SBT777101 SYNOVIAL BIOPSY MANUAL

Study Number	SBT777101-01
Study Title	A Phase 1 Study to Evaluate the Safety, Tolerability, Pharmacokinetics, Pharmacodynamics, and Activity of Single Ascending Doses of SBT777101 in Subjects with Rheumatoid Arthritis
Investigational Product	SBT777101
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SONOMA BIOTHERAPEUTICS

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LIST OF ABBREVIATIONS

Abbreviation	Definition
AE	adverse event
CAR	chimeric antigen receptor
CTCAE	Common Terminology Criteria for Adverse Events
eCRF	electronic case report form
ECU	extensor carpi ulnaris
EDC	electronic data capture
EDQ	extensor digiti quinti proprius
FFPE	formalin-fixed paraffin-embedded
MRL	MedPace Reference Laboratories
PBS	phosphate-buffered saline
PD	pharmacodynamic
PK	pharmacokinetic
RA	rheumatoid arthritis
SOA	Schedule of Assessments
Treg	regulatory T lymphocyte
US	ultrasound

1. INTRODUCTION

SBT777101 is a genetically engineered chimeric antigen receptor (CAR) regulatory T lymphocyte (Treg) cell therapy, designed to treat patients with moderate-to-severe rheumatoid arthritis (RA). As part of the SBT777101-01 clinical trial, biopsies of inflamed synovial tissue affected by disease must be obtained.

All study participants are required to undergo 2 biopsy procedures. A pre-treatment biopsy is required during screening to evaluate for the presence of inflammatory cell infiltration as well as the SBT777101-reactive antigen to inform eligibility. A second biopsy, to be obtained approximately 4 weeks after SBT777101 infusion, will be collected for pharmacokinetic (PK) and pharmacodynamic (PD) analyses. In addition, the pre- and post-treatment samples will be evaluated for exploratory biomarkers that may provide a better understanding of the intended therapeutic effect.

2. SCOPE

The purpose of this biopsy manual is to provide standard procedures for the acquisition, handling, processing, and storage of synovial tissue and synovial fluid obtained by the clinical team at each clinical study site. The utility of the data obtained from analyses of biopsy and aspirate specimens is directly related to the quality of the specimens procured. This manual was developed and adapted from written materials provided by Dr. Andrew Filer (University of Birmingham).

Note: Detailed instructions for the shipping of specimens are provided separately, in the Lab Manual.

3. PERSONNEL

Team member roles and responsibilities:

1. Proceduralist (i.e., Rheumatologist, Interventional Radiologist, orthopedic surgeon, or other designated and qualified personnel).
    a. Ensure patient has been consented to study participation and is informed of the risks associated with the biopsy procedure.
    b. Confirm that the appropriate joint for biopsy and/or arthrocentesis has been identified, either through personal examination or correspondence with the clinician who made the initial clinical assessment and determination at screening.
    c. Perform procedure(s) per institutional standard operating procedures or the instructions provided in Section 6.
2. Ultrasonographer (i.e., Radiologist, Interventional Radiologist, Ultrasound Technician, or designated qualified personnel)
    a. Perform ultrasound to guide synovial tissue biopsy and/or arthrocentesis on scheduled procedure dates (Optional).

3. Specimen Manager(s). The Specimen Manager(s) should be personnel who have participated in site protocol training activities and who are familiar with the study-specific kits for specimen collection. The Specimen Manager(s) are responsible for ensuring:

   a. Proper preparation of specimen collection kit and reagents prior to biopsy procedure
   b. Proper handling and processing of study samples
   c. Proper storage and shipping of study samples

   The Specimen Manager(s) should be notified with ample time prior to start of the biopsy procedure, in order to allow for pre-procedure planning and preparation of specimen collection supplies & equipment (Section 8).

4. SUPPLIES AND EQUIPMENT

4.1. Standard Site-Provided Supplies and Equipment for the Synovial Biopsy and Fluid Aspiration Procedure

The following list of supplies and equipment are needed for the synovial biopsy and fluid aspiration procedure and are *not* provided by the study Sponsor:

- Ultrasound machine (For US guided Biopsy)
- 3 mL, 5 mL, 10 mL, and 20 mL syringes
- Long 27G needle (for local anesthesia), and 18G needle to enlarge the incision, as necessary.
- 18G, 19G and 21G needles for synovial fluid aspiration. Needle gauge will depend on the size of the joint selected for the procedure.
- 1 % Lidocaine (max 4.5mg/kg)
- 0.9% normal saline (for joint inflation)
- Sterile alcohol swabs (for anesthesia draws)
- Sterile 0.9% normal saline (either 125 mL or 150 mL bag per biopsy, depending on size of joint)
- Antiseptic solution (viscous chlorhexidine, 4% 8oz, 1 bottle per biopsy)
- Sterile contact medium (e.g., Hibiscrub, or same as antiseptic solution)
- Sterile 4” x 4” 12-ply gauze pads (pack of 12)
- Sterile McKesson sticky XL drapes without hole (at least 8 per biopsy)
- Sterile ultrasound sheath (including gel)
- Sterile gowns and gloves. *Note: double gloving is recommended.*
- Waterproof shoe covers

- Face masks and hair covers.
- 3M Coban self-adherent wrap
- Bacitracin ointment packets for wound care x3 days post-procedure

4.2. Sponsor-Provided Supplies and Equipment for Synovial Biopsy and Fluid Aspiration Procedure

The following list of supplies and equipment are provided by the Sponsor for the biopsy and aspiration procedure:

- TEMNO, Cook, or Bard biopsy needles with coaxial introducers (16G recommended for wrist/ankle and 14G recommended for knee, each with 6cm or 10cm length)

Pre-procedure patient preparation instructions are provided in Section 5.

4.3. Standard Site-Provided Supplies and Equipment for Synovial Tissue and Fluid Processing

The following list of supplies and equipment are needed for the processing of specimens but are *not* specifically provided by the study Sponsor.

- Tabletop centrifuge
- Phosphate Buffered Saline (PBS). PBS should be kept at 4°C (refrigerated) and placed on ice when in use)
- Ice bucket or other container to keep reagents cold when in use.

4.4. Sponsor-Provided Supplies for Synovial Tissue and Fluid Processing

A complete list of supplies and equipment provided by the study Sponsor for the handling, processing, and storage of tissue and fluid samples is provided in Appendix A.

Please use the Sponsor-provided materials and kits (e.g., tubes, reagents, cryovials, controlled-rate freezing devices) and associated labels according to the instructions provided in this manual.

- Biopsy Visit Requisition Forms (examples provided in Appendix B, Appendix C, and Appendix D)
- Bulk supplies
  - CryoStor CS10 Freezing media (Freezing media should be kept at 4°C and placed on ice before use)
  - CoolCell Controlled-rate freezing container (Keep the CoolCell container at 4°C until ready to use)
  - 12 well plate
  - Sterile forceps
  - Sterile Scalpel

  - Sterile pipettes (for transfer of synovial fluids or reagents including PBS and freezing media)
  - 20 mL vial pre-filled with 10% Formalin
  - 70% Reagent Grade Alcohol (for use if the biopsy samples in 10% formalin is not shipped within 24 hr)
  - 20 mL SecurTainer (for use if biopsy samples in 10% formalin is not shipped within 24 hr)
- Visit-specific kits containing the following synovial sample collection tubes:
  - Green-top sodium heparin (NaHep) tubes
  - 2 mL Cryovials
  - 2 mL Screw-Cap tubes

5. PRE-PROCEDURE PATIENT PREPARATION

5.1. Pre-biopsy Subject Consent

The synovial tissue biopsy and arthrocentesis is described in the study Informed Consent Form, which is signed by the study investigator and subject prior to the initiation of any protocol-specific screening procedures.

5.2. Pre-biopsy Joint Evaluation

Per the Schedule of Assessments (SOA) table in Protocol SBT777101-01, subjects will have a joint evaluation at screening. This assessment is required to confirm that the investigator has determined that the subject has a joint suitable for biopsy.

5.3. Pre-Procedure Medications

5.3.1. Anti-coagulation

Anti-coagulation medications (e.g., aspirin, clopidogrel, warfarin, etc.) should be held for at least 3 days prior to the biopsy procedure to minimize risk of intra-articular bleeding.

5.3.2. Pre-procedure Medications

Local anesthesia, anxiolytics and/or sedation may be used as clinically indicated and in accordance with standard institutional practice. All medications used during the procedure should be recorded as concomitant medications on the study electronic case report form (eCRF).

6. SYNOVIAL TISSUE BIOPSY AND ARTHROCENTESIS (SYNOVIAL FLUID ASPIRATION) PROCEDURES

Sites may utilize standard institutional methods and operating procedures for obtaining biopsy and fluid material. Otherwise, synovial tissue biopsy will be obtained using a needle biopsy per instructions provided in this biopsy manual.

6.1. Optimal Tissue and Fluid Sampling

For optimal tissue and fluid sampling, avoid contamination with surrounding histologically distinct tissue (i.e., adipose, muscle, tendon).

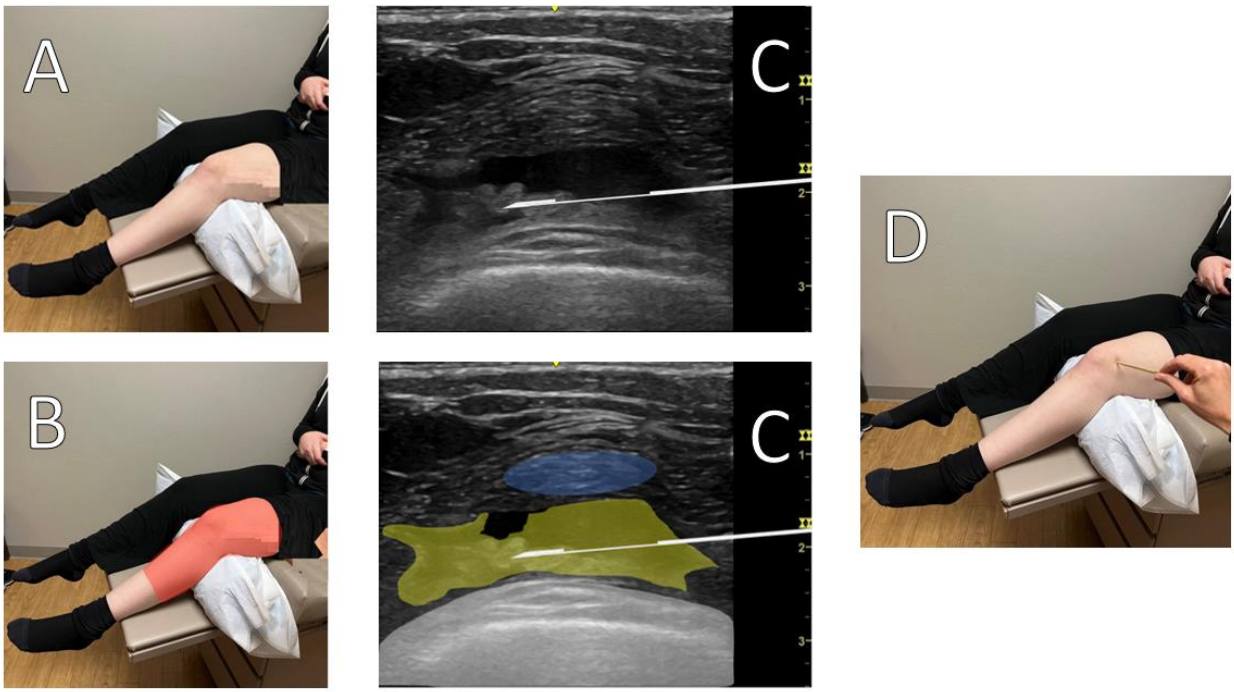
- 8-to-16 fragments per synovial tissue core biopsy (refer to Section 8.3)
- 0.3-to-4 mL synovial fluid that can be aspirated (refer to Section 8.4)

Although synovial fluid sampling is optional, aspirate specimens are strongly encouraged. Whenever possible, contemporaneous synovial fluid collection should be performed by arthrocentesis, both pre- and post-infusion of the cell product.

6.2. Needle Biopsy Procedures

6.2.1. Knee

Figure 1: Ultrasound-Guided Knee Biopsy



[A] Positioning
[B] Area to be sterilized (pink)
[C] Ultrasound for guidance; needle (white line), tendon (blue), synovium (yellow)
[D] Insertion of needle

Orientation and Positioning of the Subject

1. The subject should be reclining at 45 degrees (with the option of lying supine if the patient prefers) on an appropriate surface during the procedure (Figure 1 A).
2. To improve imaging of the supra-patella pouch, patient knee should be slightly flexed (25-30 degrees) (Figure 1 A).

Preparation

1. All participating staff with close or direct patient contact (e.g., proceduralist, assistant, ultrasonographer) should commence personal preparations for the procedure, which includes hand washing, wearing double gloves, face mask, hair net/hat, sterile gown, and shoe covers. Of note, there may be significant fluid that comes of the joint upon entry of needle and/or introducer.
2. Evaluate the equipment tray, including biopsy needles.
3. Place absorbent pads under the knee.
4. Sterilize the skin using appropriate sterilization fluid. A wide field should be sterilized, beyond the immediate area of interest (e.g., mid-thigh to mid-calf, both anteriorly and posteriorly) (Figure 1 B).
5. Position the sterile drapes below and above the knee creating a sterile field sufficient for access to the supra-patella pouch and placement of the US probe for the purposes of imaging.
6. The ultrasonographer should place the US probe within the sterile sheath. US gel should be placed within the sheath, and the probe slowly lowered into the sheath. The upper end of the sheath should be secured with a sterile tie or elastic band usually provided with the sheath. *Note: the sterile ultrasound gel should never come in direct contact with the subject's skin as this can cause tissue damage*.
7. Prepare 2 syringes with 10 mL 1% lidocaine, and one syringe with 10 mL normal saline. Have the sterile source of saline present in the operative field in case more saline is needed.
8. US examination of the lateral aspect of the knee should indicate a suitable area for needle insertion, distal to the vastus lateralis muscle insertion into the patella (Figure 1 C-D).

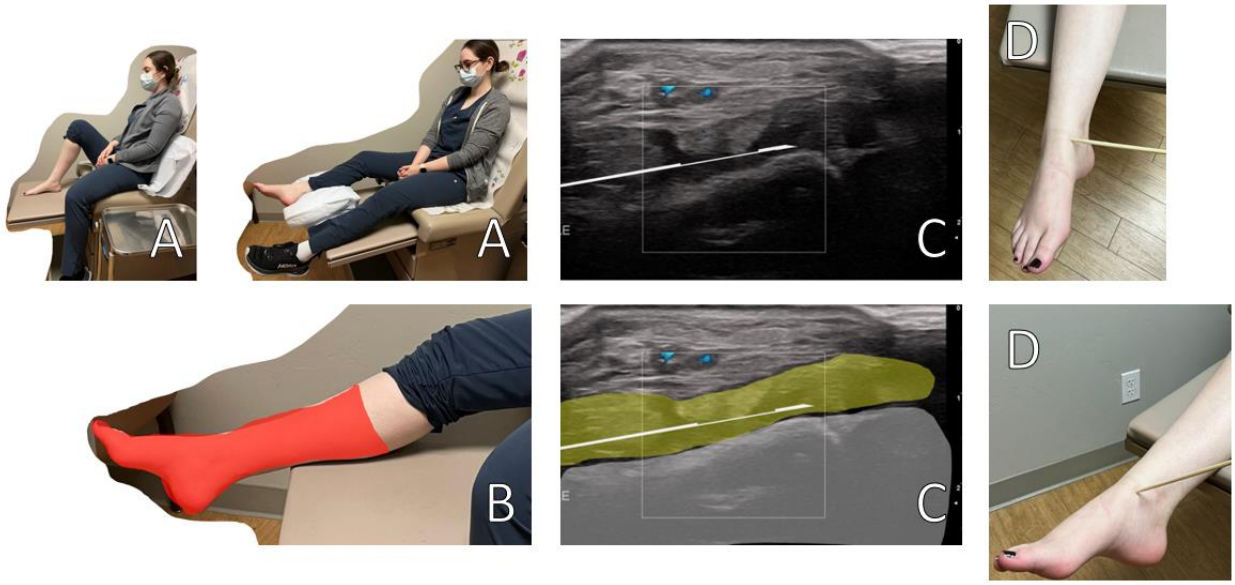
Biopsy Procedure

1. Using the initial US scan, determine point of insertion and inject 5 – 10 mL 1% lidocaine into the subcutaneous and deep tissue. Leave a minimum of 5 minutes for anesthetic effect.
2. Using US guidance, insert an 18G needle into the supra-patellar pouch and aspirate as much fluid as possible. If there is less than 0.3 mL synovial fluid present, proceed to Step 4.

3. Disconnect the syringe leaving the 18G needle in situ. Dispense the aspirated synovial fluid into a 4 mL green-top sodium heparin (NaHep) tube for further processing (Section 8.4).
4. Inject 10 mL 1% lidocaine into the joint, followed by enough normal saline to give a clear view of the suprapatellar pouch to subsequently insert the coaxial introducer and clearly identify the synovial tissue. This step may be altered according to the size of the patient and volume of synovial fluid present (e.g., some knee joints may need variable amounts of normal saline in order to expand the suprapatellar pouch).
5. Using US guidance, insert the biopsy coaxial introducer into the supra-patellar pouch. Once the introducer is in place, withdraw the sharp obturator. The biopsy needle should be primed (spring is compressed and biopsy needle retracted) before its introduction into the synovial space.
6. The biopsy needle should be extended, and the throw identified on the US images. The throw of the needle should be placed against the surface of the synovium to maximize the opportunity for capturing the lining layer. Gentle pressure should be placed on the needle to oppose the throw and synovium. *Note: if the tip of the extended needle is abutting a bony surface, backwards movement of the needle will occur, leading to poor tissue retrieval.*
7. Remove tissue from the biopsy needle by gently lifting or scraping the tissue using either another sterile needle, the flat edge of a sterile scalpel or the flat edge of sterile forceps.
    a. Always use new, clean, and sterile instruments for tissue removal from the biopsy needle to avoid contamination of the biopsy needle.
    b. Tissue specimens should be placed directly into the 12-well plate containing cold PBS (see Section 8).
8. When enough specimens are harvested, the remaining fluid should be aspirated, and up to 50 mL of normal saline (in units of 10 mL) should be used to briefly flush the joint to clear any debris or blood. Continue lavage until fluid is only blood-tinged or clear.

6.2.2. Ankle

Figure 2: Ultrasound-Guided Ankle Biopsy



[A] Positioning.
[B] Area to be sterilized (pink).
[C] Ultrasound for guidance; needle (white line), doppler (light blue), synovium (yellow).
[D] Insertion of needle.

Orientation and Positioning of the Subject

1. Position the subject comfortably semi-recumbent or supine, with biopsy joint raised on a pillow so that medial (inner) aspect is raised above contralateral limb (Figure 2 A).

Preparation

1. All participating staff with close or direct patient contact (e.g., proceduralist, assistant, ultrasonographer) should commence personal preparations for the procedure which includes hand washing, wearing gloves, face mask, hair net/hat, and sterile gown.
2. Evaluate the equipment tray, including biopsy needles.
3. Place absorbent pads under the ankle.
4. Sterilize the skin using appropriate sterilization fluid. A wide field should be sterilized beyond the immediate area of interest (e.g., toes to mid-calf, both anteriorly and posteriorly) (Figure 2 B).
5. Position the sterile drapes above, below, medial, and lateral to the ankle leaving sufficient space for access to the tibio-talar joint and placement of the US probe for the purposes of imaging.
6. The ultrasonographer should place the US probe within the sterile sheath. US gel should be placed first upon the probe footprint and the probe slowly lowered into the sheath. The

upper end of the sheath should be secured with a sterile tie or elastic band usually provided with the sheath. *Note: the sterile ultrasound gel should never come in direct contact with the subject's skin as this can cause tissue damage.*

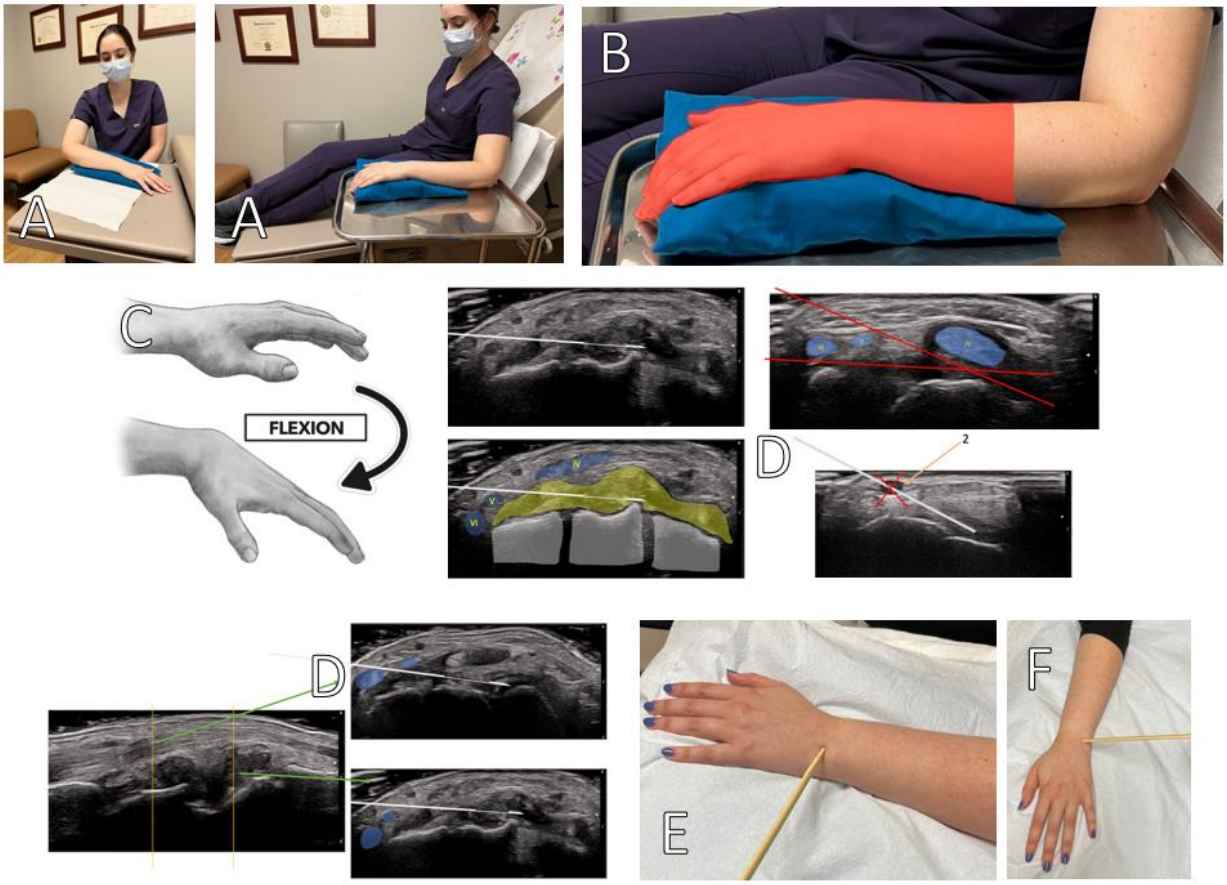
7. Prepare 2 syringes with 10 mL 1% lidocaine, and one syringe containing 10 mL normal saline.
8. US examination of the medial aspect of the ankle should indicate a suitable area for needle insertion deep to the tibialis anterior tendon, aiming laterally across the joint in line with the tibiotalar joint line (Figure 2 C-D).

Biopsy Procedure

1. Using the initial US scan, determine point of insertion and inject 3 – 5 mL 1% lidocaine into the subcutaneous and deep tissue. Leave a minimum of 5 minutes for anesthetic effect.
2. Using US guidance, insert a 21G needle and aspirate as much fluid as possible from the joint. If there is less than 0.3 mL synovial fluid present, proceed to Step 4.
3. Disconnect the syringe leaving the 21G needle in situ. Dispense the aspirated synovial fluid into a 4 mL green-top sodium heparin (NaHep) tube for further processing (Section 8.4).
4. Inject 5-10 mL 1% lidocaine, allowing 5 minutes for the anesthetic to take effect. Normal saline can be added to enable a better image to be acquired during the procedure and facilitate clear identification of synovial tissue.
5. Using US guidance, insert the biopsy coaxial introducer into the joint space. Once the introducer is in place, withdraw the sharp obturator. The biopsy needle should be primed (spring is compressed and biopsy needle retracted) before its introduction to the synovial space.
6. The biopsy needle should be extended, and the throw identified on the US images. The throw of the needle should be placed against the surface of the synovium to maximize the opportunity for capturing the lining layer. Gentle pressure should be placed on the needle to oppose the throw and synovium. *Note: if the tip of the extended needle is abutting a bony surface, backwards movement of the needle will occur, leading to poor tissue retrieval.*
7. Remove tissue from the biopsy needle by gently lifting or scraping the tissue using either another sterile needle, the flat edge of a sterile scalpel or the flat edge of sterile forceps.
    a. Always use new, clean, and sterile instruments for tissue removal from the biopsy needle to avoid contamination of the biopsy needle.
    b. Tissue specimens should be placed directly into the 12-well plate containing cold PBS (see Section 8).
8. When enough specimens are harvested, the remaining fluid should be aspirated, and 10 mL of normal saline should be used to briefly flush the joint to clear any debris or blood.

6.2.3. Wrist

Figure 3: Ultrasound-Guided Wrist Biopsy



[A] Positioning.
[B] Area to be sterilized (pink).
[C] Diagram depicting palmar flexion hand positioning
[D] Ultrasound for guidance; needle (white line), tendon (blue, with designated tendon groups IV-VI), synovium (yellow), possible angles of needle insertion to avoid tendons (red line), avoid superficial veins (red X).
[E] Possibility of accessing the radio-carpal or mid-carpal joints.
[F] Insertion of needle.

Orientation and Positioning of the Subject

1. The subject should either be in a seated position, reclining at 45 degrees, or lying supine per patient preference on an appropriate surface, with the hand placed on a table or dedicated adjacent surface. The hand should be pronated (palm facing downwards) and resting in front of the patient, so that the ulnar wrist is facing away from the patient (Figure 3 A).
2. Care should be taken not to elevate the hand or abduct the shoulder significantly as this will cause patient discomfort during the procedure.

Preparation

1. All participating staff with close or direct patient contact (e.g. proceduralist, assistant, ultrasonographer) should commence personal preparations for the procedure which includes hand washing, wearing gloves, face mask, hair net/hat, and sterile gown.
2. Evaluate the equipment tray, including biopsy needles.
3. Place absorbent pads under the wrist.
4. Sterilize the skin using appropriate sterilization fluid. A wide field should be sterilized, beyond the immediate area of interest (e.g., hand to mid-forearm, dorsal and ventral aspects) (Figure 3 B).
5. Position the sterile drapes below the wrist on the table and use a sterile drape as a cuff at the mid forearm. The wrist should be placed in slight palmar flexion to improve access and identification of the synovial recesses (Figure 3 C). This can be facilitated by placing a few sterile sections of gauze under the wrist.
6. The ultrasonographer should place the US probe within the sterile sheath. US gel should be placed first upon the probe footprint and the probe slowly lowered into the sheath. The upper end of the sheath should be secured with a sterile tie or elastic band usually provided with the sheath. *Note: the sterile ultrasound gel should never come in direct contact with the subject's skin as this can cause tissue damage.*
7. Prepare a syringe with 10 mL 1% lidocaine.
8. US examination of the wrist should be performed prior to the biopsy to identify the extensor tendons of the 4th, 5th and 6th compartments, the scaphoid lunate junction and suitable synovial tissue. A suitable path should be planned as the normal anatomical relations may be disturbed in chronic Rheumatoid Arthritis. A suitable path can usually be identified inserting the needle superior to the Extensor Carpi Ulnaris (ECU) tendon and passing inferior to the Extensor Digiti Quinti Proprius (EDQ) and long flexors (Extensor Digitum longus). The typical region for biopsy lies just superior to the scaphoid lunate ligament (Figure 3 D-F).
9. A piece of sterile gauze may be placed under the wrist to elevate the joint to facilitate better access and maneuverability when performing the biopsy procedure.

Biopsy Procedures

1. Using the initial US scan, determine point of insertion and inject 2-5 mL 1% lidocaine into the skin and subcutaneous tissue. Leave a minimum of 3-5 mins for anesthetic effect.
2. Using US guidance, insert a 19G needle and aspirate as much fluid as possible. If there is less than 0.3 mL synovial fluid present, proceed to Step 4.
3. Disconnect the syringe leaving the 19G needle in situ. Dispense the aspirated synovial fluid into a 4 mL green-top sodium heparin (NaHep) tube for further processing (Section 8.4).

4. Inject 2-5 mL 1% lidocaine into the synovial recess where biopsy is planned, allowing 5 minutes for anesthetic to take effect.
5. Using US guidance, insert the biopsy coaxial introducer into the wrist. This is best achieved by imaging the needle along its long axis. Once the introducer is in place, withdraw the sharp obturator. The biopsy needle should be primed (spring is compressed and biopsy needle retracted) before its introduction to the synovial space.
6. The biopsy needle should be extended, and the throw identified on the US images. The throw of the needle should be placed against the surface of the synovium to maximize the opportunity for capturing the lining layer. Gentle pressure should be placed on the needle to oppose the throw and synovium. *Note: if the tip of the extended needle is abutting a bony surface, backwards movement of the needle will occur, leading to poor tissue retrieval.*
7. Remove tissue from the biopsy needle by gently lifting or scraping the tissue using either another sterile needle, the flat edge of a sterile scalpel or the flat edge of sterile forceps.
    a. Always use new, clean and sterile instruments for tissue removal from the biopsy needle to avoid contamination of the biopsy needle.
    b. Tissue specimens should be placed directly into one of the wells the 12-well plate pre-filled with cold PBS (see Section 8).
8. When enough specimens are harvested, the remaining fluid should be aspirated.

6.3. Aftercare

1. Apply bacitracin ointment at biopsy site, covered with 4”x4” gauze, and wrapped with Coban.
2. A neurovascular assessment should be made of the respective extremities following the procedure and documented in the patient’s notes.
3. The subject should remain in the department for a minimum of 30 minutes post procedure.
4. The subject should be instructed to change the dressing daily for 3 consecutive days and in the same manner as initially applied and after getting wet (e.g., after shower).

Post-biopsy risks include but are not limited to; pain, soreness, redness, swelling, pseudogout flares, hemarthrosis. These risks and contact details in case of complications should be provided to the subject. Specifically, subjects should be asked to contact the team if there is significant pain, swelling or redness of the biopsied joint within the next 5-7 days.

7. SAFETY REPORTING

All adverse events (AEs) occurring as a result of the biopsy procedure must be reported into the electronic data capture (EDC) system and tracked as required in the clinical study protocol. AEs should be graded according to the Common Terminology Criteria for Adverse Events Version 5.0 (CTCAE v5.0).

8. SPECIMEN HANDLING, ALLOCATION, PROCESSING, AND STORAGE

This section describes the methods for handling, allocating, processing, and storage of synovial tissue and fluid specimens obtained from the biopsy and aspiration procedures.

8.1. Pre-Procedure Preparation of Sample Collection Supplies

The Specimen Manager should ensure that all necessary collection vials, reagents, and supplies are prepared and brought to the procedure room.

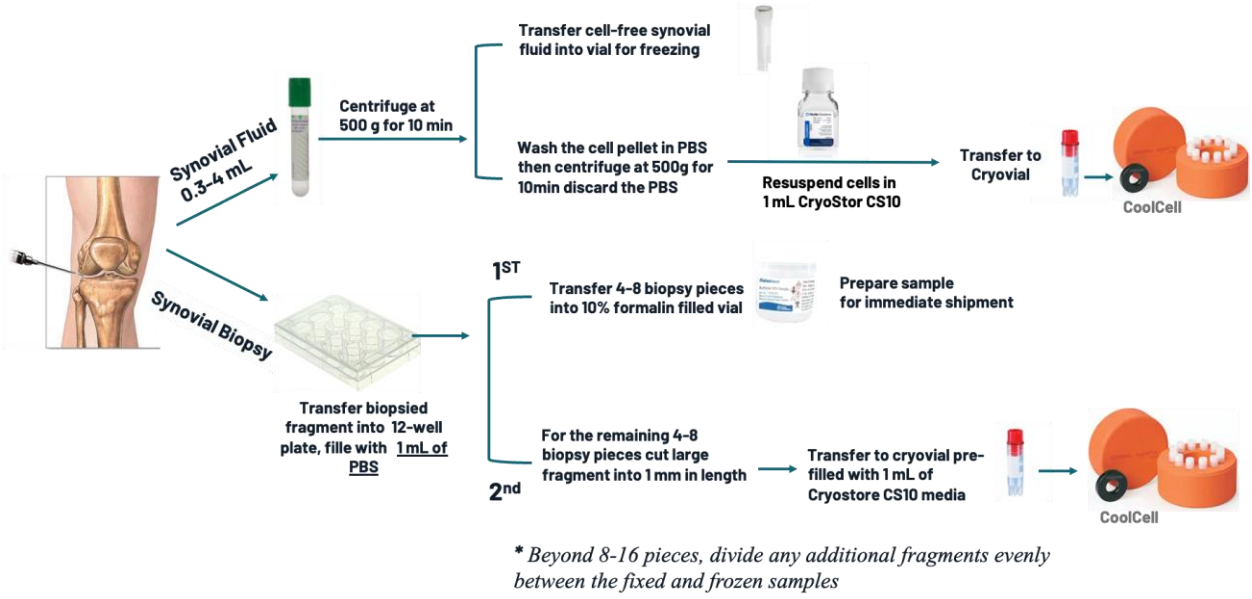
- 4 mL NaHep tube (green-top) to be used for transfer of synovial fluid from needle.
- 20 mL vial containing 10% formalin (To be used for FFPE synovial tissue fragments)
- Add 1 mL of PBS (PBS is kept at 4°C) into each well of the 12-well plate. Plate should be kept at 4°C (refrigerated) until ready for use. When ready for use, the plate should be placed on ice and transferred to the procedure room.
- Add 1 mL of Cryostor CS10 (Cryostore CS10 is kept at 4 degrees C) media to 2 cryovials. Place the cryovials on ice and transfer to a procedure room (to be used for freezing synovial tissue fragments).
- Prepare the ice bucket to maintain the 12 well plate and Cryovials cold during the procedure.
- Identify appropriate labels from the requisition form for each sample type. Labels should only be removed from requisition forms and placed on tubes after samples have been collected.

8.2. Specimen Types and Sample Processing Workflow

The following specimen types will be collected:

- Synovial tissue: Fragments are collected from the subject and first placed in the 12 well plate containing cold PBS for inspection. The fragments should then be divided between 10% formalin- or CryoStor freezing medium. Minimize the time between moving the biopsy to appropriate preservation media to prevent the initiation of cell death and subsequent degradation. Tissue biopsy samples need to be processed and transferred to the appropriate containers within 30 minutes of collection, see Section 8.3 for further details.
- Synovial fluid is collected in green-top (NaHep) tubes in the procedure room. Synovial fluid should be processed by centrifugation to obtain cell free synovial fluid and pelleted cells within 2 hours of collection (Figure 4). See Section 8.4 for further details.

Figure 4: Tissue Processing Workflow



** Beyond 8-16 pieces, divide any additional fragments evenly between the fixed and frozen samples*

8.3. Processing of Synovial Tissue

Tissue samples must be processed into a 10% formalin or freezing medium (CryoStor CS10 media) within 30 minutes of collection and as soon as possible.

Synovial tissue samples for formalin fixation are the highest priority. In situations where biopsy sampling yields less than the minimal amount of tissue for both formalin fixation and freezing, prioritization should be given to formalin fixation.

The Biopsy Visit Requisition Forms for both the formalin sample (FFPE Synovial Biopsy, Appendix B) and frozen (Cryo Synovial Biopsy, Appendix C) should be filled out completely. Labels should be placed on tubes immediately following sample collection.

1. Prior to start of procedure, the 12-well plate should be prepared as described (see Section 8.1 or Appendix A).
2. Upon removal synovial tissue fragments from the joint,
    a. Gently transfer tissue biopsy samples from biopsy needle into PBS of 12-well plate. *Note: To avoid crush artifact, tissue should be gently lifted or scraped from the biopsy needle using a sterile needle, scalpel (flat edge) or forceps.*
    b. Identify and inspect tissue fragments.
    c. Select synovial tissue samples to be placed in 10% formalin vial (FFPE Synovial Biopsy) as described in Table 1. *Note: the FFPE Synovial Biopsy samples are of highest priority. Ensure adequate synovial tissue samples are first allocated to formalin fixation.*
    d. Select synovial tissue samples for freezing in Cryovials as described in Table 2. Samples for freezing should be cut into ~1 mm in length to ensure proper freezing. *Note: Never place tissue intended as a frozen specimen into formalin.*

- A sterile scalpel can be used to cut larger fragments into ~1 mm length, (Use the 12 well-plate lid for cutting the fragments into smaller pieces when needed).
- Using a sterile needle, the flat edge sterile forceps or the flat edge of a sterile scalpel, transfer fragments into a single cryovial containing 1 mL of Crystore CS10 media (Transfer works best if all fragments are placed to one side of the cryovial).
- Gently shake or flick the cryovial to separate and suspend the tissue pieces in the freezing medium. Be sure no fragments stick to the side of the tube.

e. Remaining fragments should be distributed between formalin vials and Cryovials as described in Table 1 and Table 2, with priority given to formalin fixation.
f. Label each tube with its corresponding label from the Biopsy Visit Requisition form.

3. Record numbers of biopsies taken on the Biopsy Visit Requisition Form
4. See Sections 8.3.1, 8.3.2, and 9 for information on short term storage and shipping of the samples.

Table 1: Allocation of Large Joint (Knee/Ankle) Specimens:

	Preservation	Number of fragments	Format	Process
1	Formalin Fixed tissue (FFPE Synovial Biopsy)	6 – 8[a]	Loose in a pre-filled vial with 10% formalin	Place tissue directly into 10% formalin
2	Frozen tissue (Cryovials)	6 – 8[a]	Loose in in a cryovial filled with Cryostore CS10	Cut tissue to ~1 mm pices and place it into 2 mL cold Cryostor medium

[a] Allocate 6 fragments first for fixation and then allocate 6 fragments for freezing. Divide any additional fragments evenly between the fixed and frozen samples. Collect up to 16 fragments in total (even if using Portal and Forceps method, which would typically generate 20-30 fragments).

Table 2: Allocation of Small/Medium Joint (Wrist/Digits) Specimens

	Preservation	Number of fragments	Format	Process
1	Formalin Fixed tissue (FFPE Synovial Biopsy)	4-6[a]	Loose in 10% formalin in a pre-filled vial	Place tissue directly into pre-filled vial
2	Frozen tissue (Cryo)	4-6a	Loose in CryoStor CS10 medium in a cryovial	Place tissue into 2 mL cryovial with 2 mL cold Cryostor medium

[a] Allocate 4 fragments first for fixation and then allocate 4 fragments for freezing. Divide any additional fragments evenly between the fixed and frozen samples. Collect up to 12 fragments in total.

8.3.1. Formalin-Fixed Tissue Sample Processing – Additional Instructions

1. Tissue samples in 10% Formalin tissue (FFPE Synovial Biopsy) should be kept refrigerated until shipment to Mosaic Laboratories, L.L.C.
2. Tissue samples in 10% formalin should be shipped within **24 hr** of collection to Mosaic Laboratories, L.L.C. whenever possible. Specific shipping instructions are provided in the Lab Manual.
3. If short-term temporary storage is necessary, additional specimen processing steps for synovial tissue samples are required to ensure specimen integrity. The following steps are required if FFPE samples cannot be shipped within 24 hr of collection:
    a. Allow samples to fix in 10% formalin vial for 24 hr.
    b. Fill labeled 20 mL SecurTainer with 10 mL of 70% Reagent Grade Alcohol (provided by the sponsor).
    c. Label the 20 mL SecureTainer container.
    d. Using sterile forceps, gently transfer formalin-fixed synovial tissue samples from formalin vials to SecurTainer containing 10 mL of 70% Reagent Grade Alcohol (minimize formalin carryover into the new container).
    e. Store SecurTainer at 4°C until ready for shipment (samples should be shipped within 24 hr after transfer to the 70% Reagent Grade Alcohol).

8.3.2. Frozen Tissue Sample Processing – Additional Instructions

1. All frozen tissue specimens should be suspended in cold Cryostor freezing media. Be sure that no fragments are stuck to the sides of the tube.
2. Make sure the core (black ring) is seated in the bottom of the CoolCell container. Place the labeled Cryovial containing tissue sample into the empty CoolCell well.
3. Place the CoolCell container at ≤-70°C for at least 24 hr. Samples should then be removed from the CoolCell container and shipped to MedPace Reference Laboratories (MRL) on dry ice within 7 days of freezing. Detailed shipping instructions are provided in the Lab Manual.

8.4. Processing of Synovial Fluid

The Biopsy Visit Requisition Form should be filled out completely.

1. The fluid should be transferred from the collecting syringe into a 4 mL green-top sodium heparin (NaHep) tube. If more than 4 mL are collected, please discard extra fluid.
2. The green top NaHep tube should be inverted 8-10 times immediately after being placed in NaHep tube.
3. Centrifuged NaHep tube at room temperature at 500 g for 10 minutes within 2 hours of collection.

4. The supernatant (cell-free synovial fluid) should be transferred using a transfer pipette into new, 2 mL empty screw-cap vial as described below. (Please use screw-cap tubes provided in the kit and the provided labels only).
    a. If 0.3 mL to 4 mL is acquired, split the total volume between two tubes.
    b. If less than 0.3 mL is acquired, freeze all the synovial fluid in one vial.
5. Cell free synovial fluid aliquots must be placed on ice until transfer into a ≤-70°C freezer.
6. The cell pellet should be resuspended by gently pipetting up and down in 2 mL cold PBS using a transfer pipette.
7. Centrifuge the resuspended cell pellet at room temperature at 500 g for 10 minutes.
8. Remove PBS using a transfer pipette and discard PBS, Resuspend the new cell pellet in 1 mL cold CryoStor CS10 media by gently pipetting up and down and immediately transferred to a new 2 mL cryovial tube (Cryovials provided in the kit, please use provided labels only).
9. The controlled-rate freezing container (provided by the sponsor) should be used for viable freezing of resuspended cells. Make sure the core (black ring) is seated in the bottom of the CoolCell container. Place the labeled sample vials containing resuspended cells in empty CoolCell wells.
10. Place the CoolCell container at ≤-70°C for at least 24 hr. Samples should then be removed from the CoolCell container and shipped to MedPace Reference Laboratories (MRL) on dry ice within 7 days of freezing. Detailed shipping instructions are provided in the Lab Manual.

9. SHIPPING OF SPECIMENS

Please refer to the Lab Manual for detailed specimen shipping instructions, including:

- Shipping supplies and labels
- Pre-shipping specimen preparation

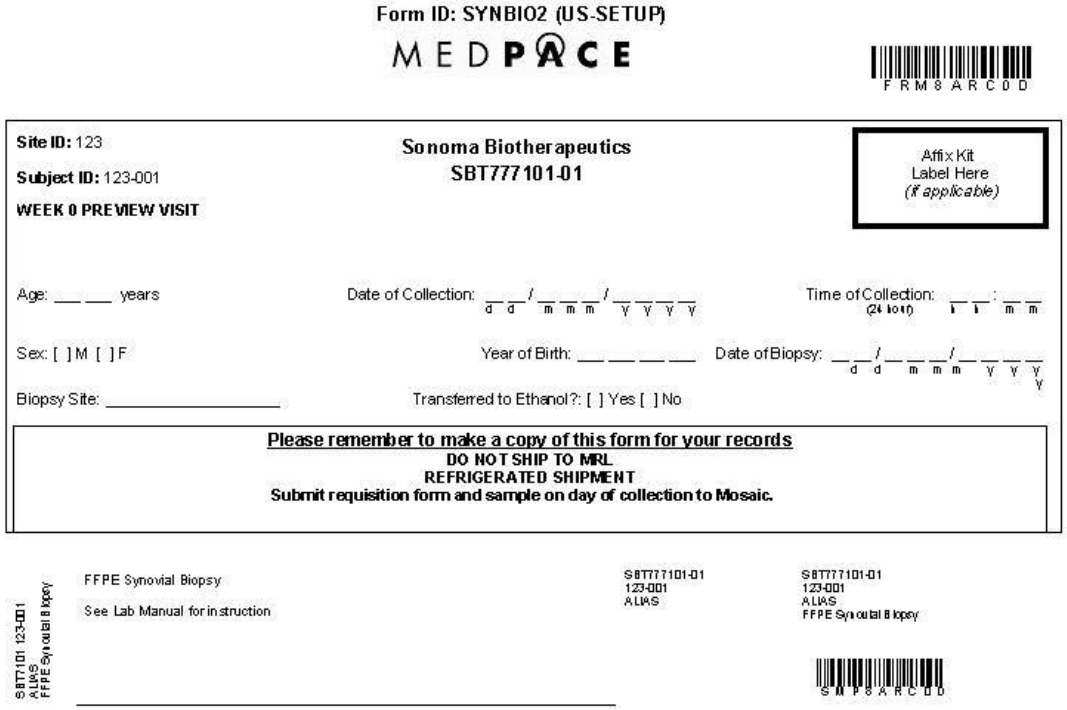
APPENDIX A. SUMMARY OF SPONSOR-PROVIDED SUPPLIES AND INSTRUCTIONS FOR SYNOVIAL TISSUE AND FLUID PROCESSING

Item	Purpose	General Instructions and Notes
Biopsy Visit-Specific Kit		
Biopsy Visit Requisition Form	Documentation and tracking of biopsy tissue and fluid samples	• To be filled out completely, the date and time of sample collection must be noted. A copy of the requisition form should be made for site records. • Original form to be placed in shipper with biopsy samples. Detailed shipping instructions provided in the Lab Manual.
Green-Top Sodium Heparin (NaHep) Tube	Collection & processing of synovial fluid	• NaHep tubes should be brought to the procedure room for synovial fluid sample collection. • Synovial fluid in NaHep tube should be processed within 2 hr of sample collection. • See Section 8.4 for synovial fluid sample processing details.
2 mL Cryovials	Collection and freezing of synovial tissue and synovial fluid cell pellet	• *Cryovials for tissue biopsy require pre-procedure preparation*. See Section 8.1 for preparation instructions. • Keep Cryovials with CryoStor CS10 media on ice during sample collection and processing. • Samples in cryovial must be frozen at <-70 °C using the CoolCell Controlled-rate freezing container for at least 24 hr prior to shipping. • Samples should be shipped to MRL within 7 days of collection. • Ship on dry ice. Detailed shipping instructions provided in the Lab Manual.
2 mL Screw cap tubes	Cell free synovial fluid	• Synovial fluid samples post-centrifugation in NaHep tubes (Cell free synovial fluid) must be transferred to 2 mL screw cap tube for freezing, storage and shipping (details in Section 8.4 numbers 5-9) • Samples should be shipped to MRL within 7 days of collection. • Ship on dry ice. Detailed shipping instructions provided in the Lab Manual.
Bulk Supplies		
10% Formalin-filled vials	Collection and formalin fixation of synovial tissue	• Formalin-filled vials should be brought to the procedure room for biopsy sample collection. • *Synovial tissue samples for formalin fixation should be prioritized over freezing when tissue samples are limited.*

Item	Purpose	General Instructions and Notes
		• Tubes should be stored at room temperature until use. • Formalin samples should be shipped to Mosaic Laboratories, L.L.C. within 24 hr whenever possible. Detailed shipping instructions provided in the Lab Manual. • ***Important****: samples that cannot be shipped within 24 hr must be transferred from the formalin-filled vials into a 20 mL SecurTainer containing 10 mL of 70% Reagent Grade Alcohol after 24 hr of formalin fixation.*
CryoStor CS10 media	Media for freezing synovial tissue, and synovial cell pellet	• Store at 4°C (refrigerated) • Keep on ice when in use
CoolCell Controlled-rate freezing container	To gradually lower the temperature of samples	• Store at 4°C (refrigerated) when empty & not in use. • Place on ice when in use. • Make sure the core (black ring) is seated in the bottom of the CoolCell container prior to loading with Cryovials. • Place the labeled Cryovials containing tissue/fluid/cell pellet sample in empty CoolCell well. • Place CoolCell with samples into ≤-70°C for at least 24 hr before shipping. • Cryovial samples should be removed from the CoolCell and placed on dry ice for shipment to MRL within 7 days.
12 well plate	Provided for synovial tissue sample handling	• *12-well plate requires pre-procedure preparation.* See Section 8.1 for pre-procedure preparation instructions. • 12-well plate with cold PBS should be brought to the procedure room for biopsy sample collection & processing. • Keep on ice when in use.
Sterile forceps	for specimen handling during tissue processing & preparation	• Forceps may be used to gently handle synovial biopsy tissue during processing. • Care should be taken when handling biopsy samples to avoid crush artefact.
Sterile Scalpel	For cutting synovial biopsy tissue samples intended for	• Scalpel may be used to gently cut synovial biopsy tissue fragments into ~1 mm pieces prior to sample freezing in CryoStor vials.

Item	Purpose	General Instructions and Notes
	Cryovial into ~1 mm fragments	
Sterile pipettes	For transfer of reagents & synovial fluid	• Pipettes should be used for all media, liquid reagent, and synovial fluid transfers.
20 mL SecurTainer	For use if the 10% formalin sample is not shipped within 24 hr of collection	• Formalin-fixed samples must be transferred into a SecrueTainer containing 10 mL of 70% ETOH if samples are not shipped within 24 hr of collection in order to prevent over-fixation. • See Section 8.3.1 number 3 for detailed instructions.
70% Reagent Grade Alcohol	For use if the 10% formalin sample is not shipped within 24 hr of collection	• Formalin-fixed samples must be transferred into a SecrueTainer containing 10 mL of 70% ETOH if samples are not shipped within 24 hr of collection in order to prevent over-fixation. • See Section 8.3.1 number 3 for detailed instructions. • Allow synovial tissue biopsy samples to fix in the 10% formalin-filled vials for 24 hrs prior to transfer; do not transfer samples to SecurTainer with 10 mL of 70% Reagent Grade Alcohol sooner than 24 hr. • Store at room temperature.

APPENDIX B. REQUISITION FORM FOR FFPE BIOPSY SAMPLE



Form ID: SYNBIO2 (US-SETUP)

MEDPACE

FRM8ARC0D

Site ID: 123

Subject ID: 123-001

WEEK 0 PREVIEW VISIT

Sonoma Biotherapeutics
SBT777101-01

Affix Kit Label Here *(if applicable)*

Age: ___ ___ years

Date of Collection: ___ ___ / ___ ___ ___ / ___ ___ ___ ___ (d d m m m y y y y)

Time of Collection: ___ ___ : ___ ___ (24 hour) (h h m m)

Sex: [ ] M [ ] F

Year of Birth: ___ ___ ___ ___

Date of Biopsy: ___ ___ / ___ ___ ___ / ___ ___ ___ ___ (d d m m m y y y y)

Biopsy Site: _____

Transferred to Ethanol?: [ ] Yes [ ] No

Please remember to make a copy of this form for your records
DO NOT SHIP TO MRL
REFRIGERATED SHIPMENT
Submit requisition form and sample on day of collection to Mosaic.

SBT777101 123-001 ALIAS FFPE Synovial Biopsy

FFPE Synovial Biopsy

See Lab Manual for instruction

SBT777101-01
123-001
ALIAS

SBT777101-01
123-001
ALIAS
FFPE Synovial Biopsy

SMPSARC0D

APPENDIX C. REQUISITION FORM FOR CRYOPRESERVED SAMPLE

Form ID: SYNBIO1 (US-SETUP)

MEDPACE

FRM8ARCOD

Site ID: 123
Subject ID: 123-001
WEEK 0 PREVIEW VISIT

Sonoma Biotherapeutics
SBT777101-01

Affix Kit Label Here *(if applicable)*

Age: ___ ___ years

Date of Collection: ___ ___ / ___ ___ / ___ ___ ___ ___ (d d / m m / y y y y)

Time of Collection: ___ ___ : ___ ___ (24 hour) (h h : m m)

Sex: [ ] M [ ] F

Year of Birth: ___ ___ ___ ___

Date of Biopsy: ___ ___ / ___ ___ / ___ ___ ___ ___ (d d / m m / y y y y)

Biopsy Site: _____

Please remember to make a copy of this form for your records

FROZEN SHIPMENT
Submit requisition form and samples frozen in weekly batches.

SBT1101 123-001 ALIAS Cryo Synovial Biopsy Alq 1

Cryo Synovial Biopsy Alq 1
See Lab Manual for instruction

SBT777101-01
123-001
ALIAS

SBT777101-01
123-001
ALIAS
Cryo Synovial Biopsy Alq 1
(2m L Conilg)

SMPSARCOD

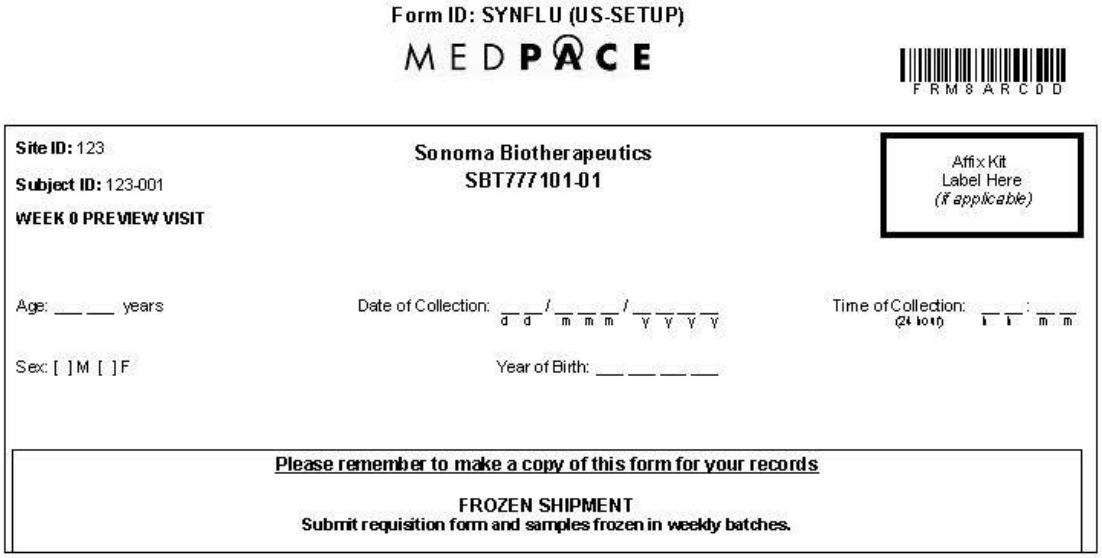
SBT1101 123-001 ALIAS Cryo Synovial Biopsy Alq 2

Cryo Synovial Biopsy Alq 2
See Lab Manual for instruction

SBT777101-01
123-001
ALIAS
Cryo Synovial Biopsy Alq 2
(2m L Conilg)

SMPSARCOD

APPENDIX D. REQUISITION FORM FOR SYNOVIAL FLUID SAMPLES



Form ID: SYNFLU (US-SETUP)

MEDPACE

FRM8ARC0D

Site ID: 123

Subject ID: 123-001

WEEK 0 PREVIEW VISIT

Sonoma Biotherapeutics
SBT777101-01

Affix Kit Label Here *(if applicable)*

Age: ___ ___ years

Date of Collection: ___ ___ / ___ ___ / ___ ___ ___ ___ (d d / m m / y y y y)

Time of Collection: ___ ___ : ___ ___ (24 hour) (h h : m m)

Sex: [ ] M [ ] F

Year of Birth: ___ ___ ___ ___

Please remember to make a copy of this form for your records

FROZEN SHIPMENT
Submit requisition form and samples frozen in weekly batches.

SBT7101 123-001 ALIAS Synovial Fluid Supernatant Aliq 1

Synovial Fluid Supernatant Aliq 1
See Lab Manual for instruction

SBT777101-01
123-001
ALIAS
Synovial Fluid Supernatant and Cells
(4m L NaHep)

Prepare from above tube

SBT777101-01
123-001
ALIAS
Synovial Fluid Supernatant Aliq 1
(2m L Sarstedt)

SMPSARC0D

SBT7101 123-001 ALIAS Synovial Fluid Supernatant Aliq 2

Synovial Fluid Supernatant Aliq 2
See Lab Manual for instruction

SBT777101-01
123-001
ALIAS
Synovial Fluid Supernatant Aliq 2
(2m L Sarstedt)

SMPSARC0D

SBT7101 123-001 ALIAS Synovial Fluid Viable Cells

Synovial Fluid Viable Cells
See Lab Manual for instruction

SBT777101-01
123-001
ALIAS
Prepare from above tube

SBT777101-01
123-001
ALIAS
Synovial Fluid Viable Cells
(2m L Corning)

SMPSARC0D