**TITLE:** MICROBIOLOGICAL AND MOLECULAR ANALYSIS OF HUMAN CARDIAC VALVES

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## ABSTRACT:

The Human Tissue Bank (HTB) of the Pontifical Catholical University of Paraná is in charge of collecting, transporting, processing, storing and distributing cardiovascular tissues for therapeutic use. The microbiological control is essential to all steps, ensuring safety and quality of the tissues and minimizing the risks of disease transmission. This study aimed to determine the sensitivity of a protocol for microorganisms detection in liquid media during all steps of tissue processing. Routine samples from HTB without previous microorganisms detection and samples artificially contaminated with *Escherichia coli* (ATCC 25922) in concentrations of $10^{-1}$ to $10^{3}$ CFU/mL were collected from all the tissue processing steps: Transport Solution (TS), in which the heart is transported (NaCl 0,9%), Pre-Antibiotics Treatment Solution (PRE), Post-Antibiotics Treatment Solution (POS) and Washing Solution (WS). Ten microliters from all the solutions were spiked in Tryptic Soy Broth, Thioglycollate Broth and Sabouraud Broth and 1.5 mL of each broth was immediately aliquoted for DNA extraction, which was performed using lysis buffer and QIAamp DNA Blood Mini Kit® (QIAGEN), according to a protocol in development by the Carlos Chagas Institute (CCI) / Institute of Molecular Biology of Paraná (IMBP). DNA was amplified using real time PCR targeting the 16S rRNA gene (7500 Real Time PCR System, Applied Biosystems®) using a pair of universal primers for the 16S rRNA gene, a specific probe (FAM) and Master Mix ClinTaq® under development by CCI / IMBP. An amplification control (*E. coli* DNA at 1ng/µL, Microseq 500, Applied Biosystems®) in ultrapure water was used to generate a standard curve, with a detection limit of 0.0001 ng/µl $R^2$ of 0.977, with a Ct (threshold cycle) between >16.71 and < 33.08. Artificially contaminated Transport and Pre-Antibiotics Treatment solutions in Thioglycolate broth showed a Ct between >25.31±0.05 < 30.39±0.03 and >23.72±0.03 < 29.03±0.06, respectively. It was possible to detect 16S rRNA gene in all routine samples, observing a Ct between >23.57±0.13 < 30.32±0.15 for ST, >24.48±0.28 < 26.10±0.22 for PRE, >23.89±0.04 < 25.68±0.10 at POS and >24.49±0.06 < 31.05±0.21 at SL. These partial results demonstrated that this method has a superior sensitivity in comparison to manual culturing methods described in the literature, a faster turnaround time, and it could be applied for the detection of other microorganisms groups and in other contexts.

**Keywords:** Cardiovascular tissues, real time PCR, sensitivity, 16S rRNA gene

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