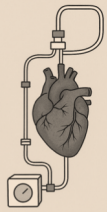

Development and testing of a PCR-based protocol to detect contamination in an isolated pig heart perfusion system

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1. Abstract

Heart transplantation is limited by donor organ shortage, and ex-situ heart perfusion (ESHP) has emerged as an important technique to preserve and assess grafts, especially after donation after circulatory death (DCD). A major limitation of ESHP is the limited perfusion time, which may be shortened by microbial contamination of the system in our experimental setup. In this thesis, perfusate samples from 14 porcine ESHP experiments were analysed for bacterial contamination using quantitative PCR (qPCR). Bacterial DNA was reproducibly detected, with contamination levels increasing continuously during perfusion. While no significant differences were found between sterile, non-sterile, and donor blood groups, pooled data revealed a significant increase over time. A positive correlation between perfusion time and bacterial load was observed. These findings demonstrate that qPCR reliably detects bacterial contamination in ESHP. The observed time-dependent increase in bacterial load highlights the potential role of contamination in limiting perfusion duration during pre-clinical experiments.

2. Introduction

Heart transplantation remains the gold standard for patients with end-stage heart failure, but donor organ shortage limits availability [1]. ESHP allows normothermic preservation and functional assessment of donor hearts, and has become essential for donation after circulatory death (DCD), where conventional cold storage is not feasible [2].

A major limitation of ESHP is the restricted perfusion time. Preclinical studies in porcine DCD models have shown that hemolysis and functional decline usually occurs after 9–12 hours, potentially linked to microbial contamination of the system [3]. Bacterial growth in the perfusate may critically affect heart function and shorten preservation time.

3. Aim and Leading Questions

To relate contamination levels to key cardiac recovery parameters, with a focus on differences between sterile and non-sterile conditions and their association with perfusion time.

4. Methods and Material

Perfusate samples from 14 porcine ex-situ heart perfusions were collected at defined time points (0 h, 1 h, 6 h, and endpoint), centrifuged, aliquoted, and stored at –80 °C. Of these, 7 were performed under non-sterile conditions (3 of which additionally received Butcher Blood), and 6 under sterile conditions. To ensure assay reliability, positive controls (bacterial DNA standards and perfusate spiked with E. coli) and negative controls (nuclease-free water, no-template controls) were included. A TATAA DNA spike-in was added to each sample to monitor extraction efficiency and detect PCR inhibition. DNA was isolated using the QIAamp DNA Blood Midi Kit (Qiagen), and concentration and purity were determined by NanoDrop. Quantitative PCR was performed on the QuantStudio™ 6 Flex System with SYBR Green chemistry using the bacterial 16S rRNA primer pair 335F769R, with all samples run in duplicate. Samples showing >1 Ct difference were repeated, and results were normalised using inter-run calibrators and the spike-in control, expressed as calibrated, normalised relative quantities (CNRQ).

5. Results

CNRQ values measured for bacterial contamination in sterile, non-sterile and donor blood experimental groups are presented in Figure 1. There was no significant difference between groups. However, higher CNRQ values could be observed in the last timepoint measurements. An increase in CNRQ values for all samples is presented in Figure 2, with a significant rise at the last time point.

There was a significant correlation between perfusion time and CNRQ at six hours (Spearman, p = 0.0303).

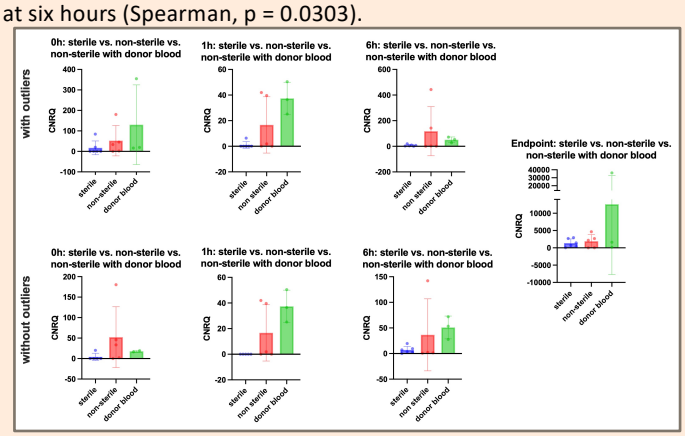

Figure 1: CNRQ values for bacterial in sterile vs. non-sterile vs. non-sterile with donor blood. The plots are shown with and without outliers. The end point has no outliers. Data are shown as mean and standard deviation. The statistical test used was Kruskal-Wallis test with multiple comparison. N=3-6/group. CNRQ: calibrated normalised relative quantity.

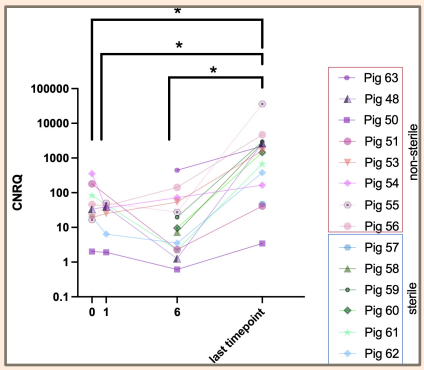

Figure 2: Representation increase of all samples. The CNRQs are given in logarithms, so samples with a value of 0 are not shown. The statistical test used was the Friedman with multiple comparisons. Adjusted p value from 0h. vs. last time point:0.0138. Adjusted p value from 1 vs. last Timepoint: < 0.0001. Adjusted p value from 6h vs. last time point: 0.0138. N=14. CNRQ: calibrated normalised relative quantity.

6. Discussion and conclusion

Bacterial contamination was reproducibly detected by qPCR and showed a continuous increase over perfusion time. Although no statistically significant differences were observed between sterile, non-sterile, and donor blood groups, the pooled data demonstrated a clear and significant rise in bacterial load as perfusion progressed. A tendency toward lower contamination under sterile conditions was observed, but bacterial overgrowth still occurred at later time points. The influence of donor blood could not be determined due to the small sample size.

Overall, these results suggest that bacterial contamination increases with perfusion time and has an influence on perfusion time.

List of References:

[1] Arnold et al., 2024, pp 1-2

[2] Tozzi, 2022; Swisstransplant, 2025, pp.12-13

[3] DBMR,2024. Perfusion protocol, internal document

Figure

Figure 1 Guarriello, 2025m, Sterile vs. Non-sterile vs. Non-sterile with donor blood. medi.

Figure 2 Guarriello, 2025r, Representation increase of all samples. medi.