0, 0, 11, 8, 20, 11, 3, 13, 5, 2 (%)
E, G, M, Q, R	22 °C	3, 0, 3, 6, 1 (%)
A, N	37 °C	13, 23 (%)

presence of contaminants) for 18 h only at 4–7 °C, and a significant variation in between.

Only 8 of the 19 TEs cultured the HVs for the presence of tuberculosis (TB). The duration of TB culture varied significantly from as short as 48 h (one TE), to 2 weeks (one TE), 2 months (five TEs) and one TE culturing for TB for 3 months.

Ten TEs carried out environmental monitoring during HV processing while nine did not.

Discussion

This data-gathering exercise was an excellent collaborative venture between EBA T&C WG and the Foundation of European Tissue Banks (FETB). Despite the level of detail requested in the questionnaire, there was an excellent response, indicating that individuals involved in HV banking were keen to examine the process being followed.

The upper age limit for HV donation varied significantly in the different TEs. When the donor age limit was compared to the individual TE's discard rate for anatomical reasons and medical reasons, there was no correlation (data not shown). The TEs with the lowest and the highest age limits did not have a significantly different discard rate for either anatomical or medical reasons. The TE with the lowest age cutoff for HV donation may wish to consider increasing the age cutoff used, if there is an unmet clinical demand. Clinical feedback on the suitability of HVs from older donors is required; this may allow the donor age limit to be safely increased.

Not all TEs provided information on the type of donor assessment carried out. Some TEs were in fact not responsible for the pre-donation part of the process. From the data provided, all but one of the TEs indicated that there was an assessment of donor suitability done prior to proceeding with tissue retrieval. Further detailed comparisons of the type/level of detail of pre-donation donor assessment that is carried out are required to establish whether this has a significant impact on HV discard rate.

Table 4 Microbiological samples taken during HV processing

TE code	Tissue sample	Rinse fluid	Transport fluid	Swabs	Cryoprotectant	Antibiotic Soln.	Percentage HV microbiology loss TOD + MOD + LD
A	Yes	Yes	Yes	No	Yes	Yes	13%
B	Yes	Yes	Yes	No	Yes	No	5%
C	Yes	Yes	Yes	No	No	No	25%
D	Yes	No	No	No	No	No	0
E	Yes	No	No	No	No	No	3%
F	Yes	No	No	No	Yes	No	0
G	Yes	No	No	No	Yes	No	0
H	Yes	Yes	Yes	No	Yes	No	11%
I	Yes	No	No	Yes	No	No	8%
J	Yes	No	Yes	Yes	Yes	Yes	20%
K	Yes	Yes	Yes	Yes	No	No	11%
L	Yes	No	Yes	No	No	Yes	3%
M	Yes	Yes	No	No	No	No	3%
N	Yes	Yes	Yes	No	Yes	No	23%
O	Yes	No	Yes	No	No	No	13%
P	Yes	No	Yes	No	No	No	5%
R	Yes	Yes	Yes	No	No	No	1%
S	Yes	No	No	No	No	No	2%

The detail of post-donation donor assessment also varied between the different TEs, with some TEs reviewing the medical notes and contacting the donor family doctor and practically all TEs indicating that they also reviewed the post-mortem results. Only one TE indicated that rather than carrying out a detailed donor history, they relied on BBV testing and post-mortem results to establish the suitability of potential HV donors.

What is unclear from the information available is whether there was any difference in HV safety assessment between TEs that carried out very detailed potential donor assessment and those TEs that carried out a less detailed one, in particular, if post-mortem results were routinely reviewed. Further information as regards any serious adverse events/reactions in recipients is needed to establish whether the level of donor assessment detail that is carried out has an impact on product safety.

Further, it is unclear whether the acceptance criteria between different TEs varied. It is quite possible that some TEs may be declining donors and/or discarding HVs that may be accepted by other TEs. Certainly, it would be important to try and compare acceptance criteria between different TEs with the aim of standardising them. This will require further detailed work.

From the data provided, the main reasons leading to HV discard included microbiological (bacterial and/or fungal) contamination, medical reasons and anatomical reasons. It would be important to try to establish whether the medical and anatomical reasons leading to HV discard are being applied uniformly by all TEs. It would also be important to investigate further the microbiological contamination discards to establish whether the HV banking process can be amended to reduce the rate of such contamination. Harmonisation of the procurement and dissection techniques used, as well as the training of personnel, together with a regular audit of the testing laboratories that TEs work with would help to decrease the discard rate of donated HVs by ensuring good uniform practice in all aspects of HV banking. This is subject to a separate study.

It is of note that only 7 of the 19 TEs reported HV discards secondary to BBV positivity in 2015 (data not shown). This may reflect either a higher prevalence of BBV positivity in the general donor population or a less detailed pre-donation donor assessment by these TEs. The latter certainly seems to be the case for at least one TE where 46% of all discards were due to BBV positivity—when contacted, TE D indicated that only a brief donor history is taken prior to tissue retrieval being progressed, relying instead on BBV testing and post-mortem results to assess the suitability of donated HVs. Further information, in particular, the presence or absence of serious adverse events and/or reactions in recipients, is required to assess whether such practice impacts on donation safety or not; the risk of accepting "higher risk" HV donors by TEs carrying out a less detailed donor assessment needs to be considered in the setting of the possibility of false-negative results when relying mainly on BBV testing results [2–4].

All TEs carried out BBV testing although different TEs used different assays. NAT (nucleic acid test) testing for HIV, hepatitis B and hepatitis C was carried out by most but not all TEs, and all except one non-European TE tested for anti-HBc antibodies. Lack of information in this data-gathering exercise about the potential rate of serious adverse events/reaction (particularly as regards transplant transmitted infection rate) from the different TEs means that we cannot comment on the suitability or otherwise of the different testing regimes, although the risk of a transplant transmitted infection should be reduced by NAT testing as the window period is significantly reduced [5].

The staff responsible for carrying out heart retrieval for HV donation varied. Unsurprisingly, heart retrieval from MODs always included medical staff; however, when the heart was being retrieved from a TOD, then the staff carrying out the retrieval included a varying combination of doctors, nurses and/or laboratory staff with one TE successfully carrying out retrieval using trained laboratory staff under medical supervision. There did not appear to be any correlation between the rate of HV discards and the type of retrieval team employed.

The order of heart retrieval varied between the different types of donors, with the heart often being the last organ to be retrieved from MODs, but the first or one of the first from TODs. It was of note that while most TEs had a higher microbiological contamination rate of HVs from TODs, this was not true in all cases (Fig. 2). Further information is required to be able to reach definite conclusions about this, as there were too many variables: venue of retrieval, the length of time after the circulatory arrest that tissue retrieval took place and the order of retrieval are all likely to have impacted the HV contamination rate. This level of detail was not part of this survey.

It is well documented that after cardiac arrest, organs and tissue may be contaminated by endogenous organisms due to the transmigration of organisms from within the body [6], although this has been refuted by more recent literature [7]. The length of time between circulatory arrest and tissue retrieval of HV was not an issue for MODs as heart retrieval took place soon after cross-clamp or circulatory arrest as part of the organ retrieval process. However, when HVs were being retrieved from a TOD, then retrieval took place at varying time points after the time of the cardiac arrest. There was significant variation in the acceptable time limits that different TEs applied. There were too many variables that might have influenced the microbiological contamination rate of HVs to be able to draw definite conclusions from this data-gathering exercise, although there was a trend that the TE with the longest warm ischaemic time (12 h after cardiac arrest) had one of the highest microbiological contamination rates of

HVs, suggesting that cooling the donor body as soon as possible is important [8, 9].

Several of the TEs did not rinse the heart at the time of retrieval. The numbers in this data-gathering exercise are too small to reach definite conclusions; however, there was a trend for a lower microbiological contamination rate of HVs in the TEs that did not rinse the heart at retrieval, something worth exploring further. Heart rinsing at the time of retrieval is carried out to remove any contaminants already present at the time of heart retrieval and also to remove any blood clots as the latter are thought to provide a good medium for bacterial growth [10]; however, it is possible that the technique used for heart rinsing may have had an adverse impact on microbiological contamination.

Antibiotic decontamination protocols in use to decontaminate HVs varied significantly between the different TEs both in this data-gathering exercise and as previously reported by other authors [11–15]. The antibiotic cocktails used, the temperature at which decontamination was carried out and the lengths of time HVs were kept in antibiotics varied widely between different TEs. In the absence of further information as regards the presence or not of serious adverse events in recipients due to the presence of residual antibiotics, it is impossible to comment whether the different protocols used were all equally effective in ensuring the microbiological safety of the HVs. This needs to be investigated further to try and establish the best practice as regards HV decontamination.

The number and type of cultures taken and the culturing methods used also varied significantly between different TEs. It is highly likely that such different techniques would have led to different sensitivity in detecting contamination. Hence, a low microbiological contamination rate quoted by some TEs could be due to low sensitivity in identifying contamination rather than a genuinely low contamination rate [16]. Caution therefore needs to be exercised when interpreting the rate of microbiological contamination in isolation without attention to the numerous variables that may be influencing that figure, including the potential of false-negative culture results due to antibiotic treatment given to the donor in the hours prior to death and also the antibiotics used to decontaminate the HVs during processing [17, 18]. This requires, further, more detailed work. It is important for individual TEs to be sure that the samples taken and the culture techniques used are sufficiently sensitive (using enriched culture media) to detect clinically significant contamination.

It is worth noting that only eight of the nineteen TEs cultured for tuberculosis (TB). Further, the length of culture for TB varied significantly between TEs. This is something that should be relatively straightforward to standardise. Testing HV donors for the presence of undiagnosed TB is important [19]. Culturing for TB for 48 h or even 2 weeks is considered insufficient. Longer culture duration is required using tissue samples in specific culture media that have been shown to be suitable for the identification of the presence of TB. An expert microbiological opinion should be sought to ensure that TB cultures are done using appropriate culture media that is incubated for a sufficient length of time.

Further, only just over half of the TEs carried out environmental monitoring during HV processing. The TEs that carried out environmental monitoring indicated that the environmental monitoring results were taken into account when deciding whether a particular HV would be released for transplant or not. Without carrying out environmental monitoring during HV processing, it is impossible to assess whether the GMP (good manufacturing practice) environment has been maintained as required during the processing, leading to concerns of the product safety [16].

Conclusions

This data-gathering exercise has highlighted that HV retrieval and processing are very variable with significant differences in processes between different TEs. There is a significant discard rate of HVs and many aspects of HV banking can and should be standardised through further collaborative work between TEs.

One weakness of this data-gathering exercise is the lack of information on the serious adverse events and/or reaction rates in recipients from the different TEs. Tissue vigilance should be encouraged by all TEs and clinicians to enhance the quality of the information available.

There are many aspects of HV banking that can be investigated further with the aim of validating and standardising practice. The main findings of this data-gathering exercise highlighted the significant variation in practice throughout the HV banking process—from the selection of the donor right through to the storing of the final product. All these factors need to be evaluated in a systematic manner to establish best practice. Following on from this initial exercise, we also looked in greater detail at the microbiological testing and decontamination protocols used by different TEs during HV banking. As a result, we organised a HV quality round in 2017 as a first step in establishing an external quality assessment scheme for HV banking, focusing in particular on the microbiological aspects of HV banking. The results of this quality round will be discussed in a separate publication.

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Cardiovascular Tissue Establishment of Milan
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NHS Blood and Transplant, Tissue and Eye Services, England
Oxford Heart Valve Bank
Scottish National Blood Transfusion Service, Tissue, Cells and Advanced Therapeutics
Thai Red Cross Organ Donation Centre
Tissue Establishment Lund, Sweden
Transplant Tissue Centre, Singapore

Compliance with ethical standards

We can confirm that this work has been carried out in compliance with Ethical Standards. There is no conflict of interest for any of the authors. Ethical approval was not required. Informed consent is not relevant for this work.

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