# STANDARD OPERATING PROCEDURE

## Heart Pilot Case Processing

UF OPPC-SOP-01

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# HEART CASE PROCESSING

**POLICY:** Use universal safety precautions when handling human samples and use appropriate personal protective equipment. Follow biohazardous and chemical safety procedures and dispose of waste tissues according to UF EH&S guidelines. Handle sharps carefully and dispose in sharps containers. Follow aseptic procedures throughout processing.

**PURPOSE:** The purpose of this Standard Operating Procedure (SOP) is to outline procedures for processing and storing heart and other human samples including serum and whole blood by the UF Organ Processing and Pathology Core (OPPC).

**SCOPE:** This SOP will be applied to all samples recovered through the UF Heart Pilot program.

**RESPOSIBILITIES:** Managers and supervisors - are responsible for making sure that technicians are properly trained and equipment and facility are maintained in good working order.
Laboratory personnel - are responsible for reading and understanding this SOP and related documents and performing these tasks in accordance with the SOPs. They are responsible for following clinical laboratory and tissue banking best practices.

**EQUIPMENT & MATERIALS:** The materials, equipment and forms in the following list are recommendations only and alternative products may be substituted for the site specific task or procedure.

- Kimberly-Clark Benchtop Protector (Fisher, Cat. No. 15-235-101)
- Leica L'Absorbs towel (Leica, Cat. No. 3803240)
- Double Edge Pathology Blades for Pathco Handle (Fisher, Cat. No. 23-720-200)
- Pathco Handle (Fisher, Cat.No. NC9552049)
- Dissection Board (Fisher, Cat. No. 36114)
- Dissecting Forceps (Fisher, Cat. No. 13-812-40)
- Dissecting Scissors (Fisher, Cat.No. 08-940)
- Microtubes with Silicone O-ring (VWR, Cat. No. 89004-302)
- Invitrogen RNALater™ Stabilizing Solution (Invitrogen, Cat. No. AM7021), store at room temperature
- D-PBS 1X without $Ca^{2+}$ or $Mg^{2+}$ (Fisher, Cat. No. MT21040CM), store at 4°C
- DMEM/F12 50/50 Media, with L-Glutamine and 15mM HEPES, (Fisher, Cat. No. MT10092CM)
- Antibiotic-Antimycotic solution, 100x, 10,000 I.U./ml Penicillin 10,000 µg/ml Streptomycin 25 µg/ml Amphotericin B (Fisher,

- 32 oz. Screw Top Polypropylene Histology Container (Fisher, Cat. No. 22-026-315)
- EDTA Powder, 500g, ACS Grade, 99.4-100.6% (Sigma, E9884-500G)
- 52 oz. Plastic Container with Lid (Fisher, Cat. No. 02-544-127)
- 10% Neutral Buffered Formalin (NBF) (Fisher, Cat. No. 23-245-685)
- Embedding LWS Uni Cassettes (Electron Microscopy, Cat.No. 62500-GR)
- Tissue-Tek Mega Cassette (Electron Microscopy, Cat.No. 62512-30)
- Disposable Base Molds, 15x15mm, 24x24mm, 30x24mm, 37x24mm (Fisher, Cat.No. 22-050-159 to 162)
- Disposable Deep Base Mold for Macro-Cassette (Electron Microscopy, Cat.No. 62353)
- Tissue-Tek O.C.T. Compound Media (VWR, Cat.No. 25608-930)
- Parafilm M™ Wrapping Film (Fisher, Cat.No. S37440)
- Kimberly-Clark Fluidshield Fog-Free Protective Mask (Fisher, Cat. No. 19-003-495)
- Disposable Lab Coats
- Nitrile gloves
- Permanent marker
- Biosafety cabinet
- Cat. No. MT30004CI), aliquot 5 ml and store at -20°C
- HyClone Fetal Bovine Serum (Fisher, Cat. No. MT35016CV), aliquot 50 ml and store at -20°C
- High-Performance centrifuge tubes, 15 and 50 ml, Sterile (VWR, Cat. No. 89039-666 and 89039-658)
- Sterile Nunc Cryotubes (Thermo Sci, Cat No. 375418)
- Weigh boats
- Pipettes and sterile filter tips (20 µl, 200 µl, 1000 µl)
- Dry ice
- 2-Methylbutane
- Magic Touch 2™ Ice Pans, 9L with lid (Bel-Art, Cat.No. M16807-9104)
- Integra Miltex Surgical Instrument Cleaner (Fisher, Cat.No. 12-460-424)
- PDI™ Super Sani-Cloth™ Germicidal Disposable Wipes (Fisher, Cat. No. 23-100-124)
- Diversey™ Virex® Tb Disinfectant (Office Depot, Cat.No. 898168)
- 70% ethanol
- Concentrated bleach (6% sodium hypochlorite)
- Biohazard sharps containers
- Label printers (cab EOS1, Brady BSP31 Label Attachment System)
- Centrifuge
- Microtube racks
- Balance, 200g

**PROCEDURE:**

## 1.0 Identification of tissue and aliquots from samples

1.1 Sample Type Nomenclature and Abbreviations:

**Table 1. Sample Type Nomenclature and Abbreviations**

| Sample Type | Sample Type Abbreviation |
|---|---|

| | |
|---|---|
| Aorta | Aorta |
| Aortic Valve | AV |
| Apex | Apex |
| Common Carotid Arteries | CCA |
| Left Anterior Descending Artery | LAD |
| Left Atrium, Appendage | LA-A |
| Left Atrium, Free Wall | LA-FW |
| Left Circumflex Artery | LCx |
| Left Main Coronary Artery | LM |
| Left Ventricle, Anterior Apical | LV-AA |
| Left Ventricle, Anterior Basal | LV-AB |
| Left Ventricle, Anterior Middle | LV-AM |
| Left Ventricle, Lateral Apical | LV-LA |
| Left Ventricle, Lateral Basal | LV-LB |
| Left Ventricle, Lateral Middle | LV-LM |
| Left Ventricle, Posterior Apical | LV-PA |
| Left Ventricle, Posterior Basal | LV-PB |
| Left Ventricle, Posterior Middle | LV-PM |
| Mitral Valve | MV |
| Right Atrium, Appendage | RA-A |
| Right Atrium, Free Wall | RA-FW |
| Right Coronary Artery | RCA |
| Right Ventricle, Anterior Apical | RV-AA |
| Right Ventricle, Anterior Basal | RV-AB |
| Right Ventricle, Anterior Middle | RV-AM |
| Right Ventricle, Posterior Apical | RV-PA |
| Right Ventricle, Posterior Basal | RV-PB |
| Right Ventricle, Posterior Middle | RV-PM |
| Septum Apical | SPM-A |
| Septum Basal | SPM-B |
| Septum Middle | SPM-M |

| | |
|---|---|
| Whole Blood | PBMC |
| Whole Blood | Plasma |
| Serum | Serum |

1.2 Aliquots from samples will be identified as follows:

| **Table 2. Aliquot Type** |
|---|
| OCT |
| Paraffin |
| Vial |

**2.0 Case number assignment**

2.1 Heart organ donors will be assigned sequential case numbers for the University of Florida processing facility.

**3.0 Aliquot labeling**

3.1 Refer to UF OPPC SOP Tissue Sample Archiving

3.2 Cassettes for formalin-fixed paraffin embedding
- 3.2.1 Line #1: Case ID + Block # + Sub-division where applicable (e.g., 6101-01A)
- 3.2.2 Line #2: Sample type abbreviation (Section 1.1)
- 3.2.3 Line #3: 2D Barcode

3.3 OCT cryomolds
- 3.3.1 Line #1 and #2: As for cassettes

3.4 Cryovials and Fresh tissue
- 3.4.1 Line #1 Case ID + aliquot number
- 3.4.2 Line #2: Sample type abbreviation (Section 1.1)
- 3.4.3 Line #3: Aliquot type (Section 1.2)
- 3.4.4 Line #4: 2D Barcode

**4.0 Data collection**

4.1 Sample processing and donor data will be recorded in the nPOD databases. Access will be limited to UF Organ Processing and Pathology Core staff and will be granted by the Administration or OPPC Director.

4.2 Use a single line to strikethrough corrections, then initial and date.

4.3 Identify and record all shipment contents.
- 4.3.1 Photograph interior of container if there is any packing abnormality (ie. melted ice, missing items).
- 4.3.2 In the event of any shipment error, contact on-call administration staff who will notify the OPO.
- 4.3.3 Complete the Recovery Feedback section on the Case Worksheet.

4.4 Completely fill out the case worksheet form during processing.
- 4.4.1 After the case has been processed, archive the case worksheet and enter all data following SOP 52 Case Data Management and SOP 55 Sample Data Management.

**5.0 Sterile media preparation**

5.1 Complete media

5.1.1 Remove and discard 50ml from 500ml DMEM/F12 media.
5.1.2 Add 50ml FBS to the 450ml DMEM/F12 for a final concentration of 10% FBS.
5.1.3 Add 5ml 100X antibiotic/antimycotic stock.
5.1.4 Label the sterile media container with preparation date, additives, and preparer's initials. Store at 4°C for up to one month.

5.2 Phosphate-Buffered saline
5.2.1 Add 5ml 100x antibiotic/antimycotic stock to 500ml 1x D-PBS (without $Ca^{2+}$ or $Mg^{2+}$).
5.2.2 Label the sterile D-PBS container with the preparation date, additive, and preparer's initials. Store at 4°C for up to one month.

**6.0 Fixation and freezing preparation**

6.1 Add 5-10 lbs dry ice pellets to an ice pan and cover.
6.2 Add 5 lbs dry ice pellets and vial racks to a second ice pan, fill pan ~5 cm with 2-Methylbutane and cover. Ensure enough 2-Methylbutane is added such that vials are 75% submerged.
6.3 Pour 10% NBF into two 52 oz. plastic containers until half full, then secure lids.
6.3.1 Label one container with case ID number, date, and "10% Neutral Buffered Formalin" for cassettes.
6.3.2 Label second container as "Waste Tissue, 10% Neutral Buffered Formalin".

**7.0 Tissue sample and blood processing**

7.1 Whole Blood
7.1.1 Mix one tube well by inversion, then aliquot 100 µl for HbA1c testing using the DCA Vantage.
7.1.2 Refer to SOP 59 Isolation of PBMC for further processing of whole blood into PBMC.
7.1.2.1 To prepare plasma aliquots: Centrifuge desired quantity of tubes at 1300 x g for 10 minutes at room temperature.
7.1.2.2 If hemolysis observed, record the degree and re-centrifuge at the same settings for an additional 5 minutes.
7.1.2.3 Aliquot 700 µl of plasma into labeled O-ring microtubes. Snap freeze in 2-methylbutane/dry ice then place on dry ice.

7.2 Serum
7.2.1 Centrifuge tubes at 1300 x g for 10 minutes at room temperature.
7.2.2 If hemolysis observed, record the degree (i.e., light or gross) and re-centrifuge at the same settings for an additional 5 minutes.
7.2.3 Aliquot 300 µl of serum into the first three labeled O-ring microtubes, then 700 µl into subsequent aliquots. Snap freeze in 2-methylbutane/dry ice, then place on dry ice.
7.2.4 Aliquots 1 through 3 will be used for autoantibody, C-peptide, and QC analysis. Refer to SOP 85 C-Peptide Determination and SOP 22 Autoantibody Screening Process.

7.3 Heart
7.3.1 If necessary, remove and discard surrounding adipose using blunt dissection technique and surgical dissection tools as needed.
7.3.2 Document external examination of the heart, artery dominance, obtain weight, and record all external gross findings according to case worksheet.
7.3.3 Take photograph of entire heart prior to sample processing.

7.4 Coronary Artery Processing

7.4.1 Section the major coronary arteries including left main, left anterior descending, left circumflex, and right coronary by making serial sections on a transverse plane at 3-5 mm intervals throughout the entire length of the artery. (Appendix 1, Figures 1 and 2).

7.4.2 Document visual signs of arterial thrombosis or atherosclerosis. Mark obtained sections as 'disease' or 'disease-free' on the Heart Case Processing worksheet (Appendix 2).

7.4.3 Three sample types will be made in each sample set in the following order: formalin-fixed paraffin-embedded (FFPE), flash-frozen tissue in vials, and fresh frozen in OCT (Appendix 1, Figure 2).

7.4.4 FFPE and OCT blocks

7.4.4.1 Alternate sections for FFPE and OCT blocks.

7.4.4.2 Place 3-5 mm thick sections for FFPE processing in labeled cassettes, then place cassettes in container with 10% NBF labeled with the case ID.

7.4.4.2.1 Record the processing start time when the first cassette is placed in fixative and end time when the last cassette is placed in fixative.

7.4.4.3 Place enough OCT media to cover the bottom surface of the cryomold, then place one 3-5 mm thick section on top of media in the center of the mold. Add OCT until the tissue is covered.

7.4.4.4 Place the cryomold in the dry ice/2-methylbutane bath for 120 seconds, then transfer the frozen block to the dry ice container.

7.4.5 Flash frozen vials

7.4.5.1 Mince tissue and place in microtubes, with 0.5g of tissue per vial.

7.4.5.2 Place the vial in the dry ice/2-methylbutane bath for 120 seconds, then transfer to the dry ice container.

7.5 Ventricle Dissection and Processing

7.5.1 Cut off apex.

7.5.2 Document abnormal gross findings.

7.5.3 Section ventricles on a transverse plane into three equal segments from the apex to the atria representing basal, mid, and apical portions of the ventricles (See Appendix 1, Figure 3).

7.5.4 Document abnormal internal gross findings and measure wall thickness of each ventricle.

7.5.4.1 Abnormal findings include hypertrophy, dilation, fibrosis, infarcts, or thrombus.

7.5.5 Take photograph of ventricles after sectioning.

7.5.6 Make FFPE and OCT samples as in 7.4.3. From each ventricle segment, make myocardium transmural tissue blocks from the anterior, lateral, and posterior surfaces of the left ventricles, the anterior, lateral and posterior surface of the right ventricle, the ventricular septum, and apex (See Appendix 1, Figure 3).

7.5.7 Make flash frozen vials as in 7.4.4 depending on amount of tissue available.

7.6 Atria and Appendage Processing

7.6.1 Open the right and left atrium.

7.6.2 Document abnormal internal gross findings and measure wall thickness of each atria.

7.6.2.1 Abnormal findings include hypertrophy, dilation, fibrosis, infarcts, or thrombus.

7.6.3 Make FFPE and OCT samples as in 7.4.3. Transmural tissue blocks will be taken from the free wall of the right and left atrium (See Appendix 1, Figure 3). Make sure to prepare FFPE and OCT samples from Appendage.

7.6.4 Make flash frozen vials as in 7.4.4 depending on amount of tissue available.

7.7 Valve Dissection and Processing

7.7.1 Record the number and appearance of leaflets apparent in mitral valves and the number and appearance of cusps in aortic valves.

7.7.1.1 Findings include fibrotic, thinned, or calcified valves.

7.7.2 Make FFPE samples as in 7.4.3.

7.8 Fixation, Decalcification and Tissue Processing

7.8.1 Fix cassettes using an automatic processor or manually for 24 hours at room temperature in 1-2 L of 10% NBF with magnetic stirring. Record fixation start time.

7.8.1.1 Formalin volume must be at least 20 times greater than tissue volume. Ensure cassettes are completely covered.

7.8.2 Decalcification

7.8.2.1 FFPE blocks may be subjected to decalcification on an as needed basis. Refer to SOP 80 Tissue de-calcification protocol Isolation of PBMC for further processing.

7.8.3 Record fixation end time and transfer cassettes to tissue processor. Follow tissue processing protocol for kidney/heart. If tissue processor is unavailable, transfer cassettes to a 52oz container labeled with the Case ID number, and the date, then fill with 70% ethanol. Place container in processing room fridge until tissue processor is available.

7.8.3.1 Set tissue processor for “Heart” program.

7.8.3.2 Embed all tissue into paraffin after processing.

7.8.4 Make serial sections from two FFPE blocks from each anatomical location and stain as follows for each tissue type. Submit stained slides for assessment by pathologists.

7.8.4.1 Coronary artery: H&E, Elastic van Gieson, Movat pentachrome

7.8.4.2 Myocardial tissue: H&E, Masson trichrome, Movat pentachrome

7.9 Sample Archiving

7.9.1 Transfer all snap frozen materials and OCT blocks to t -80°C freezer for storage.

7.9.2 Materials obtained by this program will be inventoried in the nPOD database and archived in the OPPC according to SOP 55 Case Data Management, SOP 52 Sample Data Management, and SOP Tissue Sample Archiving.

7.10 Disinfection

7.10.1 Dispose of any remaining tissue according to SOP 66 Specimen Disposal.

7.10.2 Sterilize all work surfaces using freshly prepared 10% bleach or VirexTb, then wipe surfaces with 70% ethanol to remove residue.

7.10.3 Wash all surgical tools and dissection boards using surgical instrument cleaning solution, then autoclave tools to sterilize.

7.10.4 Place dissection boards, pens, pipettes, camera, and other non-autoclavable items in biosafety cabinet and expose to UV radiation for at least 30-45 minutes to decontaminate.

7.10.5 Dispose of all biohazardous waste according to UF EH&S guidelines.

**REFERENCES:**

## 1.0 Related Documents and Procedures

1.1 Clinical Association of Pathology Anatomic Pathology Manual
1.2 UF Biological Waste Disposal Policy
1.3 Campbell-Thompson, et. al. Processing of human pancreas. JoVE, 2012.
1.4 SOP 22 Autoantibody Screening
1.5 SOP 26 Autoantibody Radioimmunoassay
1.6 SOP 52 Sample Data Management
1.7 SOP 55 Case Data Management
1.8 SOP 59 Isolation of PBMC
1.9 SOP 66 Specimen Disposal
1.10 SOP 85 C-Peptide Determination
1.11 GDL Tissue Sample Archiving

**REVISION HISTORY**

| Version | Date | Revision |
|---|---|---|
| 0 | 9/17/21 | SOP Created |
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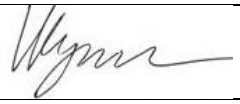
|---|---|---|---|
| Prepared by | Maria Beery | | |
| Edited by | Irina Kusmartseva | | |
| Approved by | Dr. Kusmartseva |  | 9.17.2021 |
| | Name | Signature | Date |

**Appendix 1**

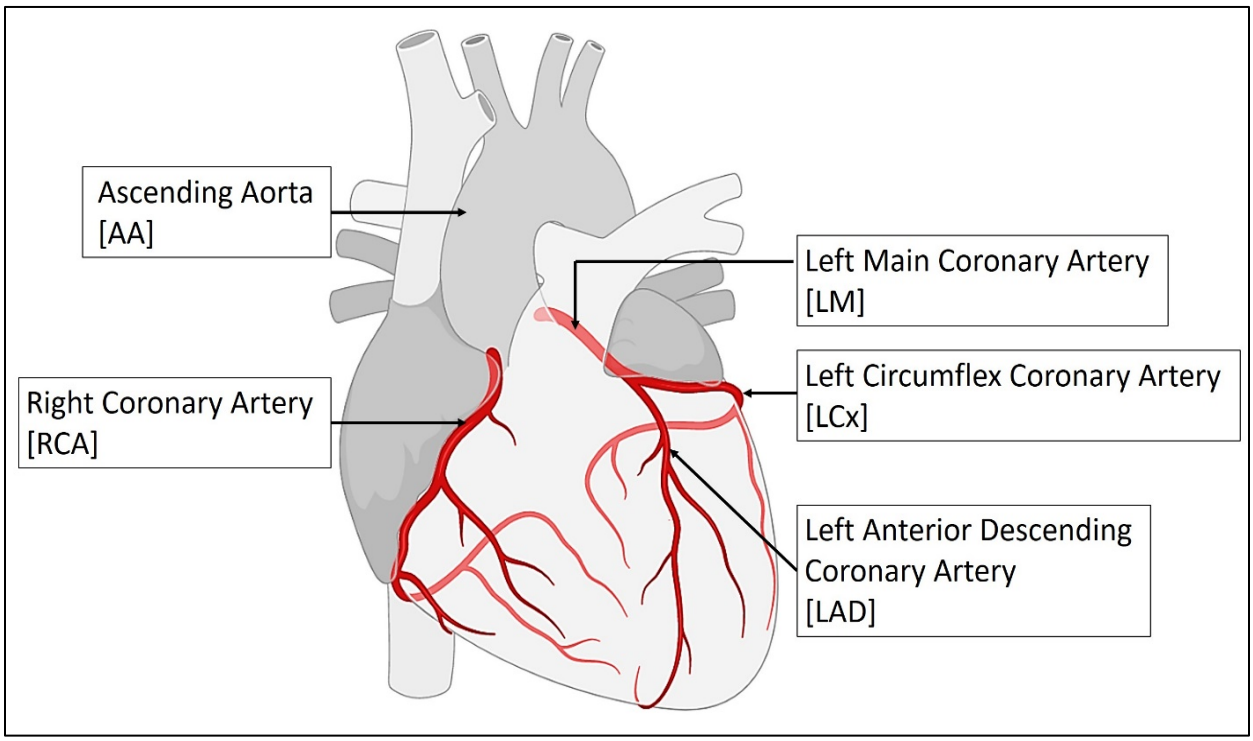



Figure 1 Anatomy of the human heart including the aorta and major coronary arteries

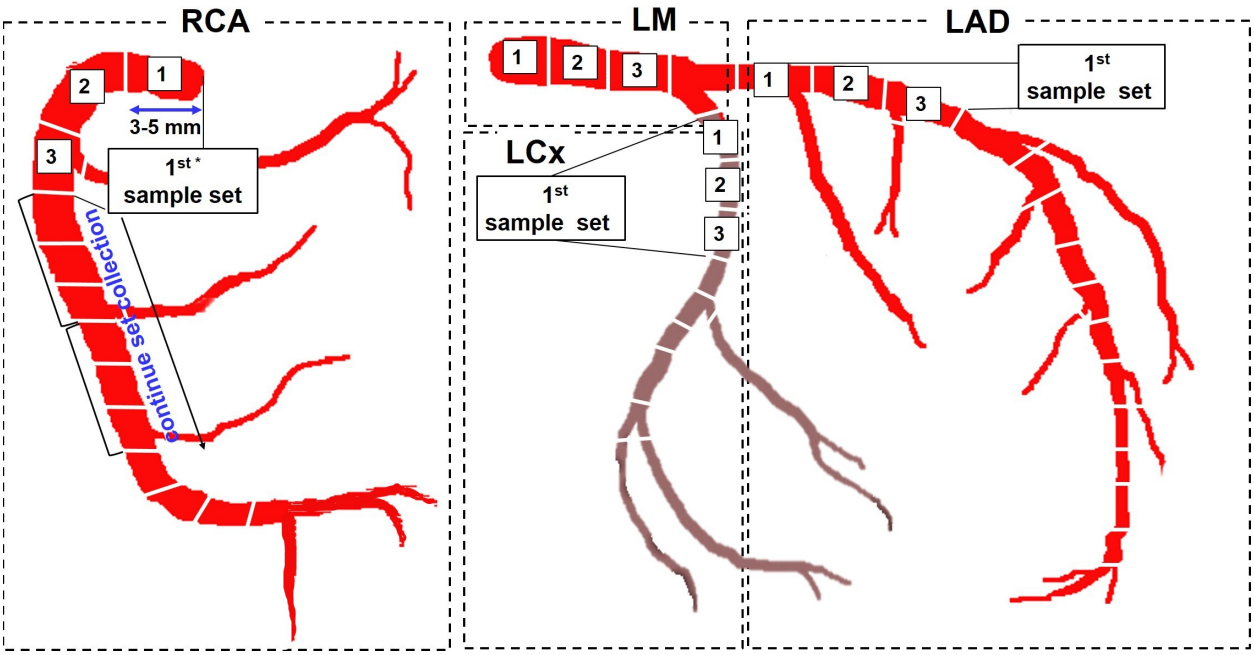



Figure 2. Scheme of arteries tissue collection and sample preparation**. RCA: right coronary artery, LM: left main coronary artery, LAD: left anterior descending coronary artery, LCx: left circumflex coronary artery. 1- FFPE, 2: flash-frozen tissue in vial, 3: fresh frozen in OCT. *Identical sample sets will be collected through entire length of each artery. **For visual representation only.

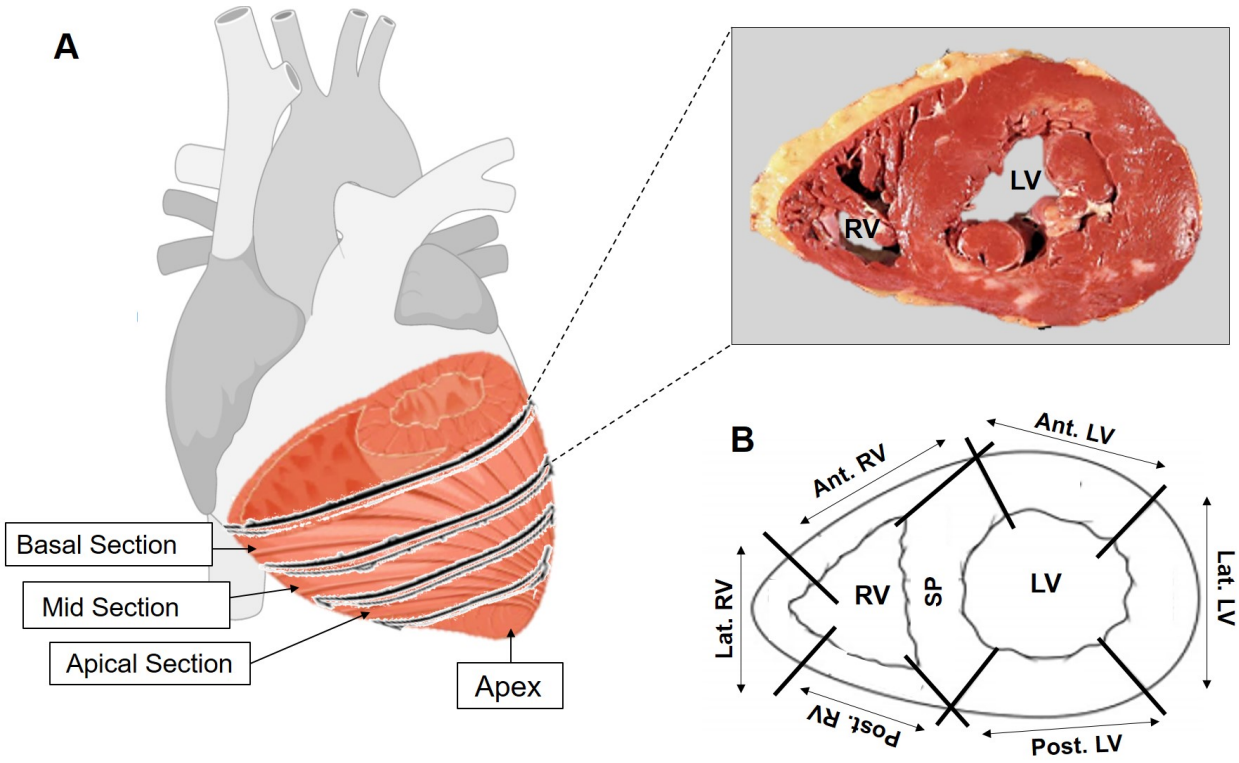



Figure 3. Anatomy of the human heart cross-section. A. Anatomical transverse cut of the heart at the ventricle level with inset depicting the left (LV) and right (RV) ventricles. B. Schematic representation of the ventricles structure. Ant. LV: anterior surface of the left ventricle, Lat. LV: lateral surface of the left ventricle, Post. LV: posterior surface of the left ventricle, Ant. RV: anterior surface of right ventricle, Lat. RV: lateral surface of the right ventricle, Post. RV: posterior surface of right ventricle, SP- septum. Solid black lines indicate where samples from the left and right ventricles and septum will be taken.