



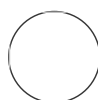
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BIDMC TMC - SOP for collection of peripheral lymphatic vessels

nkalavro¹, Ioannis Vlachos¹, Dhruv Singhal¹

¹BIDMC

Human BioMolecular Atlas Program (HuBMAP) Method Development Community
Tech. support email: Jeff.spraggins@vanderbilt.edu



nkalavro

ABSTRACT

BIDMC Lymphatic TMC SOP for peripheral lymphatic vessel collection

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We use this protocol and it's working

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Standard Operating Procedure Overview

- 1 Standard Operating Procedure to outline the process for obtaining, processing, and storing peripheral lymphatic vessel samples collected for the HuBMAP Lymphatic TMC.

The vessels are collected through a permanent removal of right axillary lymphatic vessel during lymphedema surgery from patients with malignant neoplasm of right female breast.

Donor Criteria

- 2 Patients with age >18 years from any race (White, Black, Hispanic, and Asian) are included.

Patients are excluded if they have one of the following characteristic:

1. Have a known underlying lymphatic disease.
2. Suffer from a chronic inflammatory condition (e.g. RA).
3. Have a history of filarial infection.
4. Have a history of allergy to lymphazurin blue.
5. Are actively breastfeeding.
6. Have a history of sensitivity to triphenylmethane.
7. Have a history of systemic therapeutic treatment (e.g. chemotherapy).
8. Have a history of pathology at anticipated site of lymphatic vessel harvest.

Donor metadata

- 3 The following information is recorded per study participant: Age, Sex, BMI, Hospitalization Time, Comorbidities, and Clinical history from the OMR.

Consenting

- 4 Following confirmation of the above inclusion and exclusion criteria by a study coordinator, the patient is informed about the study and provides informed consent according to the BIDMC IRB criteria.

Donors accepting to participate into the study are assigned an ascending 3-digit number (beginning with 001)

Collection of Peripheral Lymphatic Vessel

- 5 During the planned radial forearm free flap, 3 injections of 0.1 cc of ICGare injected into the fascia 2cm apart proximal to the radial styloid.
- 6 The sites are massaged for 1 minute.

- 7 Identified lymphatic vessels are harvested (2x5mm, or longer) with microsurgical scissors and microclips applied to free lymphatic ends.
- 8 A 9-0 suture (peri-adventitial) is placed at the 12 o'clock position (i.e. the side of the vessel facing the axillary vein) – on the side farthest from the heart, to mark orientation.
- 9 The distance from the vessel to the edge of the axillary vein is recorded.
- 10 The peri-lymphatic adipose tissue of the lymphatic vessel is removed as closely to the vessel as possible.

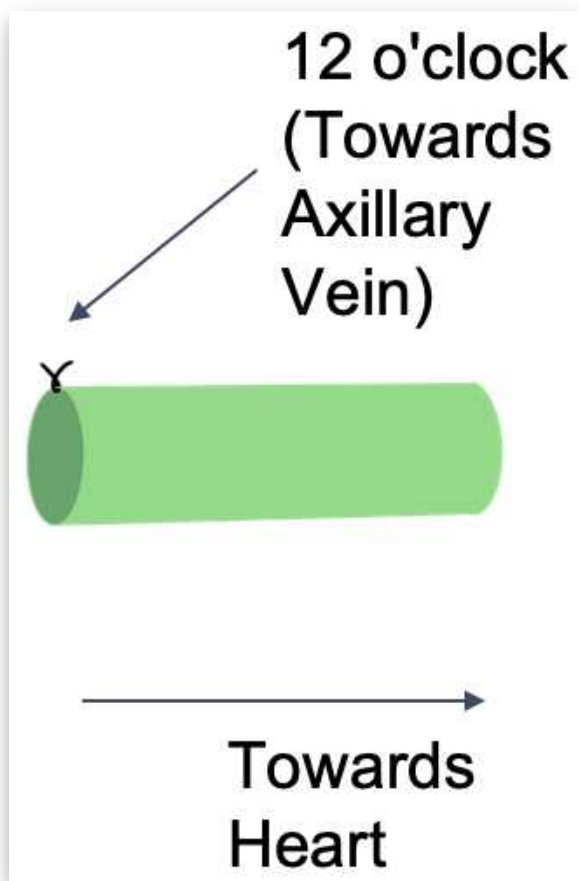


Fig 1a: Marking of lymphatic vessel with the use of a 9-0 suture (peri-adventitial) at the 12 o'clock position, on the side furthest from the heart.

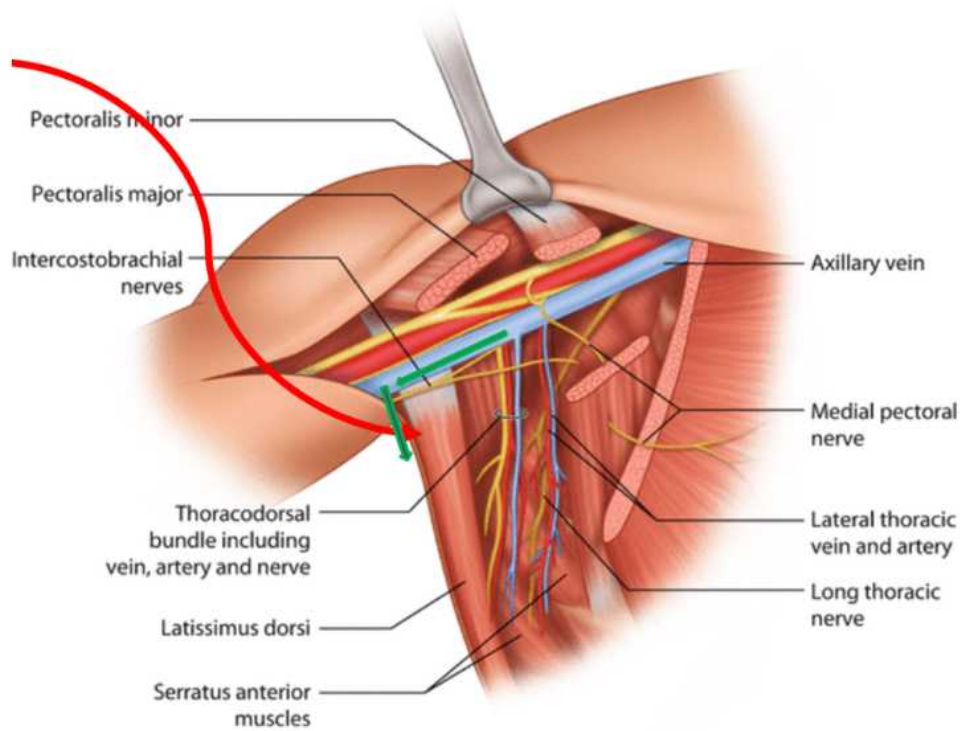



Fig 1b: Marking the distance below the axillary vein for orientation.

Sample Processing

- 11 Prepare an isopentane and liquid nitrogen bath.
- 12 In a petri dish, carefully coat fresh tissue sample with room temperature OCT. Using a spatula, place the OCT-coated tissue into an appropriately sized cryomold.
- 13 Cover the tissue completely with OCT. Label the cryomold to mark the orientation of the tissue.
- 14 Using forceps, lower the embedded tissue into the isopentane without fully submerging.

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- 15 Keep cryomold in contact with isopentane until the OCT has solidified and turned white. Once frozen, place the cryomold on powdered dry ice.
 - 16 Store frozen embedded tissue in a sealed container at -80C or liquid nitrogen for long-term storage.