

Microbiological examination of donated human cardiac tissue in heart valve banking

Jorge P. van Kats^{a,b,1,*}, Corina van Tricht^a, Alice van Dijk^a, Michel van der Schans^a,
Antoon van den Bogaerdt^a, Pieter L.C. Petit^c, Ad J.J.C. Bogers^a

^a Heart Valve Bank, Department of Cardio-Thoracic Surgery, Erasmus University Medical Center, Rotterdam, The Netherlands

^b Banco de Tecidos, Instituto Nacional de Traumatologia e Ortopedia (INTO), Rio de Janeiro, Brazil

^c Laboratory of Medical Microbiology, Vlietland Hospital, Schiedam, The Netherlands

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Abstract

Objective: Microbiological examination of donated human cardiac tissue is a necessary procedure for Heart Valve Banks to determine the biological safety of preserved allografts. Test protocols must be validated to prevent false-negative outcomes that pose a risk of infection to recipients of the tissue. The Heart Valve Bank in Rotterdam evaluated a validated, alternative entry test for donated tissues to compare the performance of its standard microbiological examinations. **Methods:** Samples of explanted heart transport medium from 275 donors were examined for the presence of microorganisms using blood culture flasks (standard test) and fluid thioglycolate medium (alternative test). Results were compared with the outcome of microbiological assessment of subvalvular myocardial fragments and the cryoprotective medium that were collected before and after treatment of the grafts with antibiotics, respectively. **Results:** Microorganisms, mainly skin flora, were detected in transport medium of 177 hearts (64%). The alternative validated culture method detected a growth in 80 transport medium samples that was not identified by the standard method. Microorganisms were only identified in the cultivated cardiac tissue fragments from 56 donors (20%). After antibiotic treatment of the tissue, microorganisms could still be encountered in cryoprotective medium samples from 55 donors (20%). Most of the contaminants in these final samples were identified as *Propionibacterium* species and *Corynebacterium* species and had already been detected in the transport medium by the alternative validated culture method. **Conclusions:** The use of blood culture flasks for microbiological assessment of non-blood liquid media and the cultivation of myocardial tissue fragments may hamper detection of certain microorganisms and therefore provide less complete information about microbiological safety. Heart Valve Banks may want to review their microbiological examination and decontamination procedures regarding the ability to detect and eliminate anaerobic skin flora, respectively.

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1. Introduction

Donated human heart valves and vascular conduits are successfully applied in reconstructive surgery of both acquired and congenital cardiac anomalies [1,2]. To ensure safety and quality of the donated tissues, a donor is first screened for a wide range of pathological entities that interfere with heart valve donation. Procurement of the heart is executed within a limited period after circulatory arrest and is followed by its transportation to a Heart Valve Bank (HVB), where the heart valves and vascular conduits are

dissected from the donated heart, processed and stored. Once serological, bacteriological and histological safety of the donor tissue has been ensured, a preserved graft may be released and subsequently applied in a surgical procedure of a recipient.

All through the procedures, aseptic techniques are applied and sterile materials are being used. Yet, it cannot be excluded that a tissue is or will become contaminated with bacteria and/or fungi that may originate from the donor itself, from the procurement environment or from the explantation staff [3–6]. Therefore, dissected heart valves and vascular conduits are treated with antibiotics to eliminate possibly present microorganisms. HVBs have developed or have adapted methods of antibiotic incubation for effective tissue decontamination [4,7,8]. These methods vary in composition and/or concentration of antibiotics and the temperature and length of incubation, and have in common that, following decontamination, a sample is tested for microorganisms to verify the effectiveness of an antibiotic treatment.

* Corresponding author. Address: Banco de Tecidos/INTO, Rua Washington Luis 61, Centro, Rio de Janeiro, RJ, CEP 20230-020, Brazil.
Tel.: +55 21 3512 4793; fax: +55 21 3512 4793.

E-mail address: svankats@hotmail.com (J.P. van Kats).

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In recent years, the HVB of the Erasmus University Medical Centre (Erasmus MC) has frequently identified isolates of *Propionibacterium* species and/or *Corynebacterium* species in the samples collected after antibiotic treatment of tissues. These organisms were not encountered when sterile saline was assessed simultaneously as a control sample, excluding the possibility of in-process contamination and indicating that these bacteria must have been present in the packed donated heart as received by the HVB. Remarkably, microbiological examination of the transport medium that is conserving the heart between procurement and transport to the HVB, and of which a sample is routinely tested in blood culture flasks, seldom demonstrated a presence of these isolates. In principle, the heart transport medium would be assessed for the presence of bacteria and fungi by filtration testing of the total volume, being the gold standard, but this is a longer and a costly procedure. Although blood culture testing has been validated for microbiological examination of blood-free liquids, technical difficulties in isolating some anaerobic microorganisms have been reported as well [9]. It was hypothesised that microbiological assessment of the transport medium of human hearts for heart valve donation using blood culture flasks may render false-negative results.

The aim of the present study was to compare routine assessment (blood culture flasks) for microbiological contamination in the transport medium of donated human hearts at the HVB of Erasmus MC with an alternative validated method to isolate anaerobic bacteria in liquid media [10]. In addition, the results of these entry tests were related with the outcomes of microbiological assessment of the in-process and the final samples that were collected in the course of tissue processing.

2. Methods

2.1. Donors

The study included hearts retrieved from human donors who had been received by the HVB over 15 months (December 2005–February 2007). The donors had been screened for transmissible diseases and were initially approved for heart valve donation. Retrieval of the hearts from multi-organ donors (MODs), heart transplant recipients (HTXs) and non-heart-beating cadaveric tissue (NHB) donors approved for bone donation took place in the surgical theatre. Hearts from the NHB donors who were not approved for bone donation were explanted in the morgue. The procurement procedure always began within 24 h of circulatory arrest.

2.2. Tissues

The hearts were excised, dissected and processed by skilled staff using aseptic techniques and sterile materials and substances. Following explantation, the hearts were enclosed in bags with 500 ml ice-cold physiological solution (i.e., cardioplegia, saline or Ringer's solution), placed in a polystyrene box with ice and transported to the HVB. At the HVB, the hearts were dissected in a Class II-A laminar airflow cabinet, placed in a classified background (class C) [11]. After macroscopic assessment, the selected tissues that were considered suitable for potential therapeutic use were treated

with an antibiotic cocktail (ciprofloxacin 3 µg ml⁻¹, amikacin 12 µg ml⁻¹, metronidazole 12 µg ml⁻¹, vancomycin 12 µg ml⁻¹, flucytosine 30 µg ml⁻¹) at 37 °C for 5–6 h. Except for the decontamination procedure, the tissues were refrigerated or placed in a cold medium (4 °C) throughout the procedure. Tissues were incubated with cryoprotective medium, packaged and cryopreserved within 48 h after circulatory arrest and subsequently stored in the vapor of liquid nitrogen (< -150 °C).

2.3. Microbiological analysis

2.3.1. Entry test

Before dissection, a 15-ml sample of the transport medium was drawn with a syringe and distributed equally over aerobic BacT/Alert FA and anaerobic BacT/Alert FN blood culture flasks (bioMérieux, Marcy l'Etoile, France) and a fluid thioglycolate medium (FTM) (BioTrading Benelux, Mijdrecht, The Netherlands). The blood culture flasks and the FTM bottle were grown at 37 °C for 7 and 14 days, respectively.

2.3.2. In-process test

Of each heart valve or vascular conduit that was approved as suitable for further processing, five small tissue fragments (100–200 mg) of the subvalvular myocardium (left and/or right ventricle wall adjacent to valve annulus) were collected before immersion in antibiotic solution. Two fragments were sub-cultured on aerobic (chocolate) and anaerobic (Schaeffer) plates, respectively. Two other fragments were placed in Brewer's broth and Brain–Heart Infusion (BHI) broth, respectively. One fragment was stored as a back up.

2.3.3. Final test

After antibiotic treatment, a graft was first washed in cold 100–150 ml Medium 199 and then transferred to another container for incubation with cold Medium 199 and 10% dimethyl sulphoxide (DMSO) with a total volume of 160 ml. After putting the graft and some of the cryoprotective medium in a freezing bag, the remaining volume of 70 ml cryoprotective medium was used for microbiologic examination. This medium was simultaneously passed over two separate 0.45-µm polyvinylidene fluoride Durapore filters (Millipore, Billerica, MA, USA). BHI broth and thioglycolate broth were added to the filter units for aerobic and anaerobic cultivation, respectively, and grown for 7 days at 37 °C.

Growth was detected automatically for blood culture flasks and scored visibly for all other tests. In case of growth (between day 1 and 7/14), or in case of no growth at day 7/14, a set of aerobic and anaerobic plates were sub-cultured and incubated for 2 days at 37 °C, followed by a determination of isolates.

2.4. Data analysis

Microbiological examinations were only performed when hearts yielded at least one possible transplantable tissue and only these hearts are included in the analysis. Where applicable, data were analysed with Student's *t*-test (means), McNemar's test (paired observations) or with Pearson's chi square test on contingency tables. Statistical significance was accepted at 5%.

Table 1

Microorganisms of low and high virulence isolated from cultures of heart transport medium (blood culture method and FTM culture method), subvalvular tissue fragments and cryoprotective medium.

Microorganism	Number of cultures ^a			
	Blood culture method (entry)	FTM culture method (entry)	Tissue samples (in process)	Cryoprotective medium (final)
Low virulence				
<i>Coagulase-negative staphylococcus</i>	61	28	4	1
<i>Bacillus</i> species	5	2	9	7
<i>Propionibacterium</i> species	2	67	21	38
<i>Corynebacterium</i> species	2	11	6	11
<i>Flavimonas</i> species	1			
Anaerobic gram positive cocci ^b			1	
Anaerobic gram positive rods ^b		1		
Total	71	109	41	57
High virulence				
<i>Streptococcus</i> species	20	16	18	10
<i>Escherichia coli</i>	6	5	2	
<i>Staphylococcus aureus</i>	6	2	2	1
<i>Haemophilus</i> species	2			
<i>Clostridium</i> species	1	2	1	2
<i>Enterobacter</i> species	1	1	1	
<i>Serratia</i> species	1	1		
<i>Aeromonas</i> species	1			
<i>Bacteroid</i> species		1	1	1
Non-fermentative gram negative rods ^c	1			
Non-fermentative gram negative cocci ^c		1		
Molds	1			1
Total	40	29	25	15
Not known		16		1

^a One or more than one type of microorganism isolated from each cultured sample.

^b Species level not determined, but was considered to be of low virulence.

^c Species level not determined, but was considered to be of high virulence.

3. Results

3.1. Microbiological assessment of transport medium

The HVB examined the transport medium from 275 donor hearts. In 98 samples of transport medium (36%), no microorganisms could be detected by either of the test method. In the remaining 177 samples (64%), one or more microorganisms could be identified by one or both the methods. A single type of microorganism was identified in 159 of all positive samples, whereas a presence of two or more different microorganisms was detected in 14 and four of all the positive samples, respectively. As can be seen in Table 1, low-virulent skin flora was most commonly isolated in the transport medium by both the test methods, and blood culture testing detected more microorganisms considered as high virulent [3] than the FTM method. In 16 samples of transport medium assessed by the FTM culture, the growth of microorganisms had been visibly recorded (turbid) but could not be confirmed by subsequent sub-culture or microscopy, possibly due to extinction of the cultured microorganism or aggregation and subsequent precipitation of substances in the culture medium (e.g., blood cells or proteins). These samples were registered as positive in further analysis.

In the transport medium of 70 donor hearts, growth was detected by both the methods; of which in 46 samples, the same microorganisms were identified as well (22× *Coagu-*

lase-Negative Staphylococci, 15× *Streptococcus* species, 5× *Escherichia coli*, 3× *Staphylococcus aureus*, various single observations of other microorganisms). The frequency of detecting the presence of microorganisms was significantly different between both the methods ($p = 0.0000$ McNemar's test). For 80 transport medium samples, no growth was detected with the blood culture method, while with the FTM culture of these samples, microbiological contamination was demonstrated, which could be predominantly identified as anaerobic skin flora (i.e., *Propionibacterium* species and *Corynebacterium* species). In contrast, the FTM culture failed to detect growth in only 27 transport medium samples that showed presence of bacteria with the blood culture test. These were mainly cultures of *Coagulase-Negative Staphylococci* and in six cases high-virulent microorganisms. Table 2 breaks down the results for microbiologic assessment of transport medium and donor type. Only for NHB donors, a significant difference between the test method and the outcome of heart transport medium assessment could be observed ($p = 0.0000$ McNemar's test).

The hearts of HTX ($n = 7$) and MOD ($n = 74$) donors were rapidly explanted after circulatory arrest (warm ischaemic time = $0 \text{ h}:00 \pm 0 \text{ h}:00$ and $0 \text{ h}:56 \pm 0 \text{ h}:58$ min, respectively; mean $\pm$ SD), while the excision of hearts after death of NHB donors ($n = 194$) had a much longer delay ($9 \text{ h}:19 \pm 3 \text{ h}:36$). In this last group, it was observed that a prolonged warm ischaemic time was related with a positive outcome of the

Table 2
Detection of microorganisms in transport medium of heart valve donors.

Donor type (n)	Positive test of transport medium (n)			
	Either method	By blood culture	By FTM culture	By both methods
All microorganisms				
HTX	2	1	1	0
MOD	43	28	30	15
NHB	132	68*	119*	55
Low-virulent microorganisms ^a				
HTX	2	1	1	0
MOD	37	23	27	11
NHB	99	39	93	27
High-virulent microorganisms ^b				
HTX	0	0	0	0
MOD	6	5	3	4
NHB	33	29	26	28

^a Only growth of low-virulent microorganisms detected in the sample.

^b Growth of high-virulent microorganisms detected in the sample and occasionally also growth of low-virulent microorganisms present.

* $p < 0.05$.

Table 3
Comparison of microbiological assessment of heart transport medium samples, tissue samples and cryoprotective medium samples.

	Negative ^a	Positive ^b	Total
Outcome heart transport medium test	98	177	275
Corresponding tissues selected for processing	135	241	376
Outcome tissue sample test			
Negative ^c	130	183	313
Positive ^d	5	58	63
Outcome cryoprotective medium test			
Negative ^c	131	178	309
Positive ^d	4	63	67

^a No growth of microorganisms detected by both test methods in heart transport medium.

^b Growth of microorganisms detected by either or both test method in heart transport medium.

^c No growth of microorganisms detected by referred test (tissue culture/filter test) in referred sample (tissue sample/cryoprotective medium).

^d Growth of microorganisms detected by referred test (tissue culture/filter test) in referred sample (tissue sample/cryoprotective medium).

heart transport medium assessment (8 h:22 ± 0 h:37 vs 9 h:46 ± 0 h:34; negative and positive outcome, respectively, $p = 0.016$ Student's *t*-test), regardless of the applied test method.

3.2. Microbiological assessment of myocardial tissue

The aortic and pulmonary valves of the 275 received hearts were dissected. The perivalvular tissue fragments were collected for evaluation of bacteriological contamination from 376 tissue grafts (106 aortic valves and 270 pulmonary valves and vascular conduits) that, following macroscopic assessment, had been accepted for further processing. In 83% of the samples, no microorganisms could be detected. In the remaining 17% of tissue fragments, a bacterial growth was demonstrated that was mostly characterised as low-virulent skin flora (Table 1). *Streptococcus* species was the most commonly identified high-virulent microorganism.

No microorganisms were detected in the tissue samples of five aortic and three pulmonary valves collected from hearts of HTX donors. Only four tissue samples (4%) taken from 39 aortic and 74 pulmonary valves gathered from MOD donors demonstrated a presence of bacteria (all low virulent only). In contrast, microorganisms were isolated from 59 (23%)

aortic and pulmonary perivalvular tissue samples taken from the 255 aortic and pulmonary valves and conduits, respectively, selected from NHB donors ($p = 0.000$, chi square test).

Out of the 98 hearts in which bacterial growth had been undetectable in transport medium, 135 valvular and vascular grafts were selected for further processing. In 130 tissues samples collected from these grafts, no bacterial growth could be detected as well, whereas in five myocardial samples low-virulent microorganisms (all skin flora) were detected (Table 3). However, in less than 25% of the 241 tissue samples, bacterial growth was detected, although the grafts had been derived from the 177 hearts of which the transport medium had been found positive in either or both the tests.

3.3. Microbiological assessment of the final sample

The cryoprotective solution that remained after antibiotic treatment and preparation for cryopreservation of the 376 selected tissue grafts was assessed for the presence of microorganisms. In 82% of these final samples, no microorganisms were detected. In the 18% of final samples that proved positive, *Propionibacterium* species and *Corynebacterium* species were the most detected low-virulent microorganisms and *Streptococcus* species was the most prevalent high-virulent microorganism identified (Table 1). Detection

of microorganisms was more common in the final samples of decontaminated tissues derived from NHB donors ($p = 0.012$ chi square test); of the 255 samples examined, 55 tested positive (22%). Only in 10% and 13% of antibiotic-treated tissues derived from MOD donors (11 of 113) and HTX recipients (1 of 8), respectively, a growth of microorganisms was demonstrated.

Of all the grafts derived from hearts with a contaminated transport medium, 74% were free from microorganisms after antibiotic treatment while, in 26% of final samples, microorganism were still detectable (Table 3). Of four tissues (3%) growth of microorganisms (*Corynebacterium* species, *Propionibacterium* species, unidentified) was detected in the final sample, whereas no contamination had been encountered in the transport medium and myocardial samples.

4. Discussion

This article addresses the microbiological examination of transport medium of donated human hearts that were received for heart valve donation at the HVB of Erasmus MC. A major part of heart transport medium samples appeared to be contaminated with microorganisms, which is in agreement with the data reported by others [4,8]. Subdivided by donor type, it was observed that contamination of transport medium was the least frequent in samples from HTX recipients, underlining the aseptic character of that procedure [4,7,8]. The highest contamination rate was observed for cadaveric donations that, as demonstrated in this analysis, can be attributed to the delayed onset of harvest of hearts from NHB donors, allowing leakage of microorganisms from other body compartments to the blood and thoracic compartment [5,6] and procurement taking place in the non-sterile environment of a morgue [4,6,8]. The short warm ischaemic interval and the explantation of the organ in the operating theatre may probably explain the lower contamination rate of the transport medium for MOD donations as compared to cadaveric donations. Organ retrieval from the abdominal compartment and a death involving trauma in a subgroup of these donors partly account for the higher initial contamination rate as compared with that of HTX recipients [3,4,12].

Skin flora was most commonly identified in the heart transport media from cadaveric and multi-organ donors [3,6,8,13]. In this analysis, a significant relationship was detected between the warm ischaemic time and contamination of the explanted heart of NHB donors. Thus, a delayed onset of explantation may facilitate the migration of microorganisms from the cutis and/or the upper airways into the blood compartment and deeper organs after death. Furthermore, leakage of skin flora from the sternal incision to access the thoracic cavity and exposure to the procurement environment and staff may also contribute to some extent to the detected contaminations [3–6].

The blood culture method detected growth of microorganisms in 27 samples of transport medium that tested negative with the FTM method. This is not surprising since the thioglycolate medium specifically selects for growth of anaerobic microorganisms and is not an enriched medium as is required for growth of fastidious high-virulent organ-

isms. This may explain why, in all cases, organisms such as *Coagulase-Negative Staphylococci* and *Streptococci* were not identified by the FTM method. Furthermore, bacteriostatic or bactericidal components possibly present in the heart transport medium may hinder growth of microorganisms in the FTM culture medium. Blood culture flasks contain active coal that neutralises such components from the culture liquid.

On the other hand, in 80 samples of heart transport medium, the FTM method identified a bacterial growth that was not detected with the blood culture method. In 75% of those samples, it related to isolates of *Propionibacterium* species or *Corynebacterium* species. In total, a presence of these species was demonstrated in 78 (28%) FTM-tested samples of transport medium. In only three occasions, these bacteria were also identified when the transport medium was assessed with the blood culture method. Earlier onset of growth of other microorganisms may have impeded growth of *Propionibacterium* species or *Corynebacterium* species in 14 samples and no growth was detected at all in 61 samples. When identified with the FTM method, *Propionibacterium* species and *Corynebacterium* species became detectable on average after 6 and 7 days, respectively, of culturing at 37 °C. It is unlikely that incubation of the blood culture flasks for a longer period than the 7 days in our protocol would have detected these slow-growing bacteria [14], because neither the microorganism nor any other appeared after continued incubation of a subset of blood culture samples up to 14 days that had been negative after 7 days of cultivation (data not shown). It is more likely that the blood culture assessment of transport medium is not appropriate to detect the growth of these microorganisms in non-blood samples.

Blood culture flasks were originally developed to detect microorganisms in whole blood samples more rapidly and with a greater sensitivity than the standard methods. More recently, these flasks are being used to assess bacteriological contamination of various human non-blood liquid samples with a low inoculum [15–18]. Therefore, some tissue banks have adopted this technique to address bacteriological and fungal contamination of tissue cultures [19,20] or tissue preservation fluids. However, the identification of various anaerobic microorganisms in non-blood liquids has been demonstrated to be slower than application of conventional broths, may require supplementation with serum and may encounter technical problems of detection in the cultivating apparatus [9]. The presented data demonstrate that application of the blood culture method to assess microbiological growth in non-blood samples fails to adequately detect slow-growing anaerobic bacteria and thereby puts an important restriction to its use in the assessment of microbiological safety.

In principle, all the tests applied during processing of donated human cardiac tissue have a similar high sensitivity and specificity. In the present situation, a combination of the blood culture testing of heart transport medium with the FTM method will improve sensitivity and specificity of the entry test as a whole and may be close or equal to filtration testing of the whole transport medium batch, although with lower costs and less labour. However, such a conclusion requires further validation of these test methods through limit testing, precision studies and bacteriostasis/fungistasis

testing, and thereby addressing an important limitation of our analysis.

Cultivation of small valvular tissue samples in culture broths and sub-culture of fragments on agar plates are methods that are being applied by many HVBs to determine microbiological safety of the dissected heart valves. In the present analysis, only 17% of the cultivated myocardial tissue fragments demonstrated a presence of microorganisms, whereas 64% of the transport medium samples of the incoming hearts tested positive. This is a remarkable difference given that both the types of sample had been collected before antibiotic treatment. Others have explained this discrepancy by dilution of contaminated transport medium during trimming to a near-undetectable inoculum in the tissue fragment [4]. In the present analysis though, a large group of heart valves demonstrated bacterial growth of the same species in the transport medium sample and final sample, while examination for microorganisms of myocardial samples obtained before antibiotic treatment was negative. Apparently, the initial contamination has a haematogenous character and it is not rinsed off during processing. Tissue fragments are only a very small part of the exposed organ surface, which makes it easy to miss a possible contamination. Given the observed low sensitivity and specificity, cultivation of myocardial tissue samples only has a limited value to verify the degree of contamination of dissected heart valves. The observation that five samples of cultivated tissue samples showed growth while the transport medium sample of these donors had been negative in both the tests applied is likely related to the limited sensitivity of the transport medium tests, as discussed above.

Out of the 78 hearts, in which the FTM examination of transport medium demonstrated a presence of *Propionibacterium* species or *Corynebacterium* species, eventually 109 tissues were harvested, of which 25% was still found to be contaminated with these bacteria after antibiotic treatment. Vancomycin as component of the applied cocktail is expected to eliminate *Propionibacterium* species and *Corynebacterium* species, but its bactericidal action may be hampered under aerobic incubation when anaerobic bacteria will not replicate and maintain a low metabolism. Furthermore, the drug may be less effective because of its poor tissue penetration and slow bactericidal action [21]. These data suggest that the HVB might benefit from an improved rinsing procedure of the heart following its recovery and a review of its decontamination protocol. A possible additional effect of prolonged, cold exposure to antibiotics (18–19 h at 4 °C) to the standard decontamination procedure is currently under investigation.

Growth of *Propionibacterium* species and/or *Corynebacterium* species was identified in 47 positive final samples. On 25 occasions, these species had also been detected in the transport medium by the FTM method, while in another 15 samples of the 47 aforementioned positive final samples, other fast-growing microorganisms in the transport medium may have hampered the detection of slow-growing *Propionibacterium* species and/or *Corynebacterium* species. Both the species were more frequently detected in the final samples derived from NHB donors than in the final samples derived from MOD and DO donors. This suggests that reduced dermal integrity, which is associated with cadaveric donation,

facilitates migration of skin flora into the blood compartment and organs of NHB donors [5,6]. There are no papers that report high numbers of donated cardiac tissues contaminated with *Propionibacterium* species and/or *Corynebacterium* species initially and after antibiotic treatment. The finding is unlikely to be attributed to different procedures for explantation, dissection, decontamination and cryopreservation. The HVB of Erasmus MC follows the guidelines of both the American and the European Association of Tissue Banks and the abovementioned procedures are in agreement with the recommendations for aseptic heart valve processing, as earlier described by Gall et al. [4]. One major difference with the other reports is that the final test to assess the presence of microorganisms is performed with the Steritest™ sterility testing system (Millipore, Billerica, MA, USA). Concentration of the cryoprotective fluid in which the valve is bathed before preservation and subsequent cultivation of the filter allows detection of very low inocula and excludes the risk of false-negative test results due to the presence of residual antibiotics or other growth-inhibiting substances [4,22,23]. Unfortunately, detection of *Propionibacterium* species and *Corynebacterium* species in the final samples leads to the discard of a large number of processed tissues, because presence of any microorganism in these samples prohibits release for implantation because of the risk of development of endocarditis [14,24,25] in the recipient.

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References

- [1] Dacey LJ. Pulmonary homografts: current status. *Curr Opin Cardiol* 2000;15:86–90.
- [2] Gulbins H, Kreuzer E, Reichart B. Homografts: a review. *Expert Rev Cardiovasc Ther* 2003;1:533–9.
- [3] Deijkers RLM, Bloem RM, Petit PLC, Brand R, Vehmeyer SBW, Veen MR. Contamination of bone allografts: analysis of incidence and predominant factors. *J Bone Joint Surg Br* 1994;79:161–6.
- [4] Gall K, Smith S, Willmette C, Wong M, O'Brien M. Allograft heart valve sterilization: a six-year in-depth analysis of a twenty-five-year experience with low dose antibiotics. *J Thorac Cardiovasc Surg* 1995;110:680–7.
- [5] Koneman EW, Davis MA. Postmortem bacteriology: III. Clinical significance of microorganisms recovered at autopsy. *Am J Clin Pathol* 1974;61:28–40.
- [6] Martinez OV, Malinin TI, Valla PH, Flores A. Postmortem bacteriology of cadaver tissue donors: an evaluation of blood cultures as an index of tissue sterility. *Diagn Microbiol Infect Dis* 1985;3:193–200.
- [7] Hunt CJ, Caffrey EA, Large SR. Factors affecting the yield of cardiac valve allografts from living unrelated donors. *Eur J Cardiothorac Surg* 1998;13:71–7.
- [8] Tabaku M, Jashari R, Carton HF, du Verger A, Van Hoeck B, Vanderkelen A. Processing of cardiovascular allografts: effectiveness of European Homograft Bank (EHB) antimicrobial treatment (cool decontamination protocol with low concentration antibiotics). *Cell Tissue Bank* 2004;5:261–6.
- [9] Melhus A, Tjernerberg I. Blood culture bottles for transportation and recovery of anaerobic bacteria from non-blood samples. *APMIS* 2000;108:453–8.
- [10] Alexander K, Bryans T. Evaluation of the sterility test for detection of microbial contaminants of allografts. *Cell Tissue Bank* 2006;7:23–8.
- [11] EC guide to good manufacturing practice (GMP). Annex 1: manufacture of sterile medicinal products. European commission, enterprise directorate-general; 30 May 2003.

- [12] Vehmeyer SBW, Wolkenfelt J, Deijkers RLM, Petit PLC, Brand R, Bloem RM. Bacterial contamination in postmortem bone donors. *Acta Orthop Scand* 2002;73:678–83.
- [13] Peruzzo AM, da Costa FD, Abrahão WM. Microbiologic control in human heart valves. *Arq Bras Cardiol* 2006;87:778–82.
- [14] Günthard H, Hany A, Türina M, Wujst J. *Propionibacterium acnes* as a cause of aggressive aortic valve endocarditis and importance of tissue grinding: case report and review. *J Clin Microbiol* 1994;32:3043–5.
- [15] Fuller DD, Davis TE. Comparison of BACTEC plus Aerobic/F, Anaerobic/F, Peds Plus/F, and Lytic/F media with and without fastidious organism supplement to conventional methods for culture of sterile body fluids. *Diagn Microbiol Infect Dis* 1997;29:219–25.
- [16] Runyon BA, Canawati HN, Akriviadis EA. Optimization of ascitic fluid culture technique. *Gastroenterology* 1988;95:1351–5.
- [17] Saubolle MA, Sewell DL, Holland MD, Golper TA. Comparison of two commercial broth-culture systems for microbial detection in dialysates of patients on continuous ambulatory peritoneal dialysis. *Diagn Microbiol Infect Dis* 1989;12:457–61.
- [18] Yagupsky P, Dagan R, Howard CW, Einhorn M, Kassis I, Simu A. High prevalence of *Kingella kingae* in joint fluid from children with septic arthritis revealed by the BACTEC blood culture system. *J Clin Microbiol* 1992;30:1278–81.
- [19] Gain P, Thuret G, Chiquet C, Vautrin AC, Carricajo A, Acquart S, Maugery J, Aubert G. Use of a pair of blood culture bottles for sterility testing of corneal organ culture media. *Br J Ophthalmol* 2001;85:1158–62.
- [20] Thuret G, Carricajo A, Vautrin AC, Raberin H, Acquart S, Garraud O, Gain P, Aubert G. Efficiency of blood culture bottles for the fungal sterility testing of corneal organ culture media. *Br J Ophthalmol* 2005;89:586–90.
- [21] Stevens DL. The role of vancomycin in the treatment paradigm. *Clin Infect Dis* 2006;42(Suppl. 1):S5–7.
- [22] Leeming JP, Lovering AM, Hunt CJ. Residual antibiotics in allograft heart valve tissue samples following antibiotic disinfection. *J Hosp Infect* 2005;60:231–4.
- [23] Waterworth PM, Lockey E, Berry EM, Pearce HM. A critical investigation into the antibiotic sterilization of heart valve homografts. *Thorax* 1974;29:432–6.
- [24] Belmares J, Dettlerline S, Pak JB, Parada JP. *Corynebacterium* endocarditis species-specific risk factors and outcomes. *BMC Infect Dis* 2007;7:4.
- [25] Lalani T, Person AK, Hedayati SS, Moore L, Murdoch DR, Hoen B, Peterson G, Shahbaz H, Raoult D, Miro JM, Olaison L, Snygg-Martino U, Suter F, Spelman D, Eykyn S, Strahilevitz J, Van der Meer JT, Verhagen D, Baloch K, Abrutyn E, Cabell CH, International Collaboration on Endocarditis Merged Database Study Group. *Propionibacterium* endocarditis: a case series from the International Collaboration on Endocarditis Merged Database and Prospective Cohort Study. *Scand J Infect Dis* 2007;39:840–8.