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Tonsil Collection and Processing Protocol

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We use this protocol and it's working

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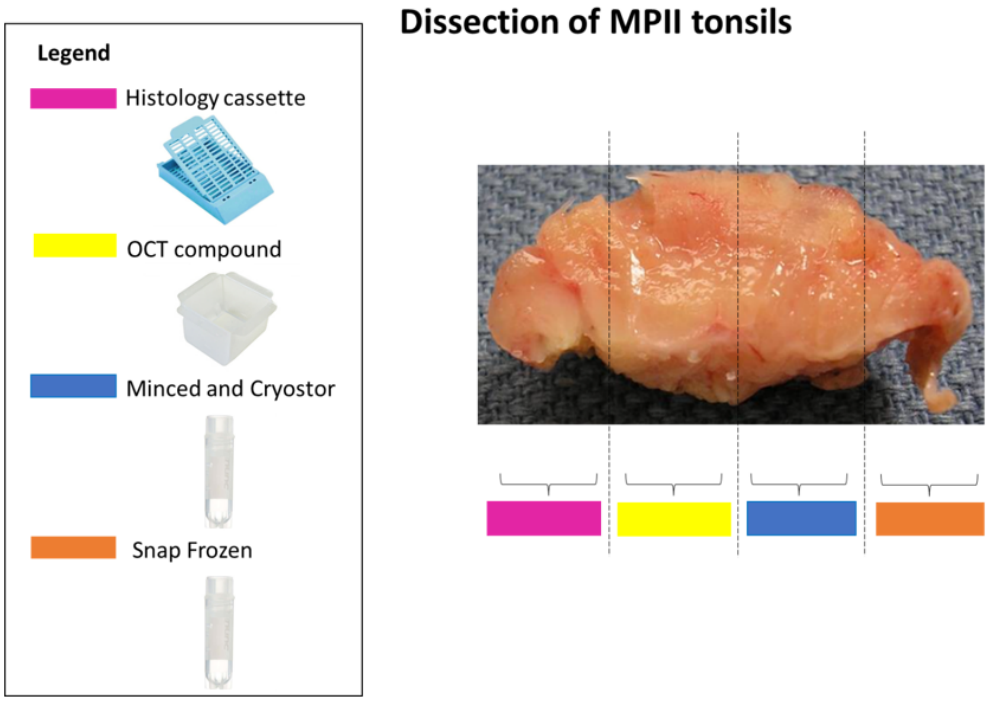
Abstract

For preserving human tonsils by formalin fixation, O.C.T. embedding and freezing, Cryostor freezing medium, and snap freezing in liquid nitrogen.

Guidelines

Illustration



Materials

Equipment/Supplies (items equivalent to those in parentheses may be used)

- Biosafety cabinet (BSC); if none is available, wear a lab coat, mask and face shield and/or safety shield.
- Chemical Fume hood, or BSC vented outside the building, containing a waste trap with aspirator to collect formalin waste.
- Pencil and permanent fine point marker
- For optional tonsil weight and photograph:

1. Laboratory scale with 2g capacity
2. Sterile plastic container to use on scale, of appropriate size to contain the tonsil
3. Sterile 15 cm/6 in ruler
4. Digital camera

- 4 °C Refrigerator
- -80 °C Freezer
- Liquid Nitrogen storage
- Liquid nitrogen
- Small dewar liquid nitrogen flask
- Dry ice
- Wet ice
- Disposable Graduated Transfer Pipettes **Thermo Fisher Catalog #**1371120
- Fisherbrand™ Nonwoven Gauze Sponges **Fisher Scientific Catalog #**22-028-558
- Scalpel #11 (Feather 089275B)
- Hardened Fine Scissors **Fine Science Tools Catalog #**14091-11
- Student Surgical Standard Pattern Forceps **Fine Science Tools Catalog #**91121-12
- Dissection board or 15 cm sterile tissue culture dish
- Specimen cups (Medegen 01063)
- Tissue Cassettes (Epredia B851739BL)
- Embedding molds (Epredia 1220)
- Falcon® 100 µm Cell Strainer, Yellow, Sterile, Individually Packaged, 50/Case **Corning Catalog #**352360
- Fisherbrand™ Aluminum Foil, Standard-Gauge Roll **Fisher Scientific Catalog #**01-213-101
- Sterile Disposable Towel Drape (Dynarex 4410)
- Sterile Toothpicks or pipet tips for positioning tissue
- Small plastic bags: closable and large enough to hold a foil-wrapped embedding mold
- Fisherbrand™ Caution: Contains Formaldehyde Labels **Fisher Scientific Catalog #**18-999-929
- Screw cap tube, 15 ml, (LxØ): 120 x 17 mm, PP, with print **Sarstedt Catalog #**62.554.002
- 50 mL conical tubes (Sarstedt 62.554.004)
- Nunc™ Biobanking and Cell Culture Cryogenic Tubes, 1.8mL, 49mm, internal thread, printed **Thermo Fisher Catalog #**377267

- Nunc™ Biobanking and Cell Culture Cryogenic Tube Accessories, assorted coder **Thermo Fisher Catalog #**375930
- Cryo freezer labels (GA. International CL-6T1-WH)
- Corning® CoolCell® FTS30, Freezing Container, for 30 x 1 mL or 2 mL Cryogenic Vials, Purple **Corning Catalog #**432006
- Mr. Frosty **Thermo Fisher Scientific Catalog #**5100-0001
- Cryogenic gloves

Reagents (items equivalent to those in parentheses may be used EXCEPT for Cryostor and O.C.T. Compound)

- Sterile Normal Saline for sample collection (typically found in operating rooms)
- 10% Buffered Formalin (final 4% formaldehyde) (Fisher Chemical SF94-4)
- PBS without Ca2 and Mg2 **Corning Catalog #**21-040-CV
- O.C.T. Compound (Tissue-Tek O.C.T. Compound 4583; no substitutions)
- Cryostar CS10 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**C2874
- 70% Ethanol
- Isopropyl alcohol (if using Mr Frosty or similar freezing container; Fisher Chemical A426P-4)

Safety warnings

Sample Processing Guidelines and Warnings:

- Basic personal protective equipment (PPE): lab coat, safety goggles, gloves; use cryogenic gloves when working with liquid nitrogen.
- Spray gloves with 70% Ethanol
- Follow your institutional guidelines for the use of human tissue in laboratories.
- Follow your institutional guidelines for the use of formalin in laboratories.
- All tubes, tissue cassettes, and molds used in processing and storage should be labeled with the sample identification code (sample ID).
- If the amount of tissue obtained is limited, the operator may not be able to perform all processing types.

Ethics statement

Ethical approval must be obtained from your Institutional Review Board (IRB) or other appropriate ethics committee before use of this protocol.

Data Collection

1 Keep a record of the sample collection and processing to track the source and quality of the samples. Record the following data for:

1.1 Sample collection:
- Subject ID
- Date and time collected
- The number of tonsils collected
- The patient position the tonsils were colleted from (left or right)
- Verify that the samples are palatine tonsils
- Any relevant collection notes

1.2 Sample processing:
- Subject ID
- Date and time of processing start
- Digital photograph of the tonsil(s) prior to dissection; see Section 6
- The weight of each tonsil
- For the Cryostor (cryopreserved) samples:

1. Date and time samples were transferred to -80C
2. Date and time samples were transferred to liquid nitrogen

- For the Formalin Fixed (NBF) samples:

1. Date and time samples were placed in NBF
2. Date and time samples were transferred to 70% Ethanol, or the total time in hours the samples were in NBF
3. Date fixed samples were embedded in paraffin

- For the O.C.T. Embedded sample:

1. Date and time samples were completely frozen in O.C.T compound

- For the snap frozen sample:

1. Date and time samples were completely frozen in liquid nitrogen

- Any relevant notes for sample processing, such as whether any specimen was not processed successfully

Sample Collection

2 Pre-collection Preparation

2.1 Prepare a sample collection record according to step 1.1.

2.2 Patients are consented according to local and organizational requirements.

2.3 Label a sterile specimen cup with the sample type (“Tonsil”) and subject identification code (subject ID). Add enough normal saline to cover the tonsil(s).

3 Palatine tonsils are excised by the surgeon during regularly scheduled surgical encounters.

4 Immediately after collection, mark the tonsil(s) on the cut (muscular side) with a skin marking pen and place the tonsil(s) in the specimen cup containing normal saline On ice .

5 Transport the tonsil(s) On ice , as well as the collection record, to the processing lab as soon as feasible.

Preparation for Sample Processing

6 Samples should be delivered to the processing laboratory On ice , accompanied by a sample collection record.

7 Prepare a sample processing record according to step 1.2.

8 In the lab, assemble the following in a BSC or behind a safety shield:

8.1 Dissection board, covered first with a sterile towel drape, and optionally sterile gauze pads to absorb blood. (Option: use a Sterile 15 cm tissue culture dish with sterile gauze inside the dish).

8.2 Sterile scissors and forceps.

9 For cryopreservation:

9.1 If using a Mr. Frosty or similar freezing container, add 250 mL isopropyl alcohol to the container.

9.2 The isopropyl alcohol may be used (frozen) up to five times; replace after the fifth use.

10 For O.C.T. Compound embedding, prepare a container of dry ice.

11 For snap frozen tissue, obtain a small dewar of liquid nitrogen.

12 Prepare the tonsils for dissection:

12.1 Transfer the tonsil(s), epithelium facing upwards, to the dissection board.

- Tonsils may be marked during collection on the cut side that was adjacent to underlying muscle. This is to help differentiate it from the epithelial side.

12.2 Digital Photograph

- Place a sterile ruler beside the tonsil to measure the length. Add a note next to tissue with the subject ID and date. Take a digital photograph.

12.3 Examine the tissue. The serosal side may have char from electrocauterization. Using a #11 scalpel, remove the char, if present.

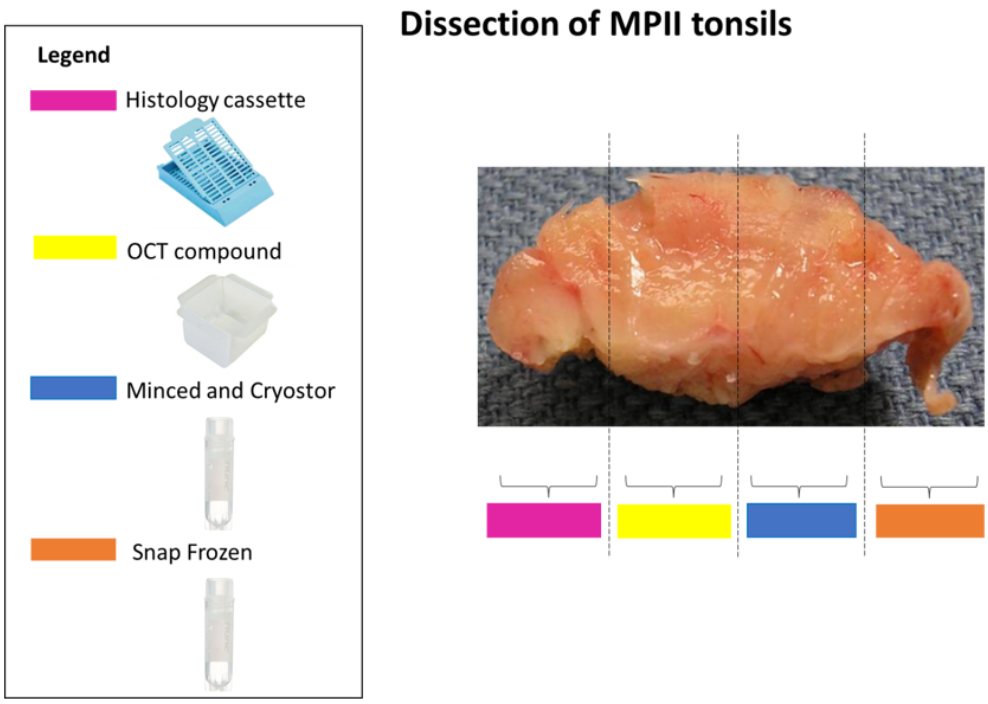
12.4 Tonsil Weight

- Place a sterile plastic container sized to contain the tonsil on a laboratory scale; zero or tare the container.
- Place the tonsil on the container and record the weight of the tonsil.
- Return the tonsil to the dissection board with the epithelium facing upwards for tissue sampling.

12.5 The tonsil(s) will be dissected as illustrated below, and according to sections "**Procedure: Formalin Fixation in Histology Cassette**", "**Procedure: Cryopreservation in Cryostor freezing medium**", "
Procedure: O.C.T. Compound Embedding and Freezing", and "
Procedure: Snap Frozen Tissue".

- It is irrelevant which parts (i.e., middle or end piece) go to which procedure.
- If the tonsil is small, reduce the amount cut for O.C.T Compound and Snap Frozen tissue.

Illustration



Procedure: Formalin Fixation in Histology Cassette

13 Follow your institutional guidelines for use of formalin.

14 Record the information on the previously prepared processing record.

15 Label a tissue cassette and specimen cup with the sample ID.; ONLY USE PENCIL on the cassette.

16 Add a formaldehyde warning sticker to the cup and label it with the date, operator initials, and fixative.

17 Using a #11 Scalpel cut approximately 1/4 of the tonsil as illustrated above.

17.1 The slice for this section should not exceed the interior dimensions of the cassette when closed, and it should not exceed 0.5 cm to ensure complete fixation within 24 hours.

18 Place the tissue cut side down in the labeled cassette and close.

19 Place the cassette in the labeled EMPTY specimen cup and store on ice. In the fume hood, pour enough 10% neutral buffered formalin (NBF) to cover the cassette. Cover and store at 4 °C for 24:00:00 . 1d

19.1 If fixation for longer than 24 hours is unavoidable, tissue may remain in NBF for up to 72 hours.

> **Note**
>
> Do not exceed 72 hours fixation.

20 After fixation, aspirate the NBF into a formalin waste trap in the fume hood.

21 Wash the cassette(s) with PBS, aspirate the wash, and repeat. Cross through the formaldehyde sticker and fixative label to indicate NBF is no longer present.

22 Replace the last PBS wash with 70% Ethanol and store at 4 °C .

23 Submit for paraffin embedding as soon as possible.

Procedure: Cryopreservation in Cryostor freezing medium

24 Record the information on the previously prepared processing record.

25 Label a 15 mL conical tube with the sample ID for Cryostor.

26 Add 5 mL PBS to the tube. Store On ice .

27 Label TWO cryovials for each tonsil with the sample ID.

28 Using a #11 Scalpel, cut approximately 1/4 of the tonsil as illustrated above and transfer to the appropriate labeled 15 mL conical tube.

29 Using sterile surgical scissors, finely mince the tissue to approximately 2mm pieces in the 15 mL tube.

30 Set up 50 mL tube with 100 µM strainer on top.

31 Pour the minced tissue in PBS onto the filter, leaving minced tissues on top of the filter.

32 Using forceps, transfer the minced tissues to the labeled cryovials, dividing the tissue evenly between them. DO NOT ADD TISSUE HIGHER THAN the 1 mL mark on the cryovials. Discard any extra tissue remaining into a biohazard waste bin.

33 Make sure the tissue is not stuck on the side or rim of the cryovials. Tap each cryovial on a hard surface to collect all tissue at bottom of the vial.

34 Add 1 mL Cryostor freeze solution to each cryovial and mix thoroughly.

35 Transfer the cryovials to a freezing container and place in a -80 °C freezer.

36 24-72 hours later, transfer the vials into liquid nitrogen storage.

Procedure: O.C.T. Compound Embedding and Freezing

37 Record the information on the previously prepared processing record.

38 Label an embedding mold with the sample ID using a permanent marker on the lip on mold.

39 Label an aluminum foil sheet, sized to wrap around the mold. Label a small plastic bag to store the frozen sample.

40 Place the mold and aluminum foil on the dry ice to chill.

41 Using #11 Scalpel cut approximately 1/4 of the tonsil as illustrated above.

42 Add a thin layer of O.C.T. Compound to mold and place the tissue, cut side down, on the O.C.T. in the center of mold. Add more O.C.T. Compound on top of the tissue.

43 Use toothpicks or pipet tips to maintain the position of the tissue and keep it from floating to the surface. Make sure there are no bubbles around the tissue. Continue adding O.C.T. Compound until entire tissue is covered.

> **Note**
>
> The goal is to have the tissue frozen in the center of the mold.

44 Place dry ice around mold to help it freeze faster. Wrap the frozen mold with the pre-labeled aluminum foil and place it in the plastic bag.

45 Store in -80 °C freezer.

Procedure: Snap Frozen Tissue

46 Record the information on the previously prepared processing record.

47 Label a cryovial with the sample ID.

48 Obtain a small dewar flask of liquid nitrogen.

49 Using #11 Scalpel cut approximately 1/4 of the tonsil as illustrated above.

50 Place the tissue sample in the cryovial and cap.

51 Immediately don cryogenic gloves and, using forceps, submerge the vial in liquid nitrogen. Allow the vial to remain in the liquid nitrogen for at least 20 seconds to ensure freezing.

52 Remove the vial from the liquid nitrogen and immediately store on dry ice until transfer to liquid nitrogen storage.