X-ray phase contrast imaging of endomyocardial biopsy samples preserved in formalin and embedded in paraffin – a comparison of tissue preparation methods

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Background: Endomyocardial biopsy (EMB) is the gold standard in heart transplantation (HTx) followup, with samples commonly fixed with formalin, and then embedded in paraffin for histology analysis. Recently, EMB samples have been scanned with synchrotron X-ray phase-contrast imaging (X-PCI) to assess graft rejection.¹ We aim to compare imaging time efficiency and image quality between formalin-fixed and paraffin-embedded samples to determine the optimal scanning methodology.

Methods: Three adult patients undergoing EMB after HTx were included. EMB samples were initially stored in formalin and imaged by X-PCI at the Paul Scherrer Institute TOMCAT beamline (Villigen, Switzerland). On site samples were scanned in glass tubes in deionised, degassed water, and then embedded in paraffin, positioned on a holder, and scanned again using a multi-scale beamline set-up. Imaging time efficiency was measured by on-site sample preparation and scan time, and image quality was assessed with signal-to-noise ratio (SNR) and pixel resolution. Post-processing comparison included fibrosis quantification (using Ilastik for segmentation and Fiji for calculating the average percentage of collagen in 3 selected areas) and graft-rejection grading (assessed by two blinded observers based on the ISHLT 2004. criteria)².

Results: Scanning F1-F3 and P1-P3 samples produced the same imaging resolution, while F1-F3 samples exhibited higher SNR values (clearer sample visibility) (**Table 1**). On site preparation and scan time were shorter with P1-P3 samples. Fibrosis quantification produced similar results in all samples, with

TABLE 1. Imaging time (including preparation and scanning), technical parameters and imaging dataanalysis between the two sample preparation methodologies.

Sample	Methodology	Imaging time efficiency		Technical image quality	Image post-processing analysis	
		On-site preparati- on time (min:sec)	Scan time (min:sec)	SNR (dB)	Average percentage of collagen in 3 selected areas (%)	Rejection grading (ISHLT 2004. criteria)
F1	Formalin	3:58	49:08	112,16	0.34	1R
P1	Paraffin	0:17	6:34	72,86	0.21	0R
F2	Formalin	4:13	37:24	119,39	0.16	1R
P2	Paraffin	0:20	12:03	54,65	0.11	0R
F3	Formalin	4:21	49:08	112,19	0.37	0R
P3	Paraffin	0:32	12:08	56,72	0.12	0R

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7th Cardiology Highlights International Update Meeting in Cardiology October 19-22, 2023 | Dubrovnik, Croatia F1-F3 showing slightly higher collagen percentage compared to the corresponding P1-P3 samples (**Table 1** and **Figure 1**). Samples F1 and F2 were graded as 1R, with others classified as 0R (ISHLT 2004.) (**Table 1**).



FIGURE 1. Left side of the figure showing X-PCI images of formalin samples and the right side of the figure showing X-PCI images of the same samples in paraffin. Both set of samples are marked with the corresponding tissue areas for the collagen segmentation and quantification (collagen shown in light blue).

Conclusion: Embedding EMB samples in paraffin is more time efficient in terms of on-site sample preparation and imaging. Results showed similar fibrosis quantification regardless of preparation methods, whereas rejection grading did not differ in clinically meaningful way. In conclusion, in initial testing using small sample number, no significant difference was found between the preparation methods.

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LITERATURE

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