Review Article

# Processing of the Explanted Heart

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## Abstract

Analysis of the explanted hearts from heart transplant recipients provides valuable clinical samples, which can be used to study the anatomy and pathology of the heart. PubMed database was employed as the article source of this review. This article summarized the processing methods of the explanted heart, including dissection, histopathologic examination, cryopreservation, and genetic testing. A standard processing of explanted hearts ensures the quality and reliability of samples. Analysis of explanted hearts facilitates the diagnostic assessment and therapy strategy of heart diseases.

**Keywords:** Cryopreservation, Dissection, Explanted heart, Genetic testing, Histopathologic examination

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## Introduction

The explanted hearts of heart transplant recipients are precious samples for studying cardiac pathology. Furthermore, examination of explanted hearts can guide posttransplant therapy and prognosis. Due to the progress of heart transplant surgery, the number of explanted heart samples increased. However, there is not a routine regulation of the processing of explanted heart samples so far. On one hand, different from other tissue samples, the explanted hearts own complex anatomy and unique pathological features. On the other hand, explanted heart samples are directly obtained from end-stage heart disease patients who undergo complex transplant procedures, the following tissue processing is quite special. In addition, genetic diagnosis shows potential development in recent years and demands strict tissue processing regulations of samples. Therefore, we must pay attention to the processing of the explanted heart, ensuring credible analysis of cardiac samples.

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In this article, we aimed to summarize the processing methods of the explanted heart. Dissection, histopathologic examination, cryopreservation, and genetic testing are described in this article in detail.

Before examining the explanted heart, we should document the baseline characteristics of patients, including age, sex and diagnosis, etc. Then obtain approval of the Ethics Committees and informed consent from all patients or their parents or guardians.

## Dissection and Measurement

The standard dissection of the explanted heart is the first key point. There should be a systematic evaluation of heart transplantation patients before dissection, including family history, symptoms, image characteristics, and interventions. Pathologists should be familiar with normal cardiac anatomy, even congenital heart defects as well. The dissection technique must allow full photographic and histologic documentation while maintaining the integrity of the specimen for the following pathological examination and preservation. The structure of the explanted heart is based on the surgical procedure. In general, explanted hearts are not intact.

Fresh tissue samples are regarded as infected, so tissue collection should be under sterile conditions. Remove the explanted heart from the patient in heart transplantation operation. Keep the heart untreated, place it on wet ice in a sealed and sterile container, do not immerse the heart in any liquid. Transfer the sample container to the pathology department or tissue bank immediately. Upon arrival at the tissue bank, the procedure is done aseptically on operating microbench [Figure 1]. Rinse the sample with 4°C sterile saline solution. Removes blood and thrombosis from the cardiac cavities and the aorta. Measure the heart weight [Figure 2]. Explanted heart is an excellent source of usable aortic and pulmonary valves for valve replacement. Before dissection, assess the suitability of aortic and pulmonary valves for homograft. Da Costa *et al*.[1] Described methods for the homograft valve preparation, preservation, and storage in detail [Figure 3]. Next, systemically examine the epicardium, myocardium, endocardium, arteries, and veins. The dissection technique differs between ischemic heart disease and cardiomyopathies. Sampson offered a general review of dissection techniques used for different cardiac conditions leading to transplantation, including ischemic heart disease, cardiomyopathy, congenital heart disease, valvular disease, and the allograft.[2] If heart transplant recipient has a severe electrophysiology abnormality, the conduction system should be examined. Yanagawa and Nakajima showed a simple dissection guide for the conduction system of the human heart.[3] Except cardiac tissue, according to actual conditions, evaluate adhesions and prior surgical interventions or any related cardiac devices, including mechanical heart valves or bioprosthetic heart valves, ventricular assist devices, pacemakers, and defibrillators.

Dissection of explanted hearts is a challenge to the pathologists. Consultation with clinical doctors is often helpful. Various dissection techniques applied are based on different cardiac pathologic conditions. An accurate and comprehensive dissection permits good subsequent histopathologic examination and preservation.

Note:

The first 1 h after removal of heart from heart transplant patients is the "golden period". Transfer samples as rapidly as possible to reduce the impact on gene expression by hypoxia, and degradation of RNA and other tissue ingredients.

## Histopathologic examination

The explanted heart provides a good sample for histologic sections. After dissection and measurement, there should be a comprehensive histopathologic evaluation. Objective diagnostic evaluation should be

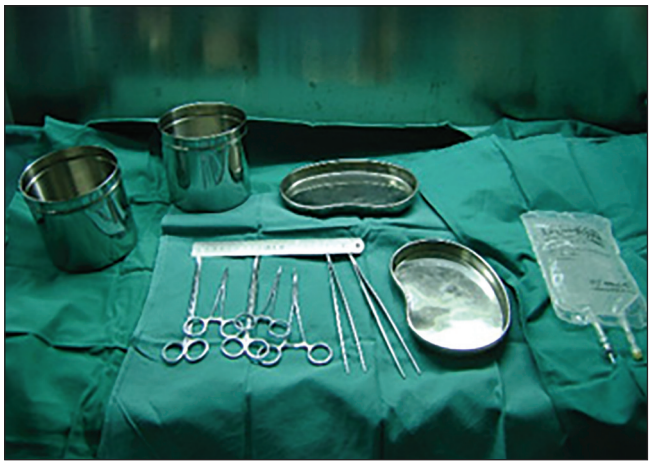

**Figure 1:** Well-prepared operating microbench. The microbench should be irradiated by ultraviolet rays for 30 min before use. All instruments used should be sterilized

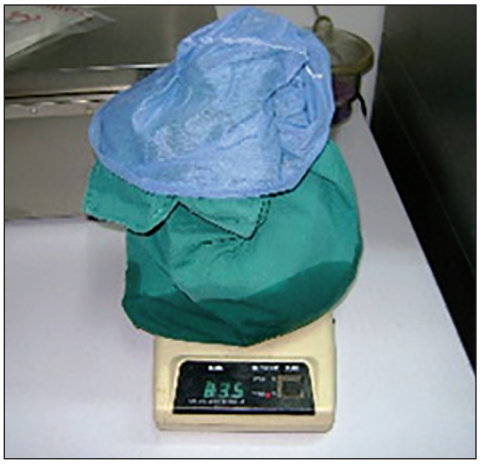

**Figure 2:** Weighing the explanted heart. Rinse and remove thrombosis in chambers, drain excessive liquid, put the heart in a sterile bag and weigh

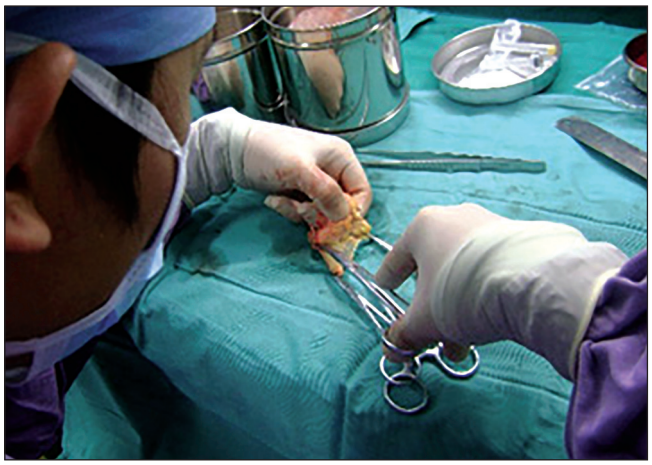

**Figure 3:** Processing of the valve. The aortic and pulmonary valves are separated and trimmed, avoid damaging the walls or the leaflets. The valve cusps must be free of atheroma and calcification

based on morphological, histologic, and histochemical changes. Histopathologic examination can identify concordance between the pretransplant diagnosis and posttransplant diagnosis of the explants.

Excise specimens after dissection, the average size of each section block is 0.6 $cm^3$. Tissue blocks are immediately fixed at 10% buffered formaldehyde solution. Sections submitted in formalin should include a minimum of four sections of the ventricles (interventricular septum, anterior wall, lateral wall, and posterior wall); a section of any valve lesions; a section of the mitral and aortic valves; representative sections of each of the coronary arteries and any additional area showing gross pathologic changes.[4] After fixation, these blocks are embedded in paraffin and cut into serial 4 μm thick tissue sections (average size of 80 $mm^2$) for histological staining. Myocardial tissue sections are stained with hematoxylin eosin and Masson trichrome according to standard protocols. Coronary vessel sections are stained with hematoxylin eosin and Elastic van Gieson. All tissue sections are examined in a blinded manner by two histopathologists. Document histologic features of each section: inflammation, interstitial fibrosis, fibrofatty replacement, hypertrophy, atrophy, cellular edema, necrosis. Classify the extent of each feature and record the results using a scoring system. If observing myocarditis or inflammatory cell infiltration under light microscope, a further immunohistochemical analysis of T lymphocytes (CD3) and macrophages (CD68) is necessary.[5,6] For suspected amyloid change sections, diagnosis should be confirmed by Congo red staining.

If the heart transplant patient has a clinical history of severe arrhythmia or conduction block, examination of the conduction system can provide a better understanding of the pathogenesis of heart disease. In general, the conduction system sections should be stained with Masson trichrome.

Routine electron microscopy of explanted heart samples is not required. Only cardiac samples with cardiomyopathy, suspected Adriamycin toxicity or abnormalities could not be identified by light microscopic examination should be given to electron microscopy. A fragment of 1 $mm^3$ is cut from every specimen and fixed in 4% glutaraldehyde for further evaluation by transmission electron microscopy.

When freezing tissue for frozen sections, freeze artifacts may occur. The best frozen sections are obtained when the tissue is frozen as rapidly as possible. Techniques for suitable freezing are isopentane cooled by liquid nitrogen and liquid nitrogen.[7] Isopentane is an agent with a high thermal conductivity which has been cooled to approximately −160°C by immersion of the liquid nitrogen. A 1.8-ml cryogenic vial of isopentane is suspended in a flask of liquid nitrogen. When the temperature of the isopentane reaches its melting point (−160°C), opaque droplets emerge at the bottom of the cryogenic vial. At this moment, submerge the biopsy tissue in this prechilled isopentane cryovial immediately. At this point, the tissue can be processed for rewarming, optimal cutting temperature embedding, and frozen sections.

## Cryopreservation

There are two preservation manners of the cardiac samples, one portion of the sample is fixed in formaldehyde solution and embedded in paraffin wax at room temperature, the other portion is cryopreserved in liquid nitrogen. Tissue samples preserved in paraffin blocks can be used for morphological staining, such as hematoxylin eosin and Masson trichrome stain. While tissue samples cryopreserved, can be used for fluorescence *in situ* hybridization, or as a molecular biological source of DNA and RNA, or for frozen sections. Compared with DNA and proteins, RNA degrades at the time of removal of the explanted heart from the patient. RNA degradation by ribonucleases already present in the cell or originating from bacteria or other environmental contamination with rapid postmortem and *in vitro* decay, and neonatal RNA generation of the stress response during tissue sampling, could affect the quality of RNA in samples. Therefore, stabilizing RNA status is an essential prerequisite for accurate quantitative analysis of gene expression, such as laser microdissection, tissue microarray, and real-time quantitative polymerase chain reaction (PCR).

In order to prevent RNA degradation and contamination of bacteria genome, the cryopreservation procedure should be under low temperature and aseptic conditions. After dissection and measurement, retain tissue block of the atria and ventricle, and any macroscopic pathologic tissues. Wash the specimen with icy sterile normal saline solution to prevent contamination of blood genome. Put the specimen on a pallet with an ice bag underneath. Separate epicardial adipose tissue from myocardium [Figure 4]. Sect the specimen into 0.5 cm × 0.5 cm × 0.5 cm blocks while avoiding destroying the integrity of the specimen. We prefer to freeze the tissue using liquid nitrogen, the tissue blocks are then filled in the 1.8-ml cryogenic vial, the cap is tightened, and the vial is immediately immersed in liquid nitrogen [Figure 5]. Tissue stored in liquid nitrogen can be used for future study.

Pieces of tissue should be cryopreserved after removal of the explanted heart of a patient. If the

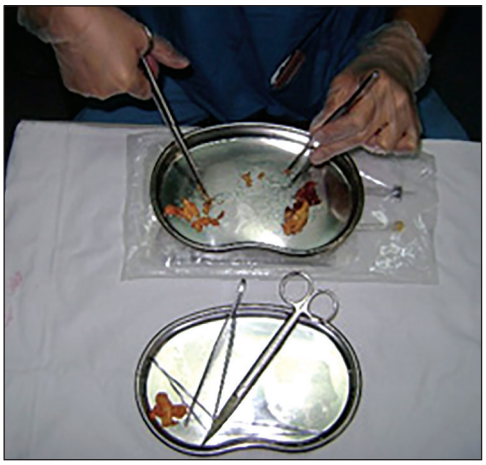

**Figure 4:** Separating epicardial adipose tissue. Epicardial adipose tissue is located between the myocardium and visceral pericardium around both ventricles. Remove it from myocardium when cutting blocks. However, do not remove the fatty or fibrofatty tissue from the myocardium of the right ventricular free wall in arrhythmogenic right ventricular cardiomyopathy samples

specimen cannot be cryopreserved in liquid nitrogen immediately, it should be immersed in RNAlater solution for protection from RNA degradation. The RNAlater solution can inactivate RNase irreversibly and prevent tissue and cells from RNA degradation at utmost. The thickness of the tissue block preserved in RNAlater solution should be <5 mm. The volume of the RNAlater solution is more than 10 times of the tissue blocks'. For tissue preserved in RNAlater solution, RNA can be kept stable at 37°C for 1 day, 25°C for 1 week, 4°C for 1 month and −20°C for long-term. After that period, when conditions permit, remove the RNAlater solution and transfer samples to liquid nitrogen for long time storage until use.

The preservation procedure affects the quality of tissue samples, which determines the probability for future use. In order to ensure optimal preservation of the tissue, some rules should be followed:

1. Plan ahead. Before preservation, all materials and instruments should be well-prepared, including sterilizing apparatus, icy sterile normal saline solution, ice bags, sterile 1.8-ml cryogenic vial (marked with a sample number and tissue region), liquid nitrogen jar
2. Avoid touching the tissue directly with the hands, keep the tissue at low temperature
3. The expression level of some mRNAs is very susceptible to ischemia times. The preservation procedure should be processed as soon as possible, shorten the time of cold ischemia (at 4°C) and enhance RNA stability
4. Tissue preserved in RNAlater solution can be stored at −80°C

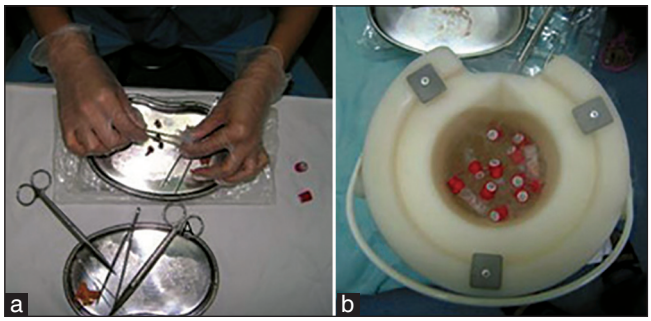


**Figure 5:** Cryopreservation of cardiac samples. (a) Cut the tissue with a sharp blade, avoid squeezing the tissue when handling with smooth forceps. (b) Tighten the cap and prevent tissue from touching liquid nitrogen

5. Cryopreservation in the lipid phase of liquid nitrogen has inherent hidden dangers. It is better to preserve tissue in the vapor phase of liquid nitrogen.

## Genetic testing

Viral infections in the myocardium play a major role in the pathogenesis of myocarditis and cardiomyopathies. Persistence of virus species aggravates myocardial injury and heart failure. An accurate diagnosis of viral genomes in the myocardium facilitates etiology finding and antiviral treatment. On the other hand, genetic testing for inherited cardiac disease may have great implications for family members and prevent sudden death. Therefore, microbiology and genetic studies of the myocardium may be helpful to viral and inherited cardiomyopathies.

Partemi described the procedure of total RNA isolation, cDNA synthesis, and real-time PCR reactions using frozen cardiac tissue stored in liquid nitrogen.[8] It should be noted that frozen human heart samples are cut into tiny slices on top of a dry ice tray to avoid thawing, and then pulverize the heart muscle slices in liquid nitrogen in a friction mortar, isolate RNA with Trizol reagent according to the manufacturer's instructions. To minimize possible nucleus-mediated RNA degradation during experimental workflow, all materials, and working surfaces were cleaned using RNase Away. After reverse transcription of RNA into cDNA, amplify viral sequence using PCR. Evaluate the data and compare the gained sequences with virus sequences from the National Center for Biotechnology Information Genebank database.[9]

Besides viral sequence detection in the myocardial tissue of viral cardiomyopathies, genetic testing for inherited cardiac disease is a potentially useful tool for clinicians to correctly identify and diagnose inheritable conditions. With the development of next generation sequencing, the investigation of the genetic basis of cardiomyopathies

will improve understanding of the pathophysiology of these diseases.

Notes:

1. When testing viral sequences in myocardial tissue, extract DNA from peripheral blood cells for viral assessment to exclude systemic viral infection
2. While testing viral genomes of explanted hearts samples, perform histologic evaluation as well. Evaluate myocardial inflammation and infiltrating immune cells
3. Because of a possible nonhomogenous focal pattern of the viral sequences in the myocardium and to prevent false-negative patients and sampling error, samples for genetic testing should be obtained from five regions of the left and right ventricular walls: Right ventricular anteroseptal region, right ventricular posteroseptal region, left ventricular anterior region, left ventricular posterior region, and left ventricular apex.[9]

## Summary

We review general considerations and guidelines for processing of explanted heart samples. Sequencing technology develops fast and demands a high quality of samples. A standard processing and preservation procedure will guarantee the reliability of specimens for future study.

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## References

1. Da Costa ML, Ghofaili FA, Oakley RM. Allograft tissue for use in valve replacement. Cell Tissue Bank 2006;7:337-48.
2. Sampson BA. Examination of the cardiac explant. Surg Pathol Clin 2012;5:485-95.
3. Yanagawa N, Nakajima Y. A simple dissection method for the conduction system of the human heart. Anat Sci Educ 2009;2:78-80.
4. Westra WH, Hruban RH, Phelps TH, Isacson C. Surgical pathology dissection. An Illustrated Guide. 2nd ed. New York, Springer; 2003. p. 94-101.
5. de Leeuw N, Ruiter DJ, Balk AH, de Jonge N, Melchers WJ, Galama JM. Histopathologic findings in explanted heart tissue from patients with end-stage idiopathic dilated cardiomyopathy. Transpl Int 2001;14:299-306.
6. Sheppard MN. Approach to the cardiac autopsy. J Clin Pathol 2012;65:484-95.
7. Bancroft JD, Gamble M. Theory and practice of histological techniques. 6th ed. London, Churchill Livingstone; 2008. p. 99.
8. Partemi S, Berne PM, Batlle M, Berruezo A, Mont L, Riuró H, *et al*. Analysis of mRNA from human heart tissue and putative applications in forensic molecular pathology. Forensic Sci Int 2010;203:99-105.
9. Hartyánszky I Jr, Tátrai E, Lászik A, Hubay M, Szelíd Z, Acsády G, *et al*. Patchy myocardial pattern of virus sequence persistence in heart transplant recipients — Possible role of sampling error in the etiology. Transplant Proc 2011;43:1285-9.

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