
CLINICAL ARTICLE

Evaluating donor tissue for bacterial contamination at the South African National Tissue Bank

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Abstract

The internationally accepted method of acquiring allograft bone is to retrieve it in a sterile environment. In South Africa, we are limited by resources and funds, making it impossible to adhere to these standards of retrieval. The purpose of the study is to evaluate the safety of the surgically clean retrieval of allograft bone outside of a theatre set-up.

The study population consisted of all the accepted donors from the beginning of 2003 to September 2008. The donors included in the study (n=749) were tested for microbial growths at various stages of retrieval and processing.

An internal audit was done on the results and the safety of the processes was evaluated. The amount of bacterial contamination of the various samples was used to evaluate the safety of the process.

We concluded that by following a strict protocol for processing and by using mandatory gamma irradiation our allograft is bacteria-free and extremely safe, making our method comparable with international standards.

Background

Tissue and bone allografts are used by a variety of specialties, but mostly in the orthopaedic fraternity. Providing quality allograft by preventing the transmission of micro-organisms to the recipient and insuring good osteo-inductive properties are of utmost importance to any tissue bank.

Internationally (in the USA, Australia, Japan, UK and other parts of Europe, e.g. Poland) the accepted method of allograft retrieval is a sterile technique that is performed in

the theatre.^{1,2,3} Due to limited resources and funds in South Africa, we use a surgically clean technique to retrieve allograft bone.

Most of the studies discourage the use of a surgically clean technique (SCT), in other words the retrieval of bone in a morgue, due to the unacceptable levels of bacterial contamination.^{4,5} Except for the risk of bacterial contamination of the allograft recipient, contamination can also lead to the degradation of proteins in bone resulting in the decreased osteo-inductive ability of the allograft.⁶

Several studies have been published on surgically clean techniques.^{1,6} These studies advocate the use of terminal sterilisation if a SCT is used to retrieve bone.⁴ Terminal sterilisation is done either by means of ethylene oxide or gamma irradiation. The preferred method is irradiation due to an increased incidence of graft dissolution when ethylene oxide is used for sterilisation.⁴

The aim of our study was to prove that the South African National Tissue Bank provides safe and bacteria-free allograft. In order to achieve this goal, we compared our bacterial contamination rates in the retrieval of allograft with international figures and evaluated the bacterial-contamination rates at different stages of the processing of our bone products.

A protocol for the surgically clean retrieval of allograft bone

The National Tissue Bank at the University of Pretoria (hereafter referred to as the NTB) adheres to strict protocols during bone retrieval. Several steps need to be followed before the end product is distributed (*Figure 1*). These guidelines are included in the standard operation-procedure documents of the NTB for donors (SOP-DONOR 001-007), for cultures (SOP-CULT 001-003) and for processing (SOP CAT 001).

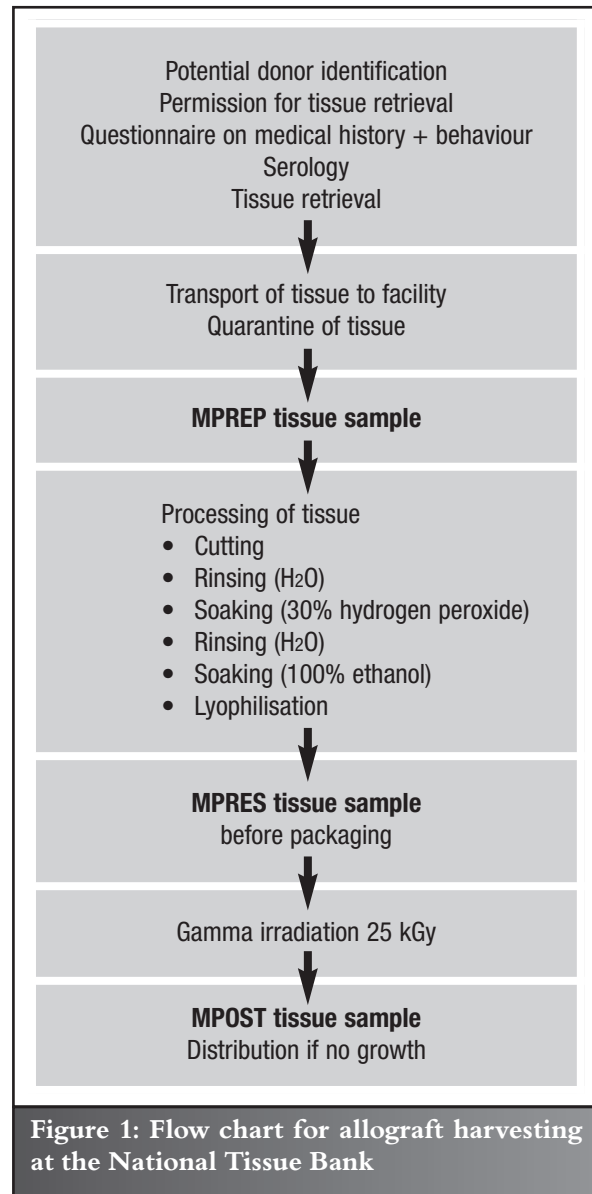
Firstly, a potential donor is identified, namely either a registered donor or a cadaverous donor between the ages of 16 and 70 years. Donor selection to verify donor suitability for transplantation purposes is set according to international standards and guidelines. The cause of death may be natural or unnatural. Permission for tissue removal is then obtained from the family by means of legally informed consent.

Donors are assessed by means of a standardised questionnaire based on their past medical history and behaviour as obtained from the family by the NTB co-ordinator. Further evaluation is done by means of serological screening and physical examination. The general contraindications and mandatory serology for donor suitability are listed in *Table I*.

The tissue retrieval is done within 72 hours on average at the forensic pathology laboratory or funeral parlour and the tissue is transported on dry ice (-45°C) and placed in quarantine storage for up to 10 days (-20°C). If donor eligibility is established during this time and the tissue found suitable, processing of the allograft tissue is commenced.

Before processing commences, a sample iliac crest bone of each donor is sent to an accredited laboratory for bacteriological culturing. This sample is referred to as the pre-processing sample (MPREP).

The processing steps depend on the type of product. The method described in this article is the most common method that includes an explanation of the processing steps during which the MPRES and MPOST samples are taken (*Table I*).



Processing includes cutting the bone to size and shape, followed by rinsing the bone with lukewarm pressurised and distilled water. The next step is to soak the bone in 30% hydrogen peroxide for 15 minutes and then repeat the rinsing with water. The bone is then soaked in 100% alcohol (ethanol) for six hours.

The last step is lyophilisation (freeze drying) of the bone for 8 to 12 hours. Lyophilisation is the rapid freezing of the bone to -40°C by direct sublimation of ice under vacuum. Moisture is simultaneously extracted from the graft during this step. Before packaging and labelling, a pre-sterilisation bone sample is sent to an accredited laboratory for bacteriological culturing. This sample is referred to as the pre-sterilisation sample (MPRES). This is only done for 1 in 10 samples.

Table I: General screening for the suitability of tissue donors

Contraindications:

- High-risk individuals
 - Drug/alcohol abuse
 - Homosexuality
 - Prostitution
- Sexually transmitted diseases such as
 - Herpes simplex
 - Syphilis
- Any form of cancer
- HIV/Aids
- Central degenerative neurological diseases (including, infection, dementia, Creutzfeldt-Jakob disease, Alzheimer's, multiple sclerosis)
- Hepatitis, unexplained jaundice, icterus
- Septicaemia and systemic viral diseases
- Leukaemia
- Haemophilia
- Unnatural/unknown cause of death
- Auto-immune disease
- Systemic mycosis
- Clinical active tuberculosis
- Metabolic bone disease
- Hansen's disease (leprosy)
- Kaposi's sarcoma
- Myasthenia gravis
- Presence or evidence of infection of donation and surrounding site
- Significant exposure, e.g. cyanide, lead, mercury, gold, carbon monoxide

Mandatory serology:

- Human Immunodeficiency Virus Antibodies [Ab/P24ag 4th gen]
- Hepatitis B Virus Surface Antigen (HBs-Ag)
- Hepatitis C Virus Antibodies (HCV-Ab)
- Syphilis antibody

Terminal sterilisation is achieved by 25 kGy irradiation. After sterilisation, a sample is sent to an accredited laboratory for bacteriological culturing, and this sample is called the post-sterilisation sample (**MPOST1**). If no growth is found the product is released for distribution.

If any growth was found, either the radiation dose might have been insufficient, the initial bacterial load might have been high, human error on the part of the testing laboratory has occurred or contamination occurred during processing. Accordingly, the process flow chart is followed again and a second sample is sent for microbial analysis (**MPOST2**). The allograft is only released after negative bacterial growth is confirmed.

Materials and methods

This study is a retrospective review (internal audit) of allograft tissue donor files at the South African National Tissue Bank, University of Pretoria between 2003 and September 2008.

Altogether 755 donor files were reviewed. Six donors were excluded from the study because the MPREP sample was not taken or not applicable. Thus the final total of donors included in the study was n=749.

We looked specifically at the MPREP (n=749), MPRES (n=116) and MPOST (n=749) microbiology reports supplied by an accredited laboratory.

All the donors were cadaveric and the retrieval protocol explained above was followed. All the samples sent for microbial analysis consisted of iliac crest bone.

Data was analysed for positive or no bacterial growth. The positive growths were processed to a contamination percentage in terms of the MPREP, MPRES and MPOST1/MPOST2 results, which represents the bacterial contamination during the three different stages of processing allograft bone. The individual organisms were also summarised and interpreted according to their relevance.

Results

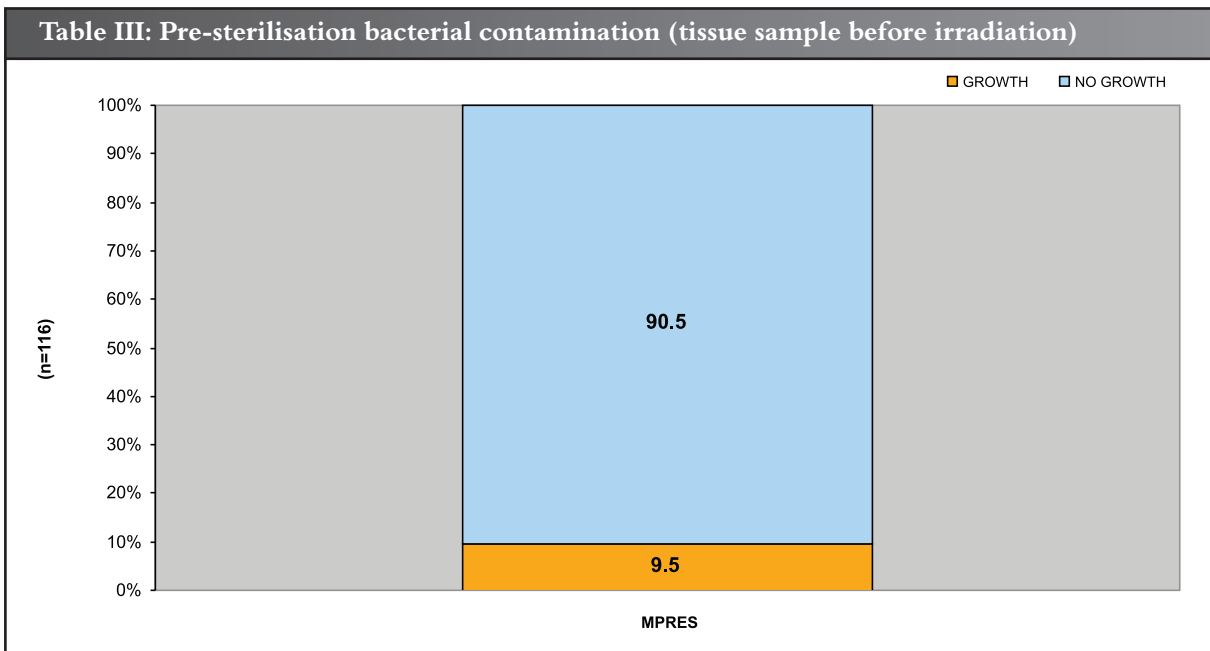
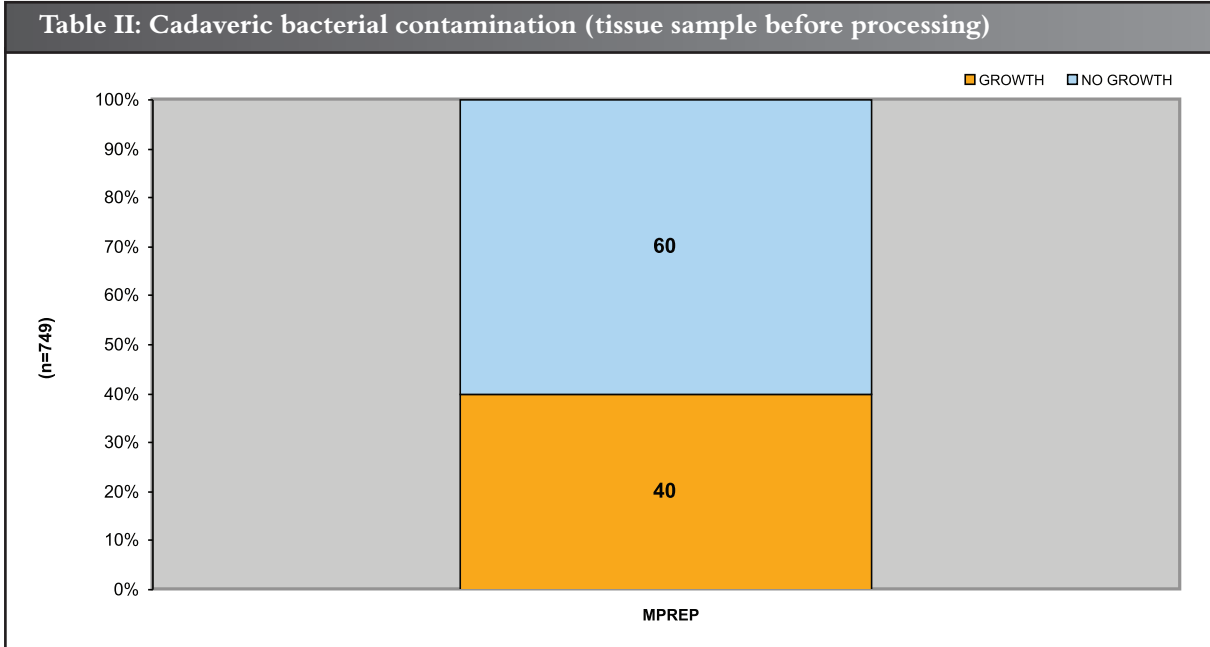
Between January 2003 and September 2008, 749 donor files were included and reviewed in the study.

A total of 299 of the 749 donors had a positive bacterial culture on the MPREP samples. Thus the cadaverous contamination rate was **40%** (Table II). More than 50% of contaminants were due to *Staphylococcus* and *Streptococcus* species. A single organism was found in 67.3% and two organisms in 27.4% of the positive MPREP samples. Only 5.3% positive donor samples could be attributed to three or four organisms.

**The allograft is only released after
negative bacterial growth is confirmed**

On the MPRES samples, 11 out of the 116 donors had a positive culture. Thus the pre-sterilisation contamination rate was **9.5%** (Table III). Of these organisms 91% were found to be *Staphylococcus* and *Streptococcus* species. Five of the positive MPRES samples had a positive growth on the MPREP results. Only two out of these five had a similar organism on the MPRES and MPREP results and in both cases it was a *Staphylococcus* spp.

Regarding the MPOST1 samples, 11 out of 749 had a positive growth; thus the post-sterilisation contamination rate was **1.47%** (Table IV). Two of the 11 samples showed two organisms namely 1) *Staphylococcus* and *Streptococcus* spp., and 2) *Streptococcus* and *Corynebacterium* spp.



The other nine had only single organisms including three with a *Streptococcus* spp, four with a *Staphylococcus* spp, one with a *Coagulation negative Staphylococcus* and one with a *Bacilli's* spp.

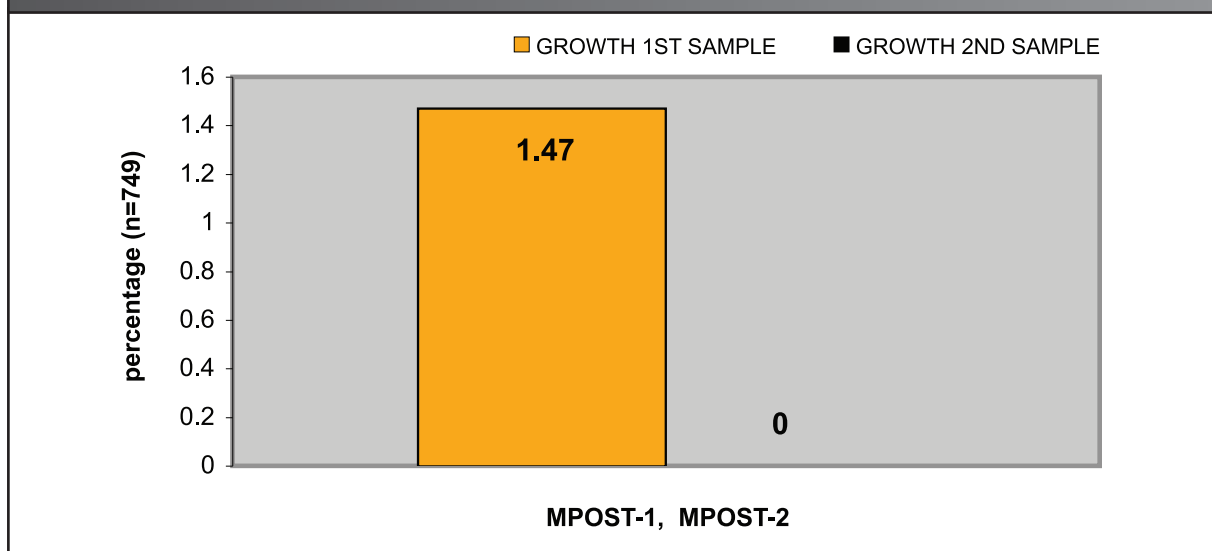
Of these positive MPOST cultures, four out of 11 donors had a positive MPREP result. Two out of this four presented with the same organism, i.e. with a *Streptococcus* spp. Of these positive MPOST results, only two out of 11 had a MPRES sample taken and on both occasions showed no growth.

A second sample was sent for micro-bacterial culturing (MPOST2). All 11 bacteriological results showed no growth. The final bacterial-contamination rate after irradiation was thus 0% (Table IV).

Discussion

Our findings of a cadaverous bacterial contamination rate of 40% compares reasonably well with other published findings.

Table IV: Post-sterilisation bacterial contamination (tissue sample after irradiation)



Journeaux and co-workers⁴ showed a 35% and Bohatyrewicz and co-workers⁶ showed a 48% cadaverous bacterial-contamination rate. Both, however, had small numbers in their studies, namely 34 and 26 cadaverous specimens respectively. Even ideal conditions like the retrieval of femur head bone in a hip arthroplasty environment yielded a bacterial contamination rate of 13% and for multi-organ donors a rate of 24%.⁴

Many publications refer to overall bacterial-contamination rates or discard rates.⁴ Thus there is also no standardisation of what is a contaminated donor.⁴ To suggest that these donors should be disregarded will waste resources, especially with the decline in our accepted donor numbers, which decreased by 35% over the last three years.

A major factor increasing the risk of bacterial contamination in the morgue is a time delay between death and bone retrieval, suggesting harvesting within 12 to 24 hours.^{6,7} For our set-up, harvesting within an average of 72 hours seems to be reasonable.

Other factors increasing bacterial contamination in the morgue includes the prevalence of micro-organisms with higher pathogenicity and whether an autopsy was performed prior to retrieval with bowel and other viscera involved.⁶ This is specifically true for the pelvic area where retrieval can be technically difficult.⁴ The majority of pathogens in our study were skin pathogens and the majority of positive cultures yielded only a single bacterium per donor harvested in the morgue.

Other useful strategies to prevent bacterial contamination are to procure samples on the left and right sides separately and to start tissue retrieval at the tibias first, followed by the femurs and then the pelvis.⁴ The use of dry ice for transportation is also not proven to decrease bacterial contamination, but is recommended.⁴

Our findings of a pre-sterilisation bacteria-contamination rate of 9.5% support the view of other publications, like that by Sommerville and co-workers,⁸ which contend that one negative microbiological culture is not enough to exclude contamination. This also supports our protocol to perform samples at different stages of processing allograft bone to ensure quality control and to assess the bacterial burden of our donors. We believe that bacterial cultures are more sensitive than conventional swabbing techniques.

We know from previous work that some gram (-) bacilli can survive radiation.⁹ More recent studies showed that a minimum of 10 kGy irradiation could destroy the bacillus strains.¹⁰ The quality process implemented at the NTB requires a minimum of 25 kGy to ensure minimal sterility insurance of 10^{-6} . We had one *Bacillus* spp. in the MPOST1 results. All the other samples in this specific batch showed no growth, meaning a contaminant was the reason for the positive growth.

None of the 11 positive cultures in the MPOST1 group showed any growth on the second culture (MPOST2). This suggests an error at the testing laboratory on the first samples, thus making our final post-sterilisation bacterial-contamination rate 0%. This supports our policy of sterilising allograft bone with gamma irradiation.

There are some limitations to our study. Firstly, we did not exclude the fact that contamination in the processing of our allograft bone could be the reason for some of the positive cultures found in the study. Secondly, this study did not comment on the contamination rate of spores, prions or viruses in our allograft bone.

Finally, this study concludes that our allograft is safe and free of bacterial contamination and that our protocols are comparable with the guidelines of the American, European and Australian tissue banks regarding the clean surgical retrieval of bone.

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No benefits of any form have been received from a commercial party related directly or indirectly to the subject of this article.

This article was not submitted to an ethical committee for approval. The content of this article is the sole work of the authors. No benefits of any form have been derived from any commercial party related directly or indirectly to the subject of this article.

References

1. Komiya K, Nasuno S, Uchiyama K. Status of bone allografting in Japan: Nation-wide survey of bone grafting performed from 1995 through 1999. *Cell and tissue banking* 2003;**4**:217-20.
2. Bohatyrewicz A, Bohatyrewicz R, Mazur R. Bone allograft harvesting following multiorgan procurement. *Transplantation Proceedings* 2002;**34**:707-8.
3. Holt G, Arthur A, Frame D. Human skeletal allograft collection: room for improvement? *Scot Med J* 2004;**49**:146-8.
4. Journeaux SF, Johnson J, Bryce SL. Bacterial contamination rates during bone allograft retrieval. *J Arthroplasty* 1999;**14**(6):677-81.
5. Bohatyrewicz A, Bohatyrewicz R, Dobiecki K. Is retrieval of bone material from multiorgan donors effective enough to cover demand for biostatic bone tissue grafts in Poland? *Transplantation Proceedings* 2006;**38**:297-300.
6. Bohatyrewicz A, Bohatyrewicz R, Klek A. Factors determining the contamination of bone tissue procured from cadaveric and multiorgan donors. *Transplantation Proceedings* 2006;**38**:301-4.
7. Kumta SM, Kendal N, Lee YL. Bacterial colonization of bone allografts related to increased interval between death and procurement: an experimental study in rats. *Arch Orthop Trauma Surg* 1997;**116**:496-7.
8. Sommerville SMM, Johnson N, Bryce SL. Contaminated femoral head allograft: incidence, bacteriology and donor follow up. *JBJS Br* 1998;**80B**(2S):135.
9. Gardner JF, Peel MM. *Introduction to sterilization and disinfection*. New York: Churchill Livingstone; 1986.
10. Nather A, Yusuf N, Hilmy N. *Radiation in tissue banking. Basic science and clinical applications of irradiated tissue allograft*. World Scientific Publishing, Singapore 2007:128.

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