Brazilian Journal of TRANSPLANTATION

https://doi.org/10.53855/bjt.v27i1.565_ENG

ORIGINAL PAPER

The Use of Microbiological Quality Control Techniques Adopted in a Human Tissue Bank

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Section editor: Ilka de Fátima Santana F. Boin

Received: Feb 01, 2024 | Accepted: Feb 29, 2024

ABSTRACT

The Brazilian Ministry of Health's Consolidation Ordinance No. 4 of September 28, 2017, provides the requisites for the evaluation of services for human tissue banks (bancos de tecidos humanos [BTH]) in terms of the microbiological control of environments (ISO 5 classification processing room) and products (culture for aerobic, anaerobic, and fungal pathogens), with a view to guaranteeing harmlessness in tissue transplants. **Objectives:** To highlight the techniques for microbiological control of distributed tissues adopted by a BTH over 5 years, as well as the results obtained from the application and use of these techniques. **Methods:** Using standard operating procedures (SOPs), the methodology, and frequency of collections, tests and room cleaning were established in advance. Microbiological cultures were taken from the tissues during all the collections and processing. During tissue processing, fingerprint samples on the processor's gloves and in the processing room were seeded on blood agar plates. The processing room was cleaned weekly with biguanide and quaternary ammonium. The processing bench was sanitized with sterile 70% alcohol and the microbiological environmental control was carried out every 6 months by a qualified outsourced company. **Results:** In the period analyzed, the techniques proved effective in 46 (96%) cases, with contamination identified in only two (4%) processed and collected samples. The effectiveness and results were documented. **Conclusion:** Effective biological quality control methods are legitimately required, but they can be improved, raising the need to develop safe protocols for the quality of services and the safety of transplanted tissues.

Descriptors: Tissue Donation; Tissue Transplantation; Biological Quality Control; Innocuous Products; Human Tissue Bank.

O Uso de Técnicas para Controle de Qualidade Microbiológico Adotadas em um Banco de Tecidos Humanos

RESUMO

A Portaria de Consolidação nº 4, de 28 de setembro de 2017, do Ministério da Saúde, dispõe sobre a avaliação dos serviços destinados aos bancos de tecidos humanos (BTH) quanto ao controle microbiológico dos ambientes (sala de processamento classificação ISO 5) e dos produtos (cultura para patógenos aeróbicos, anaeróbicos e fungos), visando a garantia de inocuidade nos transplantes de tecidos. **Objetivos:** Evidenciar as técnicas para controle microbiológico dos tecidos distribuídos, adotadas por um BTH em 5 anos, assim como os resultados obtidos pela aplicação e uso dessas técnicas. **Métodos:** Por meio de procedimentos operacionais padrão (POP), foram estabelecidas previamente a metodologia e a frequência das coletas, dos exames e da limpeza das salas. Foram coletadas culturas microbiológicas dos tecidos em todas as captações e processamentos. Além disso, durante o processamento do tecido, amostras das digitais nas luvas do processador e na sala de processamento foram semeadas em placas ágar sangue. Semanalmente, a sala de processamento foi limpa com "biguanida + quaternário de amônia", sendo a bancada de processamento higienizada com álcool 70% estéril e o controle ambiental microbiológico realizado semestralmente, por empresa terceirizada e qualificada. **Resultados:** No período analisado, as técnicas se mostraram eficazes em 46 (96%) casos, tendo sido identificada contaminação em apenas duas (4%) amostras processadas e coletadas. A eficácia e os resultados foram documentados. **Conclusão:** Métodos eficazes de controle de qualidade biológico são legitimamente obrigatórios, porém passíveis de aperfeiçoamento, suscitando a necessidade do desenvolvimento de protocolos seguros para qualidade dos serviços e inocuidade dos tecidos transplantados.

Palavras-chave: Doação de Tecidos; Transplante de Tecidos; Controle de Qualidade Biológico; Produtos Inócuos; Banco de Tecidos Humanos.

INTRODUCTION

Human tissue banks (HTB) are public or private institutions specialized in health services and mainly responsible for capturing, processing, storing and distributing human tissues for transplants and research from living and/or deceased donors (autografts/allografts)[1,2]. Among the many types of tissues that can be transplanted, the following stand out: skin, amniotic membrane, corneas, vessels, valves, bones, tendons, menisci, fascia, and cartilage, among others[2,3]

The products made available by HTB are mainly intended to meet the clinical demands of patients on the waiting list managed by the National Transplant System (SNT), with repair, regeneration and/or tissue failure needs, with these tissues being exempt from the need for histocompatibility with the donor[3-5]. Therefore, such institutions must maintain safety and quality control in all processes that permeate their products, from donor screening to delivery of tissues to the transplant doctor and also, after transplants, with the monitoring of any reaction and/or adverse event in the recipient.[5-7]

Resolution of the Collegiate Board (RDC) No. 707, of July 1, 2022, aimed at good practices in HTB, which revoked RDC No. 55, of December 11, 2015, aimed at evaluating services regarding microbiological control of environments, ISO 5 classification processing room and products, culture for aerobic, anaerobic and fungal pathogens, seeks to establish techniques that guarantee the safety of human products offered for transplants[6,7]. Furthermore, it allows HTB to establish specific microbiological control guidelines, subject to the authorization and supervision of a doctor and clinical director responsible for the institution[6-8].

Therefore, due to the lack of standardization of techniques and guidelines specific to at HTB across the country, there is a need for greater dissemination of the processes developed by these institutions to assist in the decision-making of other HTB, amid the challenges involving the processes established by legislation.

OBJECTIVE

Highlight the microbiological control techniques for distributed tissues adopted by a HTB in 5 years and the results obtained by the application and use of these techniques.

METHOD

This is an experimental study with interventions on samples of human bone tissue (product/femoral heads) and the environment (sterile processing room – ISO 5 classification) located in a HTB located in a tertiary hospital in the interior of the state of São Paulo, where they were processed with analyzes of different techniques, as well as their previously determined processes and periods/frequencies.

To optimize and establish the production routine, types of analyses to be used and frequency of collections, an internal team, coordinated by a medical clinical director, was established through an organizational chart and then guiding documents were created to the themes that established all the processes involved in this study, in light of the ISO 9001/2015 Quality Management System and High-Level Management1, being: Quality Manual (QM); Management Procedures (MPs); Standard Operating Procedures (SOPs); Quality Records (QRs); Quality Indicators (IQs); Training of the outsourced cleaning team with the Hospital Infection Control Commission (HICC); training models with a record of effectiveness.

All tissues captured and used in the research came from living patients who were elective for total hip arthroplasty surgery in the same hospital where the HTB is located. Patients were selected by convenience sampling (non-probabilistic), and authorization to include samples in the study was obtained after the patient's consent through the Free and Informed Consent Form. Bone tissue samples collected from 01/14/2018 to 12/18/2022 were used for the study.

Through the documents described, the methodology and frequency of samples collected, examinations and cleaning of rooms were established, with appropriate team training, as described below.

Terminal cleaning

Weekly, or before and after all processing, the processing room was cleaned by the cleaning team with a sterile 0.4% biguanide + quaternary ammonium solution, and the processing bench was sanitized with sterile 70% alcohol by the HTB technician (Fig. 1a).

Fabric processing

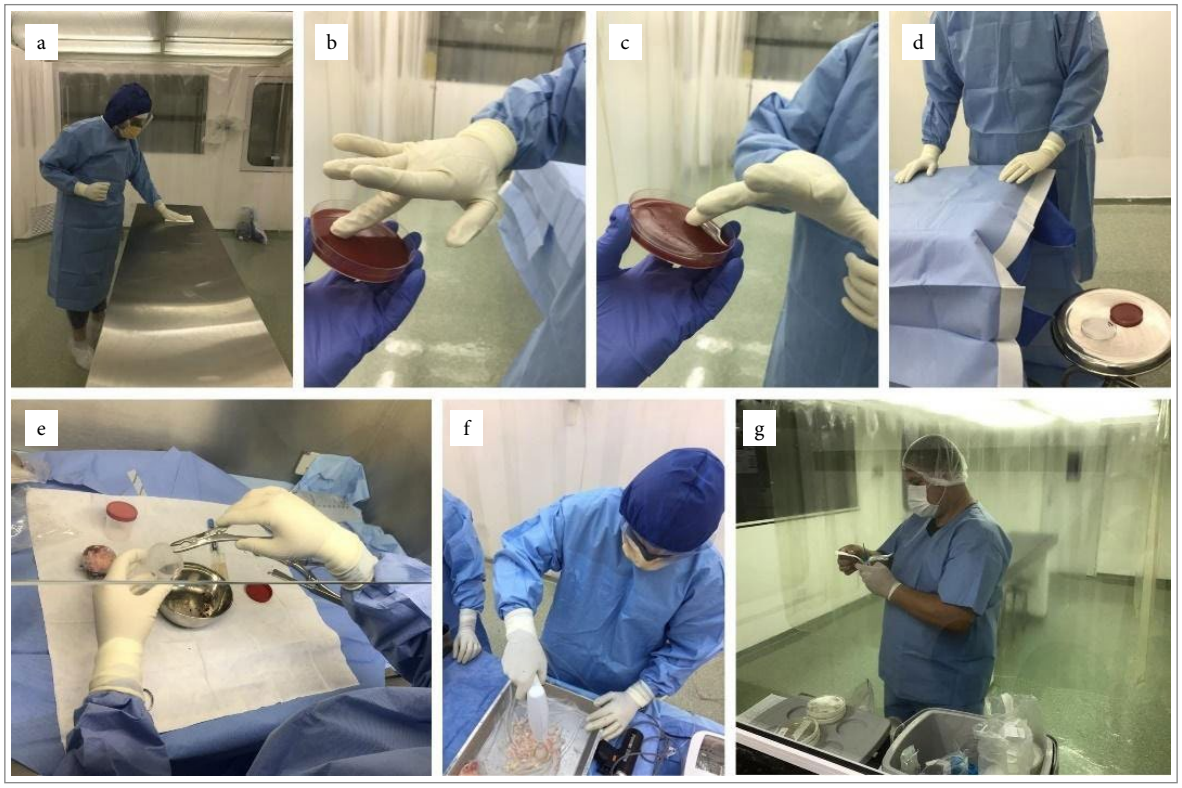
At the beginning of tissue processing, samples of fingerprints from the hands of the processor, who was wearing sterile gloves (Figs. 1b, 1c), were collected and seeded on blood agar plates, and, throughout processing, a plate containing medium of blood agar culture was exposed beneath the laminar flow environment of the processing room (Fig. 1d).

Microbiological control of processing

In all captures and processing, three bone fragment samples and three lavage samples [10 mL of 0.9% saline solution used to wash the bone tissue during processing] were collected to perform microbiological cultures, including bacteria aerobes, anaerobes and fungi (Figs. 1e, 1f).

Room environmental control

Microbiological environmental control (bacteria and fungi) of the processing room was carried out every six months by an outsourced company that collected and qualified the room as follows: a) Laminar flow analysis (ISO 5) – samples collected after processing (worst case scenario) and after first cleaning (best case scenario); b) Passive air analysis – three samples were collected with replicate organism direct agar contact plates. (RODAC) exposed to the environment for 4 consecutive hours, within laminar flow; c) Analysis of the processing bench – three samples were taken from the surface of the bench with RODAC plates; d) Analysis of the processing room, outside the flow curtain (ISO 7) – passive air analysis of four samples at different points in the room for 4 hours of exposure and analysis of the floor and walls with collection of four samples with RODAC plates (Fig. 1g).



Source: Authors' photographic collection, 2024.

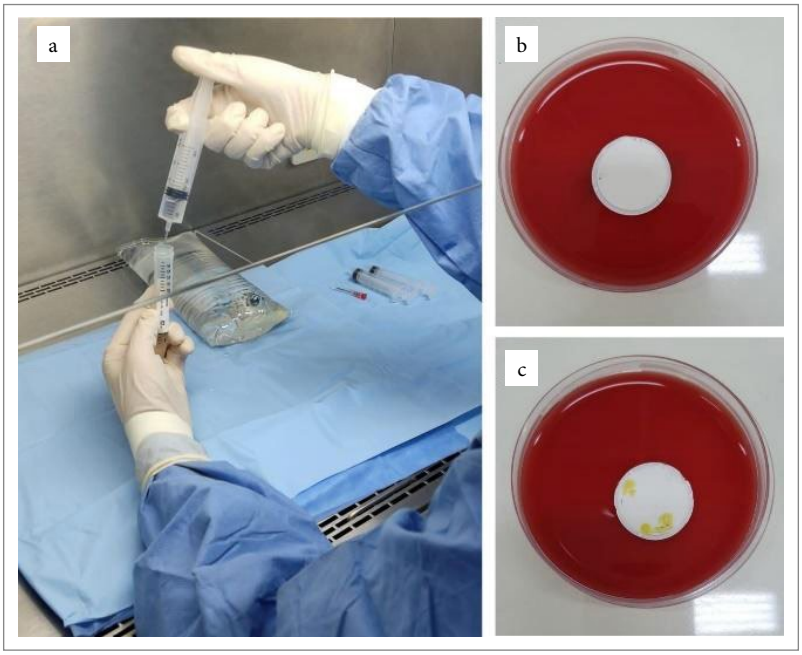
Figure 1. Records of the methodological processes used to analyze the environment and products produced by BTH, 2024.

Bioburden

Microbial load (bioburden) of all samples was quantitatively/qualitatively examined to determine the number of microorganisms present on a given surface and/or product[9,10]. For this purpose, a fragment of bone tissue was stored for 24 hours at 37°C in 50 mL of sterile 0.9% Saline solution. Subsequently, the liquid was passed through a closed system containing a sterile membrane/filter (K18-230/lot: 210410-338) with a 0.22 µm pore size (Kasvi, *São José dos Pinhais*, Brazil). The membrane was removed from the system and placed on the surface of a 5% sheep blood agar plate (Plastilabor, *Rio de Janeiro*, Brazil) identified and sent to the same laboratory where it was incubated in a bacteriological oven for 24-48 hours at 35°C[10] (Fig. 2).

All collected samples were sent to the Clinical Analysis Laboratory of the same hospital. The microbiological analyses of the samples were carried out by investigating the growth of aerobic, and anaerobic bacteria and fungi. The bone tissue samples and solutions were placed in tubes containing brain heart infusion (BHI) broth (Plastilabor, Rio de Janeiro, Brazil) and incubated for 24 hours in an oven at 37°C with 5% CO_2. After this period, they were sown on 5% sheep blood agar, Mac Conkey agar and mannitol agar plates (Plastilabor, Rio de Janeiro, Brazil) and incubated for 24-48 hours in a bacteriological greenhouse. The search for anaerobic bacteria was carried out by inoculating the material in fluid thioglycolate broth (Plastilabor, Rio de Janeiro, Brazil),

which was incubated in an oven for 24 hours and, after this period, seeded on Brucella agar plates (Plastilabor, Rio de Janeiro, Brazil) and again incubated in an oven for 48 hours in an anaerobic jar with an oxygen inhibitor. Regarding the research on yeast-like and filamentous fungi, during the processing of the material, they were sown directly on Sabouraud agar in a tube, remaining incubated for a period of up to 42 days.



Source: Authors' photographic collection, 2024.

Figure 2. Carrying out the bioburden exam for microbiological analyses. a) Preparation of the closed system for the passage of the liquid containing the sample through the membrane; b) Test membrane seeded on the plate ready to incubate; c) Test membrane showing contamination after incubation.

For cultures of bacteria or fungi that showed growth of microorganisms, the identification and quantification of colonies of microorganisms were carried out using mass spectrometry using the Microflex LT MALDI TOF equipment (Bruker, Billerica, USA) and the sensitivity test by microdilution in broth, carried out using the VITEK® 2 Compact equipment (bioMérieux, Rio de Janeiro, Brazil); sensitivity tests were performed using disk diffusion. Antimicrobial susceptibility results were interpreted in accordance with the most recent guidelines from the Brazilian Committee on Antimicrobial Susceptibility Testing/European Committee on Antimicrobial Susceptibility Testing[11,12]. The results were tabulated and presented below.

RESULTS

During the period analyzed, the techniques proved to be effective in 46 (96%) cases, with contamination being identified in only two (4%) processed and collected samples, as described in Table 1.

Table 1. Identification of the sample, location of detection and genera/species of microorganisms found during tissue processing.

ID	Sample	Microorganism
08/2019	bone fragment	Fungus/*Candida albicans*
08/2021	Processor's hand	Bacterium/*Staphylococcus epidermidis*

Source: Prepared by the authors.

Regarding the 46 samples that did not show contamination in any of the analyses, the importance and effectiveness of the processes established by BTH stand out, especially regarding the choice of protocols, types of techniques used, training of the internal team (surgeons and processors) and external cleaning team (outsourced cleaning team), frequency of sample collection and greater safety of the products offered, as 96% of the samples were harmless.

It is certain that well-established and operationalized guidelines can be capable of improving internal processes, adding quality to the products offered and satisfaction to customers, transplant doctors and recipient patients.

DISCUSSION

When it comes to analyzing and discussing the effectiveness of the processes that permeate the capture and production of human tissues, it is important to evaluate, in depth, the results of microbiological tests as quality indicators. Therefore, analyzes of microbiological contamination of tissues are urgently needed in a negative way, but with a very important and critical role in the safety and harmlessness of the distributed allografts[12,13]. In this sense, it is worth highlighting that this negative evidence can be transformed into points for immediate and future improvements to be adopted by BTH[1].

There are many factors, within HTB, that may be associated with tissue contamination, in addition to capture and processing; for example, contamination in surgical wounds in the immediate and/or late postoperative period of patients undergoing orthopedic surgery[13]. Recent studies describe the prevalence of different bacteria, such as *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, among others, in cultures associated with these surgeries[14,15].

Another important data to be analyzed, due to sample contamination, is the number of people who participated in the collection and/or processing. Studies have shown that the number of people who participated in the collection/processing can increase the contamination of pathogens in tissues[12,16], especially in surgical centers in which the tissue was collected in the processing room in which it was processed; although small, there is the possibility of contamination through the surgeon/processor's skin flora, if they do not perform satisfactory aseptic techniques[16-18]. Furthermore, Paolin et al.[12] states that a team must contain up to three people and that for each extra person in the collection/processing team, the risk of contamination increases by 1.28 times[12].

The duration of collection and transport of samples can also be a risk factor for contamination, as well as the time of year, which can be explained by the state of conservation of the sample, given the temperature of the donor's body at the time of removal or lack of care when transporting tissues[12-16,18].

Other variables related to the donor can determine the possible factors that affect the risk of contamination during collection, such as gender[12], type of donor (living or deceased)[18] and temperature[16]; however, such correlations were not addressed in this initial study.

Finally, the results of this study can be an excellent starting point for improving tissue harvesting and processing procedures in all HTB in the country. Minimizing contamination risks in products produced by HTB is an objective that must be pursued with aseptic techniques and well-established protocols, in order to meet the needs of institutions, transplanters and recipient patients.

CONCLUSION

Effective microbiological quality control methods are legitimately mandatory to highlight quality indicators. Furthermore, they can provide a reduction in the risk of tissue contamination through the continuous improvement of the aseptic techniques used in collection and processing. However, the standardization of exams is not specific, encouraging the development of safe protocols for the quality of services in each HTB.

CONFLICT OF INTEREST

Nothing to declare.

AUTHOR'S CONTRIBUTION

Substantive scientific and intellectual contributions to the study: Corsi CAC, Scarpelini KCG, Bento RL, Assunção-Luiz AV; **Conception and design:** Corsi CAC, Scarpelini KCG, Bento RL, Assunção-Luiz AV; **Data analysis and interpretation:** Corsi CAC, Scarpelini KCG, Bento RL, Assunção-Luiz AV; **Article writing:** Corsi CAC, Assunção-Luiz AV; **Critical revision:** Garcia FL, Martins LGG; **Final approval:** Garcia FL, Martins LGG.

DATA AVAILABILITY STATEMENT

All dataset were generated or analysed in the present article study

FUNDING

Not applicable

ACKNOWLEDGEMENT

The authors thank to Banco de Tecidos Humanos do Hospital das Clínicas da Faculdade de Medicina de Ribeirão Preto - Universidade de São Paulo

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