

# Bioburden Levels from Saphenous Vein Grafts Recovered for Transplant

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## Revised Abstract

Saphenous vein allografts are most commonly used for limb salvage procedures. Our laboratory evaluated bioburden levels before and after the processing and decontamination of saphenous vein grafts. We evaluated 294 donated saphenous veins to determine incoming bioburden levels, as well as the efficacy of the decontamination procedure. **Methods:** Veins are cultured for aerobic and anaerobic bacteria, and fungus upon receipt (pre-culture), and again after decontamination with an antimicrobial solution and preparation for cryopreservation (post-culture). **Results:** 203 (69%) of the pre-cultures yielded no growth, while 91 (31%) were positive, with 18 of the positive cultures (20%) yielding more than one organism. 290 (99%) of the post-cultures yielded no growth. Organisms isolated are listed in the Table below:

Organisms isolated from saphenous vein graft cultures.

Culture Result	No. pre-cultures	No. post-cultures
Negative	203	290
Mixed culture	18	1
Coagulase -negative staphylococci	64	2
Bacillus sp.	19	0
Gram -negative bacilli	13	0
Anaerobes	11	1
<i>Staphylococcus aureus</i>	3	0
<i>Enterococcus faecalis</i>	2	0
Viridans streptococci	2	0
<i>Streptococcus pyogenes</i>	2	0
Diphtheroids	2	0

Bioburden levels ranged from <100 colony forming units (cfu) /mL to >100,000 cfu/mL. Eighty-five (93%) positive pre-cultures yielded  $\leq$ 100 cfu/mL, while 5 (6%) grew 100 - 10,000 cfu/mL. Only 1 (<1%) pre-culture grew >100,000 cfu/mL. Eleven of the 18 mixed cultures grew two organisms, 6 grew three organisms and 1 grew greater than three organisms.

**Conclusions:** Evaluation of pre- and post-processing bioburden levels of saphenous vein allografts are important in trending recovery techniques, environmental conditions, culture collection and transport devices. Culture results are also necessary to determine the eligibility of a tissue for transplant. With over one million tissue transplants taking place each year, assessment of tissue quality and safety is essential.

## Introduction

The prevalence of tissue allograft-associated infection is low, but exact numbers are unknown. Reporting of such infections has been incomplete due to the lack of a national surveillance system. Non-viable allografts, such as bone, cartilage and tendons can be disinfected and sterilized prior to placement. Grafts containing viable cells, such as heart valves, veins and corneas, can be treated with antibiotics, but cannot be sterilized. While all tissue is screened and tested prior to transplant, transmission of infection can occur via several different pathways. Newly infected donors may be tested during a sero-negative window or donors with systemic acute disease may be misdiagnosed. Post mortem endogenous contamination can occur at the time of tissue recovery, or contamination can come from the tissue processing environment itself.

Our laboratory cultured donated saphenous veins after initial processing, and again after antibiotic treatment. This study evaluates the incoming bioburden and type of bacteria isolated prior to decontamination of 294 these veins from 2008 to 2009.

## Materials & Methods

Tissue is shipped from recovery agencies to our facility in an isotonic solution for surgical processing of the vein. Anaerobic cultures are collected prior to processing by generously swabbing the tissue with a swab and immediately inoculating the anaerobic transport device (Starswab Transport System, Starplex, Ontario, Canada). Prior to further decontamination procedures, aerobic, anaerobic and fungus cultures are set up. **Aerobic pre-treatment culture** – a SBAP is inoculated with 250  $\mu$ l of isotonic solution from the specimen cup. The plate is streaked by making a series of passes through the inoculum in three planes. The culture is incubated in a CO<sub>2</sub> incubator at 35°C for 48 hours. **Fungus pre-treatment culture** –a Bactiflask containing Sabouraud-Dextrose agar with chloramphenicol (Remel, Lenexa, KS) is inoculated with 250  $\mu$ l of isotonic solution from the specimen cup. The culture is incubated in a non-CO<sub>2</sub> incubator at 30° for 28 days. **Anaerobic pre-treatment culture** - an anaerobically reduced SBAP and an aerobic SBAP are inoculated with the swab from the Starplex transport in the anaerobic chamber. The reduced SBAP is incubated in the anaerobic chamber for seven days. The aerobic SBAP is incubated at 35°C in the CO<sub>2</sub> incubator for 48 hours. **Aerobic and anaerobic post-treatment cultures** – an Oxoid Blood Culture bottle previously inoculated by the vein processing technologist is incubated at 35°C for 7 days. **Fungus post-treatment culture** –a Bactiflask containing Sabouraud-Dextrose agar is inoculated by processing technologist. The culture is incubated in a non-CO<sub>2</sub> incubator at 30° for 28 days.

## Results

**Table 1. Organisms isolated from positive saphenous vein cultures.**

Culture Result	No. positive pre-treatment cultures	No. positive post-treatment cultures
Coagulase -negative staphylococci (CNS)	64	2
<i>Bacillus</i> sp.	19	0
Gram -negative bacilli	13	0
Anaerobes	11	1
<i>Staphylococcus aureus</i>	3	0
<i>Enterococcus faecalis</i>	2	0
Viridans streptococci	2	0
<i>Streptococcus pyogenes</i>	2	0
Diphtheroids	2	0

**Table 2. Gram negative bacilli isolated from 13 saphenous vein pre-treatment cultures.**

Organism	#cultures positive
<i>Klebsiella pneumoniae</i>	2
<i>Enterobacter</i> spp.	2
<i>Escherichia coli</i>	5
<i>Citrobacter</i> spp.	3
<i>Shewanella putrefaciens</i>	1

**Table 3. Anaerobic bacteria isolated from 11 saphenous vein pre-treatment cultures.**

Organism	#cultures positive
<i>Propionibacterium acnes</i>	9
<i>Clostridium perfringens</i>	2

## Discussion

Despite aseptic collection of donor veins, rigorous screening of transplant tissues is required to avoid transmission of infection. Retrospective studies may shed light on not just the prevalence of allograft-associated infections, but also the source of the bacterial contamination. Our results show the most common contaminant to be CNS, followed by *Bacillus* spp. and mixed skin flora. 93% of positive pre-cultures yielded  $\leq$ 100 cfu/mL. Bacterial translocation from the GI tract does not seem to be a significant cause of contamination of saphenous veins, with only 13(4%) cultures growing enteric bacilli. Less than 1% of veins were grossly contaminated with >100,000 cfu/mL, indicating adherence to aseptic recovery techniques. 75% of all pre-treatment cultures were negative, while 99% of post-treatment cultures were negative.

## Conclusions

While overall contamination of saphenous allograft donor veins tested was 25%, most positive cultures (98%) yielded a bioburden of less than 100,000 cfu/mL, and 99% of all cultures were negative after decontamination treatment. Continued evaluation of incoming bioburden levels, and identification of the organisms will help monitor trends to determine the efficacy of the antibiotic decontamination process, as well as trending recovery techniques, environmental conditions, culture collection and transport devices. Above all, this information is necessary to determine the eligibility of a tissue for transplant. With over one million tissue transplants taking place each year, assessment of tissue quality and safety is essential.