

## Microbiologic Control in Human Heart Valves

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**Objective:** To evaluate, from a microbiological point of view, the valves processed by the Human Heart Valve Bank of Santa Casa de Misericórdia of Curitiba for use in cardiovascular surgeries.

**Methods:** The processing of 1,671 valves in the period from June 1999 through June 2004 was evaluated. Samples were collected from the valves and the solutions involved in the process and spread on culture media such as fluid thioglycolate medium, trypticasein soy broth and Sabouraud broth for incubation during 14 days, using a modified methodology based on Farmacopéia Brasileira 1998 (Brazilian Pharmacopeia) and USP 1990 (United States Pharmacopeia). The samples in which growing was observed were submitted to microbial identification.

**Results:** Of a total of 1,671 samples, 92% were considered adequate for use from a microbiological point of view, as they did not show microbial contamination. Only 8% were not released for clinical use because of contamination at some stage of the valve processing.

**Conclusion:** On analyzing the results, we noticed the importance of microbiological control in human grafts to avoid using microbiologically contaminated valves in patients undergoing cardiovascular surgery.

**Key words:** Heart valves, homografts, allografts.

Human beings have always been very concerned about quality. When quality is related with health products, it entails even greater care and knowledge, especially as regards invasive materials such as allografts.

The use of allografts was first reported in 1948, when human tissue obtained from a corpse was implanted by Gross et al. who used arterial segments<sup>1,2</sup>.

The first implantation of a human valve in the aortic position took place in July 24 1962 and was carried out by Dr. Donald Ross at Guy's Hospital<sup>3</sup> in London.

The American Association of Tissue Banks<sup>4</sup> was founded in 1976 to ensure the quality of the tissues processed and control their distribution.

In Brazil, Irmandade da Santa Casa de Misericórdia of Curitiba – Charity Hospital has a Human Heart Valve Bank that receives tissues from the Transplant Centrals which control the recovery and use of valves within Brazil. This bank is registered at the Ministry of Health in compliance with the provisions of Ordinance n.333/GM of March 24 2000<sup>5</sup>.

In order to provide patients with quality valve replacements, the Valve Bank processes and stores human valves recovered from healthy donors following the methodology recommended

by the Quality Manual of the Human Heart Valve Bank of Santa Casa de Misericórdia of Curitiba.

The microbiological control was carried out at all the stages of the processing to quantify and qualify the microorganisms found during the handling of the valves, selecting those valves which were found to be free of contamination.

The objective of this paper was to report the microbiological results for the transportation solution until the cryopreservation of each valve in the period from July 1999 through July 2004.

### Methods

The microbiological control was carried out during the processing of 1,671 valves removed from the hearts of healthy donors.

After the recovery (carried out by a medical team according to pre-established procedures), each heart was immersed in saline solution (transportation solution = TS) and sent to the Human Heart Valve Bank of Santa Casa (BHSC) at a temperature between 2 and 8°C.

The processing of the valves followed the instructions of

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the protocol of the Human Heart Valve Bank of Santa Casa de Misericórdia of Curitiba and was carried out in a class 100 laminar flow. For the inoculation of the samples, we prepared three different culture media (using a modified methodology based on the Brazilian Pharmacopeia 1988 and on the United States Pharmacopeia 1990<sup>6,7</sup>): fluid thioglycolate medium for growing aerobic and anaerobic bacteria, trypticasein soy broth (TSB) for the growing of aerobic bacteria and Sabouraud broth for the growing of fungi<sup>6-9</sup>.

The samples were collected at the following stages:

**Stage 1** – Preparation of the sterilizing solution (SS): with 400 ml of RPMI (Gibco) and antibiotics (cefotixin: 240 µg/ml, lincomycin: 120 µg/ml, polymyxin B: 100 µg/ml and vancomycin: 50 µg/ml). After the preparation of the solution, 3 ml were taken, and 1 ml was spread on each of the 5 ml of each media. Once the solution was ready, it was stored in the refrigerator until the time of use. RPMI: Roswell Park Memorial Institute<sup>10</sup>.

**Stage 2** – Inoculation of the transportation solution: before the dissection, 3 ml of the solution were aseptically taken from the transportation solution and 1 ml was spread on each of the culture media. Then the valve was dissected. After the dissection, three fragments (samples) of the muscle and wall of each valve with approximately 0.5 cm x 0.5 cm each were removed. Then each valve and its respective fragments were placed separately in containers with 200 ml of the SS (prepared in Stage 1), at between 2<sup>o</sup> and 8<sup>o</sup>C during 24 hours.

**Stage 3** – After 24 hours, the valves were frozen in a freezing solution (FS) made up of 200 ml of RPMI + 25 ml of DMSO (dimethyl sulfoxide - Merck) and 25 ml of FBS (fetal bovine serum - Gibco) previously prepared. Three milliliters were aseptically taken from the FS and 1 ml was spread in each of the culture media.

**Stage 4** – Inoculation of solid samples (called the Sterilization Stage: S): the valves and fragments were taken from the SS and washed with saline solution. The fragments were spread in the different culture media (a piece of muscle and a piece of the wall of each valve were spread on each medium used).

**Stage 5** – Inoculation at the freezing stage (F): each valve

was then introduced into a bag with 100 ml of FS (previously prepared at Stage 3). After each valve had been in contact with the solution and before the bag was sealed, 3 ml of the solution were taken, and 1 ml was spread on each one of the culture media. The culture media were incubated for fourteen days. The tioglycolate fluid and the TSB media were kept at a temperature of 35°C and the Sabouraud broth was kept at a temperature of 22°C<sup>6-9</sup>. In the positive samples of tioglycolate and TSB fluid media, we carried out isolations in Mac Conkey<sup>9</sup> (Gram negative) and sheep blood agar<sup>9</sup> (Gram positive and negative) media; identification was carried out in an automated fashion using a Dade Behring MicroScan WalkAway 40. For positive samples of Sabouraud broth, isolation was carried out in Agar Sabouraud<sup>9</sup> and identification was performed manually using germinative tubes and microscopic examination.

## Results

Of a total of 1,671 samples analyzed, 1,535 (92%) were considered adequate and 136 (8%) were considered inadequate from a microbiological point of view (Fig.1).

Adequate samples were those which did not present microbial growth at the different stages, with absence of growth after the sterilization stage for those samples with positive TS.

The samples considered inadequate therefore were those which presented microbial growth in one or more stages after the sterilization stage.

Of the 1,535 adequate samples, 1,266 had negative results at all the stages and in 269 only the transportation solution was positive, with negative results after the sterilization process (Fig.1).

For the 136 inadequate samples, the stages with positive results were (fig.1): transportation solution (TS): 87; sterilizing solution (SS): 4; freezing solution (FS): 17; sterilizing stage (S): 78; freezing stage (F): 79.

The most frequently found microorganisms at each stage were:

- Sterilizing solution: *Rhodotorula sp.* (4) (graph 1).
- Freezing solution: *Bacillus diptheroids* (3) and

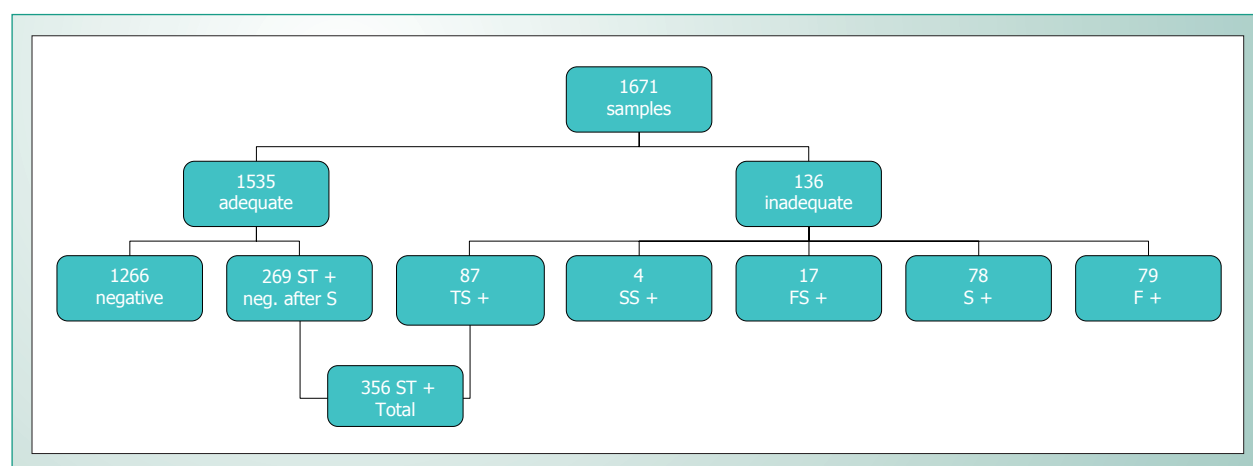


Fig. 1 - Flowchart showing the results obtained at the different stages of the process.

*Acinetobacter lwoffii* (3) (graph 1).

- Freezing stage: *Staphylococcus aureus* (16), coagulase-negative staphylococcus (11), *Pseudomonas sp* (9) and *Candida albicans* (8) (graph 1).
- Sterilizing stage: *Candida albicans* (11), *Pseudomonas sp* (10), *Escherichia coli* (8) and *Staphylococcus epidermidis* (8) (graph 1).

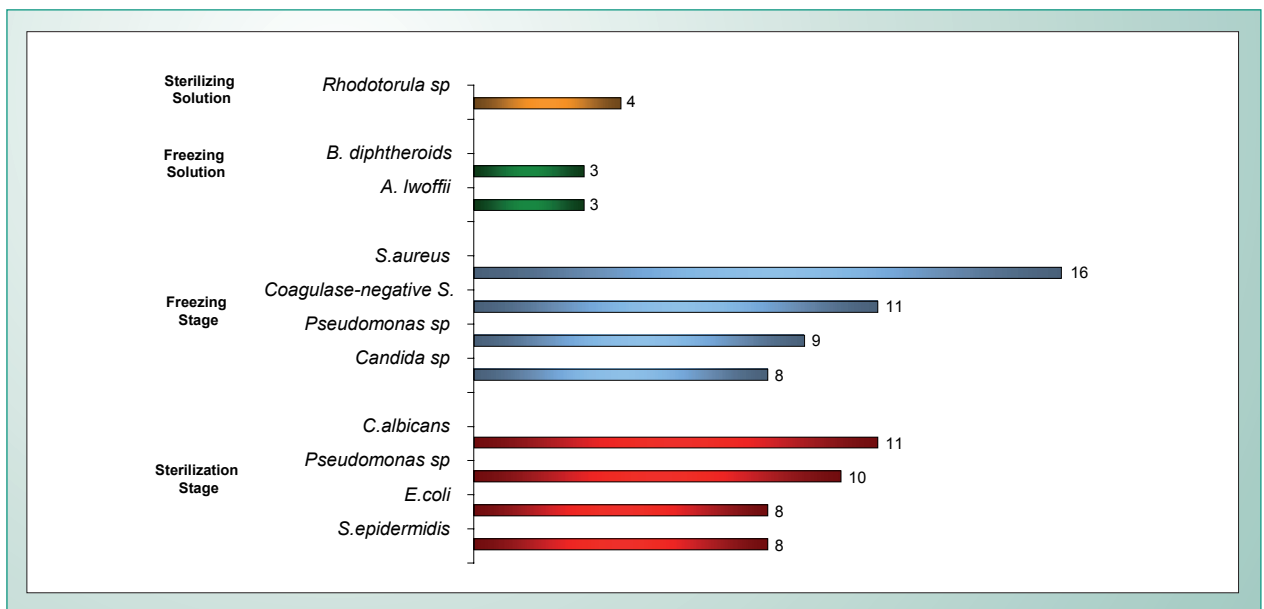
By adding the 269 positive samples of the transportation solution of the adequate analyses and the 87 of the inadequate analyses we obtained a total of 356 TS that tested positive in that the most frequent microorganisms were: *Staphylococcus aureus* (50) *Staphylococcus epidermidis* (45) and coagulase-

negative staphylococcus (44) (graph 2).

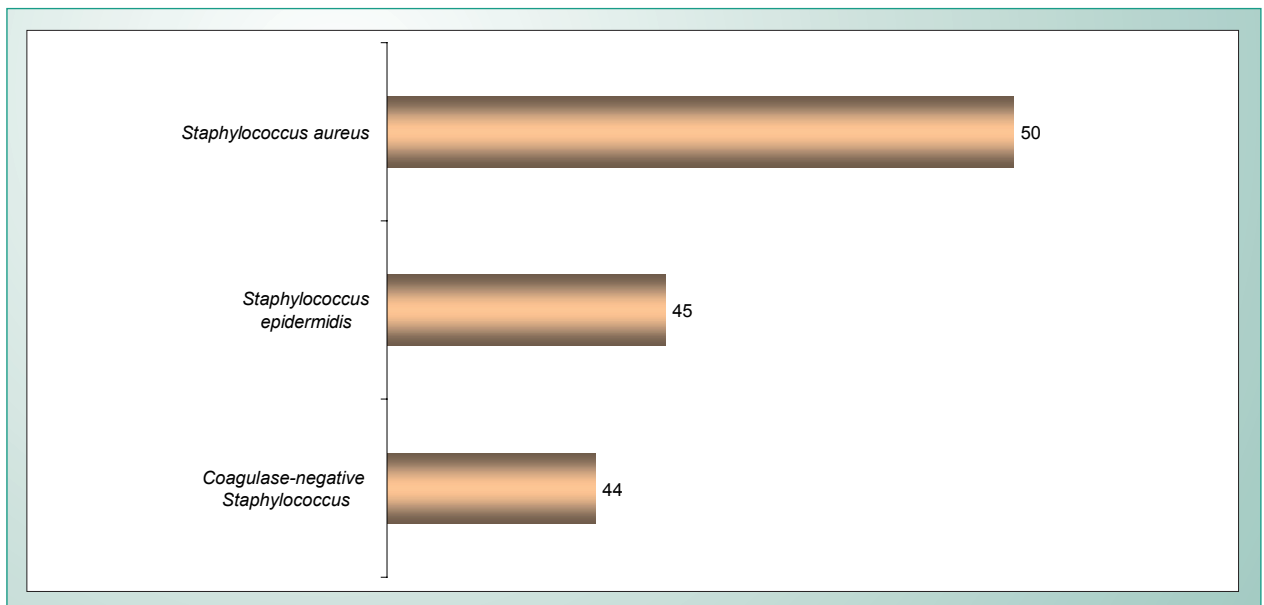
### Discussion

According to the results obtained, we can consider that the methodology used in the processing of the valves is correct, since 1,266 samples were negative at all the stages (from the recovery of the organ through the freezing of the valve), indicating asepsis and good practices throughout the procedure<sup>11</sup>.

From the 356 positive TS samples, only 269 were considered suitable for clinical use from a microbiological point of view, since they presented negative results at the stages following the sterilization stage, which shows the efficiency of



Graph 1 - Most frequent Microorganisms at the Sterilization Stage, Freezing Stage, and in the Freezing Solution and Sterilizing Solution.



Graph 2 - Most frequent Microorganisms in the Transportation Solution (Adequate and Inadequate Samples).

Site	Valve Bank - Curitiba	International Institution
Period	06/1999 – 06/2004	1992
Number of grafts analyzed	1,671	232
% microorganisms TS	21.3	17.7
Most frequent microorganisms TS	Staphylococcus aureus Staphylococcus epidermidis Negative-coagulase Staphylococcus	Neg-coagulase Staphylococcus Propionibacterium acnes Pseudomonas fluorescens
% microorganisms S	4.7	7.8
Most frequent microorganisms S	Candida albicans Escherichia coli Staphylococcus epidermidis	Neg-coagulase Staphylococcus Xanthomonas maltophilia Candida albicans
% microorganisms F	4.7	1.7
Most frequent microorganisms F	Staphylococcus aureus Neg-coagulase Staphylococcus Pseudomonas sp	Neg-coagulase Staphylococcus Bacillus diphtheroids Aspergillus niger
% microorganisms SS	0.24	0.43
Most frequent microorganisms SS	Rhodotorula sp	Aspergillus fumigatus
% microorganismos FS	1.0	0.43
Most frequent microorganisms SC	Bacillus diphtheroids Acinetobacter lwoffii	Neg-coagulase Staphylococcus

**Table 1 - Effectiveness of the sterilizing solution and reproducibility of the method in both facilities**

the sterilizing solution.

The positive transportation solution may indicate the contamination of the organ itself, contamination during its removal, during handling throughout the procedure or during the collection of the sample. However, it is not possible to say exactly where the contamination took place.

If the TS tests positive for the *Bacillus* or *Clostridium* genus, the valve is considered inadequate for use, even though at the other stages it comes to present negative results after sterilization, because these bacteria are sporulated, which makes antibiotic action more difficult.

According to the protocol of the Valve Bank, any positive samples after sterilization are considered inadequate for clinical use from a microbiological point of view, regardless of the microorganism identified, because it shows that the valve has not been sterilized.

In some cases, the presence of bacteria and fungi is expected after the sterilization stage, due to the low concentration of antibiotics and to the non-use of antifungal agents<sup>12</sup> because

of their toxicity to cells.

An international institution presented similar results using the same antibiotics (Rebeyka IM. Policy and Procedure Manual. Hospital for Sick Children Cryopreservation Laboratory. Toronto; 1992; Ap H) which shows the efficacy of the sterilizing solution in both clinics, with the reduction of contamination after the sterilization stage and the occasional growth of fungi in the sterilizing solution and at the sterilization stage, since no antifungal agent was used in any of the procedures, thus showing the reproducibility of the method (Tab. 1).

Finally we can say that the method used for microbiological control in the processing of valves has so far shown to be effective and reproducible, providing patients undergoing cardiovascular surgery with a replacement free of microbial contamination.

#### Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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