European Journal of Clinical Microbiology & Infectious Diseases (2019) 38:1481–1490
https://doi.org/10.1007/s10096-019-03576-1

ORIGINAL ARTICLE

Validation of microbiological testing in cardiovascular tissue establishments: results of a second international quality-round trial

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Received: 11 January 2019 / Accepted: 30 April 2019 / Published online: 12 May 2019

Abstract

The performance of many laboratories can be evaluated by participation in external quality assessment (EQA) schemes. EQA allows for comparison of a laboratory's performance with a source outside the laboratory—either a peer group of laboratories or a reference laboratory. Such EQA schemes do not exist in tissue banking despite the fact that tissue establishments (TE) perform very complex procedures. This paper describes the first ever EQA scheme in the field specifically assessing microbiological aspects in heart valve (HV) banking. Twenty-two TEs participated. Three HV tissue samples were sent to each participating TE—two contaminated with non-pathogenic micro-organisms and a third negative control. The aims were to isolate and identify the micro-organisms present and then to successfully decontaminate the HV tissue using the routine standard operating procedures of the TE. Eight of the TEs were able to isolate and identify all contaminating micro-organisms present, and of these, five also successfully decontaminated the tissue; 13 TEs failed to establish the identity of one or more of the contaminants; five TEs appear to have introduced contamination during the processing or testing of the tissue; and eight failed to successfully decontaminate the HV tissue. This initiative provides TEs with an international benchmark for tissue product microbiology testing. It has identified significant variation in practice and in the ability of different TEs to identify the presence of contamination. There is now work ongoing with the aim of setting up a regular EQA scheme for HV banking.

Keywords Heart valves · Banking · Transplantation · Microbiology · Contamination

Introduction

For the cardiothoracic surgical community, organ and tissue donation and transplantation are an important aspect of medical care. Donated tissues are regularly transplanted each year, providing life-saving (e.g. heart valves (HVs), arterial grafts) and life-enhancing (e.g. veins, pericardium) treatment for thousands of patients [1].

Previous work by this group in 2016 identified significant differences in the methods used by Tissue Establishments (TEs) around the world in all aspects of HV banking, from donor selection and testing, tissue processing, and HV handling before implantation. It is unclear whether such variations in methodology are materially important to the safety and clinical effectiveness of the HVs.

It was therefore decided to investigate in more detail microbiological aspects of HV banking, including the microbiology testing and decontamination protocols used.

The method used to send out HV tissue purposefully contaminated with micro-organisms to different TEs around the world for them to isolate and identify the contaminating micro-organisms is based on the previously validated and published work of two of the authors (2,3).

Methods

Thirty TEs from around the world were contacted, of which 25 (21 European, 4 Non-European) participated. HV tissue for this project was sourced from the European Homograft Bank (EHB) in Brussels. All donations were anonymised and had

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the necessary consent. All HVs used were confirmed to be sterile on microbiological culture. The HVs were cut into strips to ensure sufficient HV tissue for all participating TEs and split up into three separate groups, sample A, sample B, and sample C (see later).

Sample A was contaminated with *Sphingomonas paucimobilis* (Gram-negative rod); sample B was the sterile-negative control; and sample C was contaminated with *Roseomonas mucosa* (Gram-negative coccobacilli) and *Staphylococcus hominis* (Gram-positive cocci).

Three separate HV tissue samples were prepared for each TE, one each from samples A, B, and C. The preparation of the samples was done at the EHB, using a clean room facility. The samples were packaged in compliance with the European Directives for transportation of biomaterials (94/55EC). Two lots of samples were concurrently also sent to reference microbiology laboratories (one in Edinburgh and one in Rotterdam) as independent confirmation of the isolation and identification of the contaminating micro-organisms. The technique used was based on a previously published method that had been validated for different levels of contamination (high, medium, and low), the transport medium used, temperature of storage (room temperature), and transport duration (1 to 14 days) [2, 3].

The samples were sent out by registered delivery using a courier company that guaranteed delivery within 24 h for European destinations and within 72 h for non-European destinations. All participating TEs were asked to contribute a nominal charge to help cover the transport costs. The time of the organisers and equipment was provided free of charge.

Results were assessed against four microbiological criteria relevant in tissue banking: sampling methods, correct isolation and identification of micro-organisms, culture methods, and decontamination protocols. Any deviations from these principles will be discussed.

Results

Receipt of samples

Delivery of most samples was delayed, with only two samples being delivered within 24 h within Europe, and a longer delay for the non-European samples, the maximum delay being 6 days. Four of the TEs also reported some damage and leakage of one or more of the sample tubes delivered. Of the 26 samples sent out, 24 were safely delivered to 23 TEs (one TE requested 2 sets of samples), whilst two samples were never received (held up at customs).

Most TEs managed to process the samples as soon as they were received. However, four TEs delayed processing the samples by 3 days, and three delayed processing by 5, 10, and 23 days respectively for operational reasons. Twenty-two TEs completed the exercise and returned their results.

Microbiology reference laboratory results and interpretation

Sample A

The reference laboratory in Rotterdam received the tissue samples within 24–36 h and processed them immediately. Using Bactec and then subculture onto plate culture media, they were able to successfully isolate and identify the *Sphingomonas paucimobilis*; however, on repeat sampling after 72 h and again after 2 weeks, there was no growth of the micro-organism. The micro-organisms were still identifiable by Gram stain. This laboratory also tested the sample for the presence of antibiotics and found it to be positive.

The reference laboratory in Edinburgh received the tissue samples after 48 h. Using chocolate and blood agar culture techniques, they were not able to culture, isolate, and identify the *Sphingomonas paucimobilis*. On reviewing these results, it was concluded that the presence of antibiotics in sample A was interfering with the growth of the *Sphingomonas paucimobilis* so that an acceptable result for sample A would be either “no growth” or identification of the *Sphingomonas paucimobilis*.

Sample B

Both microbiology reference laboratories confirmed that this was a sterile sample. This sample was confirmed as a suitable negative control for this project.

Sample C

Both microbiology reference laboratories confirmed the presence of both *Roseomonas mucosa* and *Staphylococcus hominis*.

The Rotterdam microbiology laboratory again checked for the presence of antibiotics, and whilst there were antibiotics present in this sample, they were not interfering with the growth of either of the two contaminating micro-organisms. This sample was therefore suitable for this project.

Results of participating TEs

The number and type of culture samples taken to look for potential contamination pre- and post-decontamination varies between the different TEs.

A summary of the pre-decontamination samples taken for culture by the 22 different TEs is shown in Table 1. At the pre-decontamination stage, most ($n = 19$) TEs took tissue biopsies for culture, often ($n = 12$) combined with a transport-fluid

Table 1 Summary of the pre-decontamination samples taken by TEs for culture

Number of TEs	Tissue biopsy	Transport fluid	Antibiotic cocktail	Swabs	No samples taken
11	+	+			
1	+	+	+		
1	+			+	
6	+				
1		+			
2					+
Total: 22	19	13	1	1	2

sample or other sample (swab/antibiotic cocktail ($n = 2$)). Six TEs took only tissue biopsies and 1 TE sampled only transport fluid. Two TEs did not routinely take any pre-decontamination samples.

Table 2 summarises the culture samples taken post-decontamination by the different TEs. Such samples serve as a final check for the potential presence of contamination. Twenty TEs sampled the cryoprotectant in which the HVs were preserved, and 15 combined that with tissue biopsies; one TE took a tissue biopsy and sample of the antibiotic cocktail and one TE only sampled the antibiotic cocktail.

Pre-decontamination culture results

A summary of the pre-decontamination culture results from the individual TEs is presented anonymously in Table 3—each TE is represented by a number assigned randomly. Table 3 provides details where the result returned by the TE is not the expected result, i.e. TEs that appear to have contaminated their samples and TEs that did not manage to isolate and identify the micro-organisms present.

Decontamination protocols used

The antibiotic cocktails used and the temperature and duration of decontamination varies significantly between different TEs, with no two TEs using the same protocol. A summary is provided in Table 4. It is worth noting that all TEs use antibiotic cocktails consisting of at least three different antibiotics from different antibiotic classes.

Post-decontamination microbiology culture results of participating TEs

Having decontaminated the HVs using their standard protocol, the TEs were asked to repeat microbiology culture of the HVs to check whether the HVs had been successfully decontaminated.

All TEs reported “no growth” post-decontamination of the HVs in sample A and sample B. Only 12 TEs reported “no growth” post-decontamination of the HVs in sample C. One TE (4) did not carry out post-decontamination testing of sample C due to the level of bioburden detected on the pre-decontamination cultures for this sample, which led to automatic discard of this HV. Eight TEs reported positive culture post-decontamination, i.e. failed to successfully decontaminate the HV tissue—please refer to Table 5.

Culture media used by participating TEs

The participating TEs were asked to provide details of the culture media used to isolate any contamination that may be present. There is significant variation in the type of media used and also in the protocols used when culturing tissue samples by the different TEs. Table 6 provides a summary of the ability of the different culture media used by the different TEs in

Table 2 Summary of the post-decontamination samples taken by TEs for culture (final check for potential of contamination)

Number of TEs	Tissue biopsy	Cryoprotectant	Antibiotic cocktail	Rinsing fluid
12	+	+		
2	+	+	+	
1	+	+		+
1	+		+	
2		+		
2		+		+
1		+	+	
1			+	
Total: 22	16	20	5	3

isolating the different types of micro-organisms that may be present. All TEs use culture techniques that are able to isolate aerobic and anaerobic bacteria present, although 12 TEs use culture techniques that would not support the growth of fastidious or nutritionally deficient micro-organisms. Nine TEs do not use culture techniques that are able to identify the presence of fungi.

Discussion

Both the data-gathering exercise in 2016 carried out by the same group on HV banking, and this EQA in 2017, have been met with enthusiasm by TEs around the world with the number wishing to participate and eventually participating exceeding our expectations.

The courier company chosen to deliver the samples to the TEs unfortunately failed to live up to their guarantees of delivery of samples. However, from the results reported, the delay in sample delivery did not appear to have adversely affected the ability of TEs to correctly isolate and identify the micro-organisms present even when the sample was not processed until day 23 (TE 5) after preparation. The TEs reporting damage to the sample tubes did not have an issue with contamination—in fact, none of the TEs who appear to have contaminated their samples reported any damage to their sample tubes.

Sampling error (i.e. failure to identify contamination that is present) in tissue banking is high (approximately 50%) if testing for the presence of contamination is done through swabs and tissue biopsies only [4–6]. The sensitivity for isolating a contamination that is present can be increased to > 90% if samples of the filtered transport fluid are also tested as well as a tissue biopsy [7, 8]. Most TEs in fact do sample both tissue biopsies and transport fluid. However, this is not true of all TEs. In this EQA 6 TEs only tested tissue biopsies, whilst one TE did not routinely take any pre-decontamination samples for culture. When deciding whether a particular HV is safe for clinical use or not, it is important to be aware of whether there was any evidence of contamination on the tissue prior to attempting decontamination and the ID of such contaminating micro-organisms. HVs that have evidence of significant degrees of contamination particularly if contamination is with highly virulent micro-organisms that may be difficult to eradicate with antibiotics should be discarded irrespective of the culture results in the post-decontamination stage. There are a number of publications which underline the risk of false negative culture results (i.e. HV still contaminated with undetected micro-organisms) post-decontamination of tissue products when the pre-decontamination culture results are not taken into account [9–11].

In the post-decontamination stage, as a final sterility test of the HV tissue, most TEs take a sample of the cryoprotectant (DMSO) to check for the potential presence of contamination. DMSO has a toxic effect on micro-organisms and may therefore interfere with the successful isolation of contamination present leading to false-negative culture results similar to the [12] effect of antibiotics, unless cultures are prolonged (2 weeks) and in validated antibiotic-neutralising media. In order to minimise the risk of such false-negative culture results in the post-decontamination stage, it is important to include other samples besides the cryoprotectant when checking the sterility of the product, including tissue biopsies and rinsing fluid.

In this EQA, potential contamination of the HV tissue from the donor or during the retrieval process is not relevant as the HV tissue used was known to be sterile and was “processed” in a controlled clean room environment before being artificially contaminated. In view of this, if a TE has reported the presence of micro-organisms other than the ones used to purposefully contaminate the HV tissue as part of this EQA, then these micro-organisms must have been introduced either during the HV processing by the individual TE or during sample culturing by the microbiological laboratory. From information provided in the previous data-gathering exercise that was carried out in 2016, TEs indicated that they process HVs in a grade A environment with either a grade B or grade C background (i.e. in controlled environments with laminar flow; refer to Eudralex Volume 4, Annex 1) [13]. This means that the risk of contamination during HV processing by the TE is low and usually by skin-flora from the operators, although cross-contamination remains possible. On the other hand, contamination of the culture samples in the microbiology laboratory, either from the personnel or from the environment, is more likely. Microbiology laboratories may use open environments and non-sterile benches; the technicians processing the samples work in uncontrolled environments (e.g. near water taps), with no laminar flow or air-particle monitoring [14–17].

Sample A was contaminated with *Sphingomonas paucimobilis*. However, it was retrospectively identified that in sample A there were residues of antibiotics that were interfering with the growth of the micro-organisms and even potentially killing the *Sphingomonas*. In view of this, this micro-organism could only be isolated if sample culture was carried out within 24 h of the tissue samples being prepared, although the Gram-negative rods could be identified microscopically for several days later, and whilst these Gram-negative rods were not formally identified, it is felt likely that they were the same micro-organism rather than a new contaminant. This means that cultures from sample A taken more than 24 h after the samples were prepared would likely result in a negative culture result, as demonstrated and confirmed by the two microbiology reference laboratories. Any bacteria other than *Sphingomonas* isolated by the TEs at more than 24 h from sample preparation must be a contaminant introduced by the TE or the microbiology testing laboratory.

Of the three TEs reporting a positive culture result for sample A, none of them reported damage to the sample tubes during transport. One of these 3 TEs reported the presence of a Gram-negative rod (likely the *Sphingomonas*) but did not proceed to identify it. This was a correct result (cultured at < 24 h), and not a contaminant as the TE thought, and therefore considered a correct result for the purposes of this EQA, although ideally the micro-organism should have been fully identified. One TE reported the presence of a *Bacillus* species; the source of this contamination is likely to be *Bacillus* spores found in the environmental air; this type of contamination is most likely to have taken place in the microbiology laboratory. The third TE reported the presence of *Staphylococcus cohnii*, a skin commensal mostly found on the lower limbs that is likely to have originated from the personnel handling the specimens, with the microbiology laboratory being the most likely source.

None of the TEs checked for the presence of antibiotics, which raises concern about the possibility of false-negative culture results. Many donors will have received antibiotics before death which can lead to false-negative culture results, unless the samples are cultured within a few hours in a blood culture medium that contains toxin and antibiotic-neutralising resins. However, most TEs rinse their tissue several times to remove any antibiotics that may be present, although most TEs have not confirmed or validated the effectiveness of removing or neutralising the antibiotics using this method.

Sample B was a negative control sample, also confirmed by both reference microbiology laboratories. Therefore, any bacteria isolated in this sample would be a contaminant introduced either by the TE or the microbiology laboratory. Twenty of the 22 TEs reported "no growth" for this sample, whilst the remaining two TEs reported positive culture results: one TE (11) identified the presence of *Staphylococcus hominis*. This same TE reported "no growth" for sample C (which contained *Staphylococcus hominis*) leading to the possibility that this TE may have swapped their culture samples. The second TE reported the presence of *Propionibacterium acnes*, a skin commensal which may have been introduced as a contaminant either by the TE or the medical microbiology laboratory.

Sample C was contaminated with *Roseomonas mucosa* and *Staphylococcus hominis*, also confirmed by both reference microbiology laboratories. Eight TEs isolated and correctly identified both contaminating micro-organisms (although one TE did not fully identify the *Staphylococcus hominis*, reporting the presence of coagulase negative *Staphylococci* (CNS) instead); these samples being processed between 48 and 552 h after the samples were initially prepared.

A further seven TEs identified only the *Roseomonas* spp. in their culture; these samples were processed between 48 and 192 h after they were initially prepared (16: 48 h; 20 and 21: 72 h; 1, 12, and 18: 144 h; 15: 192 h). A possible reason for these TEs not identifying the *Staphylococcus hominis* could be heavy *Roseomonas* growth (reddish colonies) on the culture plates, which would make recognition of the *Staphylococci hominis* (small yellow-orange colonies) difficult.

One TE correctly identified the presence of the *Roseomonas* spp. in sample C but also reported the presence of *Bacillus pumilis*; the only likely source of this contaminant are spores from the environmental air, most likely from the diagnostic microbiology laboratory. The presence of the *Bacillus pumilis* and *Roseomonas* are likely to have made identifying the *Staphylococcus hominis* also present difficult.

One TE reported the presence of *Staphylococcus schleiferi* for sample C; this is either a wrong identification or a contaminant from the microbiology laboratory given it is not a common CNS.

One TE reported the presence of *Staphylococcus hominis* together with *Stenotrophomonas maltophilia* for sample C. The latter could either be a wrong identification or possibly a contaminant, most likely introduced by the medical microbiology laboratory, as this micro-organism is normally found in water systems and the microbiology laboratory is the only site during HV processing where there would be direct contact with a water system.

Four TEs reported "no growth" for sample C—one of these four did not routinely carry out pre-decontamination tissue cultures (although did culture the samples sent for the purposes of this EQA) and two of the TEs probably used media that were insufficient to identify all potential contaminants. The fourth TE may has swapped sample C with sample B, explaining their return of "no growth" for this sample (see earlier).

When considering the decontamination protocols used, the different TEs used very variable protocols with variable antibiotic cocktails at variable temperatures for variable lengths of time. The important question is whether the antibiotic cocktail used is likely to be effective against the skin, mucosal and environmental contaminants that are likely to be present on HVs.

In order to have effective antibiotic decontamination of HVs, the antibiotic cocktail used needs to have broad effectiveness against aerobic and anaerobic bacterial and fungal micro-organisms.

The concentrations of antibiotics used by the different TEs vary significantly. When compared with the doses normally used clinically to treat patients, all TEs were using very high concentrations of antibiotics. Only three TEs used antibiotic concentrations that did not exceed the dose used in clinical patients (i.e. the therapeutic dose) by threefold. The smallest antibiotic doses used by five of the TEs is around 10 times a therapeutic dose, and the remaining 17 TEs used antibiotic doses that were more than 20 times higher than therapeutic doses, in some cases up to more than 50 times higher. Such

Table 3 Summary of culture results submitted by the participating TEs

	TE code	Wrong/insufficient results	No. of TEs with correct results	No pre-decontamination sampling	Insufficient media	Other potential cause
Sample A			20[a]			
Contamination	2	*Bacillus* spp.				Possible air contamination ML[b]
	10	*Staph. cohnii*				TE or ML[b] contamination
Sample B			20[c]			
Contamination	11	*Staph. hominis*				Cross-contamination or sample B and C switched
	10	*Propionibact. acne*				TE or ML[b] contamination
Sample C			8[d]			
No growth	2	No growth		+		
	11	No growth			+	
	13	No growth			+	
	28	No growth				Unknown
Only one micro-organism identified	14	*Roseomonas* spp.				Possible ML[b] error
	16	*Roseomonas* spp.				Possible ML[b] error
	1	*Roseomonas* spp.				Possible ML[b] error
	21	*Roseomonas muc*				Possible ML[b] error
	20	*Roseomonas muc*				Possible ML[b] error
	12	*Roseomonas gilardi*				Possible ML[b] error
	23	*Roseomonas* spp.				Possible ML[b] error
One micro-organism identified + contamination	3	*Roseomonas* spp. + *Bacillus pumilis*				Possible air contamination ML[b]
	4	*Staph. hominis* *Stenotrophomonas*				Possible environmental contamination ML[b]
Contamination	10	*Staph. schleiferi*				Possible false identity ML[b]

TE, tissue establishment; *No.*, number; *ML*, microbiology laboratory

[a] One TE correctly identified the presence of non-fermentative Gram-negative rods in sample A but did not fully identify this micro-organism; the other 19 TEs reported “no growth” for sample A; 1 TE (13), concerned that they had been unable to identify any micro-organisms in the samples, also carried out a Gram stain of the samples (Gram stain not being part of their normal protocol)—the Gram stain identified the presence of Gram-negative bacilli in the sample. TE 13 reported that the samples had been received after 6 days and the tube of sample A was leaking

[b] ML—probable error (e.g. not recognised *Staph. hominis* amongst the *Roseomonas*) or contamination of the culture samples occurred in the diagnostic medical microbiology laboratory

[c] Twenty TBs reported “no growth” including TE 13 which again carried out a Gram stain of the samples (Gram stain not being part of their normal protocol), the Gram stain being negative on this sample, i.e. no micro-organisms present

[d] Eight TEs identified both the *Roseomonas* and the *Staph hominis*; TE 13 again carried out a Gram stain on the sample (Gram stain not being part of their normal protocol)—the Gram stain confirmed the presence of micro-organisms

doses are very difficult to rinse or neutralise leading to concerns about potential false-negative culture results after antibiotic decontamination and may have toxic effects on the tissue, something that is a concern for all antibiotics, but is a particular concern when using aminoglycosides and amphotericin [18] at high doses.

The temperature at which decontamination was carried out also varied between different TEs (4 °C, room temperature, or 37 °C). Bacteria that grow on humans, both commensal and pathogenic micro-organisms, are “mesophilic”, i.e. they grow best at a temperature of 20 to 40 °C. Below 20 °C, such micro-organisms grow much slower; their enzymes cannot mediate chemical reactions, so eventually, the viscosity of the cell-interior brings all activity to a halt. All human bacteria (and human cells) slow down and eventually die at low temperatures. Most bacteria do not grow at all, or minimally so, at 4 °C which means that betalactam antibiotics (all penicillins and cephalosporines) cannot be effective at low decontamination temperatures [19]. Even protein-attacking antibiotics are only effective against growing bacteria when protein synthesis is taking place and not at low temperatures. Any static/cidal effect on the micro-organisms at low temperatures therefore is not being caused by the antibiotics but by the temperature/medium and other factors in the liquid that the HVs are in.

Table 4 Summary of the decontamination protocols used by the 22 TEs including the following information: number of TEs using a particular class of antibiotics, the number of TEs carrying out decontamination at a particular temperature and the duration in antibiotics during decontamination, and how many TEs rinse off the antibiotics or not.

	Micro-organism cover of antibiotic cocktail				Temperature of decontamination			Duration in antibiotics			Rinsing of antibiotics	
	Gram +ve	Gram −ve	Anaerobic	Fungi	4 °C	22 °C	37 °C	12 h	24 h	48 h	Yes	No
No.* of TEs** with sufficient cover	19	14	9	13	15	3	4	4	14	4	15	7
No.* of TEs** with no cover	3 insufficient	8 insufficient	13	9								

Gram +ve—organisms that stain positively (blue) in a Gram stain (i.e. all staphylococci and streptococci). Gram −ve—organisms that stain negatively (red) in a Gram stain (i.e. all aerobic rods such as *E. coli*, *Klebsiella* spp., *Pseudomonas* spp. a.o.)

[a] No.—number

[b] TE—tissue establishment

The length of time the HVs are left in the antibiotic cocktail for decontamination also varied between the different TEs. Killing of bacteria is mostly done within the first hour of contact with antibiotics at 35 °C with no additional benefit beyond 6 h at this temperature [20]. This temperature has been shown to lead to a 100% antibiotic cidal effect for several antibiotic cocktails; this is not the case for decontamination carried out at 4 °C (10).

Post-decontamination, 13 TEs reported negative cultures, whilst 8 TEs reported persistently positive cultures with the same micro-organisms that had been present in the pre-decontamination cultures. The most likely cause for persistent growth after antibiotic decontamination is the lack of antibiotic activity at the low temperatures used during decontamination by seven of the TEs.

A further identified reason for failure of decontamination is that *Roseomonas* is resistant to cephalosporins, piperacillin/tazobactam, and polymyxin/colistin. Five of the 8 TEs that failed to successfully decontaminate the HVs use only polymyxin or polymyxin in combination with a cephalosporin. Further, one TE left the HVs in antibiotics for only 5 h (at 37 °C); this is possibly too short a period to be fully effective against slow-growing and slimy bacteria in tissue.

Finally, consideration needs to be given to the potential of residual antibiotics leading to false negative post-decontamination culture results. Hence, the importance of proper rinsing or neutralisation [21] before carrying out repeat tissue culture.

When selecting the media used to culture tissue samples, TEs need to ensure that the media will be able to isolate all potentially contaminating micro-organisms, including aerobic, anaerobic, and also fungal contaminants. Fastidious bacteria are common on skin, mucosa, and in the environment: for example *Corynebacteria* and *Propionibacterium* from the skin, *Stenotrophomonas* from the environment, and *Neisseria* and *Streptococci* from the mucosa, amongst others. In order to isolate such potential contaminants, it is necessary to use enriched media (such as blood culture media). Enriched media are also required in order to be able to successfully isolate many streptococci and other mucosal inhabitants as well as non-fermenting Gram-negative bacteria from the environment (Pseudomonas-like). A different medium needs to

Table 5 Culture results post-antibiotic decontamination for sample C for TEs that failed to fully decontaminate the HVs

TE code	Culture results	Temperature of decontamination (°C)	*Staph. hominis* Ab. coverage	*Roseomonas* Ab. coverage	Ab. incubation time (hours)	Possible reasons for positive cultures post-decontamination
1	*Roseomonas*	22	Sufficient	Sufficient	24	Low temperature
2	*Staph. hominis*	4	Sufficient	Dubious	12	Low temperature
6	*Roseomonas*	4	Sufficient	Dubious	24	Low temperature
8	*Roseomonas*	37	Sufficient	Sufficient	5	Too short for slow slimy-growing bacteria
11	*Rhodococcus equi* + *Staph. hominis*[a]	4	Sufficient	Dubious	40	Low temperature + wrong identification
16	*Roseomonas*	4	Sufficient	Dubious	24	Low temperature
19	*Roseomonas*	22	Sufficient	Sufficient	18	Low temperature
21	*Roseomonas*	4	Sufficient	Sufficient	24	Low temperature

Ab., antibiotic

[a] *Roseomonas* is a Gram-negative coccobacillus that is resistant to cephalosporins and piperacillin/tazobactam and also resistant to polymyxin. It can be difficult to differentiate *Roseomonas* from *Staph. hominis*

Table 6 Summary of ability of culture media used to identify different types of micro-organisms

TE[a] code	Gram +ve bacteria		Gram −ve bacteria		Anaerobes	Fungi
	Robust[b]	Fastidious[b]	Robust[b]	Fastidious[b]		
1	+	+	+	+	+	+
2	+	+	+	+	+	+
3	+	−	+	−	+	+
4	+	−	+	−	+	+
5	+	+	+	+	+	−
6	+	−	+	−	+	+
7	+	−	+	−	+	+
8	+	+	+	+	+	−
9	+	−	+	−	+	+
10	+	−	+	−	+	−
11	−	−	+	−	+	−
12	+	−	+	−	+	+
13	+	−	+	−	+	+
14	+	+	+	+	+	−
15	+	−	+	−	+	+
16	+	−	+	−	+	−
17	+	+	+	+	+	+
18	+	+	+	+	+	+
19	+	+	+	+	+	−
20	+	−	+	−	+	−
21	+	+	+	+	+	−
22	+	+	+	+	+	+
Total	21+/1−	10+/12−	22+	10+/12−	22+	13+/9−

[a] *TE*, tissue establishment

[b] Robust vs fastidious micro-organisms: robust micro-organisms are the ones that are able to grown on general media without needing the addition of special nutritional supplements or conditions, as different to fastidious micro-organisms

be added to allow growth of anaerobes (an oxygen free medium with extra iron). Blood culture media is also able to neutralise toxins and antibiotics that may be present [22, 23] so that growth of micro-organisms may be possible even when antibiotics are present in the tissue (although this still needs to be validated).

Contamination from the air, especially by spores, is very common. Since many such spores are fungal, culture at a lower (20 °C) temperature is required to isolate such micro-organisms. When looking for potential contamination of HVs, it is therefore important to use media that supports fungal growth to ensure no contamination of the tissue occurs during processing.

Table 6 provides a summary of the suitability or otherwise of the culture media used by the TEs in isolating the different types of potentially contaminating micro-organisms. As can be seen from this table, more than 50% of the TEs did not use culture media that would allow the isolation of fastidious bacteria or fungi. This would particularly be a concern if the antibiotic cocktail used for decontamination did not provide sufficient broad coverage that is effective against all types of micro-organisms, including against potential fungal contaminants. On the other hand, there are reports of positive bacterial results post-implantation without resultant clinical infection [24, 25], suggesting that low numbers of commensals (but not environmental micro-organisms and spores) that may be present on HVs are not necessarily detrimental to the recipient, although it is impossible to confirm this in a research trial, since avoidance of infection transmission remains the aim.

An important question to consider is whether culturing at temperatures other than 30–37 °C is necessary or not. Only fungi and bacteria that survive at human body temperature (30–35 °C) can be pathogenic. At temperatures < 30 °C, some micro-organisms die straight away. However, there remains a concern that toxins and spores from such micro-organisms can cause problems in the recipient, leading to the conclusion that TEs should aim to identify any potential contaminants that may be present to be able to minimise the risk to recipients as much as possible.

One weakness of this work is that almost no TE carry out recipient follow-up. It is important to establish whether the reported differences in the decontamination procedures used are leading to clinical problems or not.

Conclusions

This was an excellent collaborative initiative following on from our previous data-gathering exercise on HV banking. There is evidence of significant differences in practice between different TEs. Whilst a number of TEs were able to successfully isolate and identify all contaminating micro-organisms present and then successfully decontaminate the HV tissue, a fair number of TEs failed to isolate and identify one or more of the contaminants present due to the use of culture media that did not support all types of potential contaminants that may have been present or incorrect identification of the micro-organisms. Further, some TEs, or their microbiology laboratories, appeared to have introduced contamination during the processing or testing of their samples. Also, a number of TEs were unable to fully decontaminate the HV tissue, likely due to insufficient antibiotic cover and/or the incubation temperature at which decontamination was carried out, and none of the TEs checked for the residual presence of antibiotics.

Following on from this initiative, we now aim to work towards establishing a regular external quality assessment scheme for HV banking, and potentially other tissue products, with the aim of standardising tissue banking and improving the clinical safety of tissue products.

Acknowledgements We would like to thank all participating TEs for providing the information for this benchmarking exercise—in alphabetical order: Allograft Heart Valve Bank, Poland; Banco de Tecidos do Instituto Português do Sangue e da Transplantação; Banco de Tejidos Argentina; Banque de Tissus Humains, Paris; Bloemfontein Homograft Bank, South Africa; Blutzentrale Linz, Austria; Corliffe, OHG, Hannover Germany; Croatian National Homograft Bank; Establissement Francaise du Sang, Banque de Tissus et Cellules, Lyon; Establissement Francaise du Sang, Banque de Tissus et Cellules, Marseille; Euro Heart Valve Bank, The Netherlands; European Homograft Bank, Brussels; Fondazione Banca dei Tessuti di Treviso, Italia; Hema Quebec Tissue Bank, Canada; Homograftpankki, Finland; Institut fur Klin. Transfusionsmedizin, Braunschweig Germany; NHS Blood and Transplant, Tissue and Eye Services, England, UK; Oxford Heart Valve Bank, UK; Royal Brompton Heart Valve Bank, London, UK; Scottish National Blood Transfusion Service, Tissues, Cells and Advanced Therapeutics; The Tissue Bank, Irish Blood Transfusion Service; Tissue Bank Lund, Sweden.

Compliance with ethical standards

We can confirm that this work has been carried out in compliance with Ethical Standards.

Conflict of interest There is no conflict of interest for any of the authors.

Ethical approval was not required. All donated heart valves used for this project were ones that had been identified as being unsuitable for clinical transplantation where informed consent was also available.

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