PRO-00879A Sample Collection Protocol

Detection of *Borrelia* Bacteria in Early Stage Lyme Borreliosis
using the T2Lyme Panel

Sponsor: T2 Biosystems, Inc.
101 Hartwell Avenue
Lexington, MA 02421

Study Monitor: MDC Associates
180 Cabot Street
Beverly, MA 01915

CONFIDENTIAL

Protocol Title:	Sample Collection Protocol for the Detection of *Borrelia* Bacteria in Early Stage Lyme Borreliosis using the T2Lyme Panel
Protocol Number:	PRO-00879A
Revision Number:	4.1
Revision Date:	October 1, 2018
Sponsor:	T2 Biosystems, Inc. 101 Hartwell Avenue Lexington, MA 02421
Study Monitor:	MDC Associates, LLC 180 Cabot Street Beverly, MA 01915 Contact: Fran White Phone: (978) 927 3808 Fax: (978) 927 1308 Email: regulatory@mdcassoc.com

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I. Study Overview

The objective of this study is to demonstrate the clinical performance of the T2Lyme Panel on the T2Dx instrument. The assay will be compared to *Borrelia* culture from erythema migrans (EM) biopsy and/or detection of the C6 antigen in serum collected prospectively from patients suspected of early Lyme disease demonstrated by EM lesion or constitutional abnormalities consistent with a tick bite and sickness from a tick bite. The data from this study will be used to support a *de novo* application for the T2Lyme Panel to the U.S. Food and Drug Administration (FDA) for product approval.

The T2Lyme Panel is an investigational use *in vitro* diagnostic (IVD) designed to qualitatively detect and identify the major causative agents of Lyme disease (*Borrelia burgdorferi, Borrelia afzelii, Borrelia garinii,* as well as an inclusive *Borrelia* spp. detection channel) from K_2EDTA human whole blood samples.

This protocol (PRO-00897A) outlines subject enrollment, sample collection and shipping procedures. Samples collected will be shipped to defined laboratories for reference testing and sample storage.

II. Intended Use

The T2Lyme Panel is a qualitative T2 Magnetic Resonance (T2MR®) assay for the direct detection of bacteria species from EDTA human whole blood of patients with signs, symptoms and clinical history consistent with early Lyme disease. The T2Lyme Panel detects *Borrelia* spp., and specifically differentiates *Borrelia burgdorferi, Borrelia afzelii,* and *Borrelia garinii.* The T2Lyme Panel is used as an aid in the diagnosis of early stage Lyme disease.

III. Study Timeframe

The goal is to collect and test as many patients as possible in the initial Lyme season of 2018. If needed, study will continue into the 2019 season.

IV. Study Sites

Point-of-care site(s) including, but not limited to, physician's offices, urgent care centers, hospital clinics/ER in areas endemic to Lyme disease will be contracted to collect required study samples. Sites will be contracted to recruit individuals suspected of having early Lyme disease in accordance with protocol defined

inclusion criteria. Trained collection site staff members or technicians will consent and enroll eligible patients prior to obtaining required samples.

- Arm 1
  - The five (5) K2EDTA tubes, single serum tube, and tissue samples collected at the study sites will be delivered by overnight express delivery to a single, defined laboratory for T2Lyme, serology and culture of tissue biopsy. The reference laboratory will receive and document receipt of each sample.
- Arm 2
  - Three (3) K_2EDTA tubes and the single serum tube of blood collected at the study sites will be delivered by overnight express delivery to a single, defined laboratory for T2Lyme, serology and PCR testing. The remaining five (5) K_2EDTA tubes collected will be delivered by overnight express delivery to T2 Biosystems for blood culture studies.

Sponsor may add additional sites as needed to complete study within needed timelines. All study staff members will be trained on study protocol, processes and study forms. Training will be documented.

V. Study Design

Patients meeting eligibility criteria will be consented for the study prior to collecting samples. The study will consist of two arms; one in which subjects have a clinician-documented erythema migrans (EM) lesion and a second arm where subjects do not have an EM, but present with an appropriate epidemiologic history and clinical signs and symptoms consistent with early Lyme disease.

<u>Arm I – Early Lyme disease with EM lesion</u>

All patients must be diagnosed by a clinician as having an EM lesion.

PATIENTS MUST NOT HAVE RECEIVED ANY TREATMENT FOR LYME DISEASE WITHIN 30 DAYS PRIOR TO STUDY PARTICIPATION.

After obtaining informed consent, a trained staff member/phlebotomist will collect the following samples:

- Five (5) 4mL K_2EDTA vacutainer tubes
- One (1) serum separator tube
- Two (2) 2mm punch biopsies from the leading edge of the largest EM lesion
  NOTE: Every effort must be made to obtain two (2) 2mm punch biopsies. However, if only one (1) punch biopsy is available, participant will still be included in the study.

The collected samples will be sent to the reference lab for T2Lyme, culturing of tissue biopsy, PCR testing from tissue culture, and serology testing.

Arm II – Early Lyme disease Without EM lesion

All patients must be diagnosed by a clinician as having an appropriate epidemiologic history and signs/symptoms (excluding EM) of early Lyme disease

PATIENTS MUST NOT HAVE RECEIVED ANY TREATMENT FOR LYME DISEASE WITHIN 30 DAYS PRIOR TO STUDY PARTICIPATION.

A trained staff member/phlebotomist will collect the following samples:

- Eight (8) 4mL K_2EDTA vacutainer tubes (three (3) additional tubes will be used for blood culture testing)
- One (1) 5 mL serum separator tube

Five (5) of the 4mL K_2EDTA tubes will be sent to T2 Biosystems for blood culture. The remaining blood samples (3 K_2EDTA tubes and one serum separator tube) will be forwarded to the reference lab for T2Lyme, PCR testing from whole blood, and serology testing.

All Patients (Arm 1 and 2)

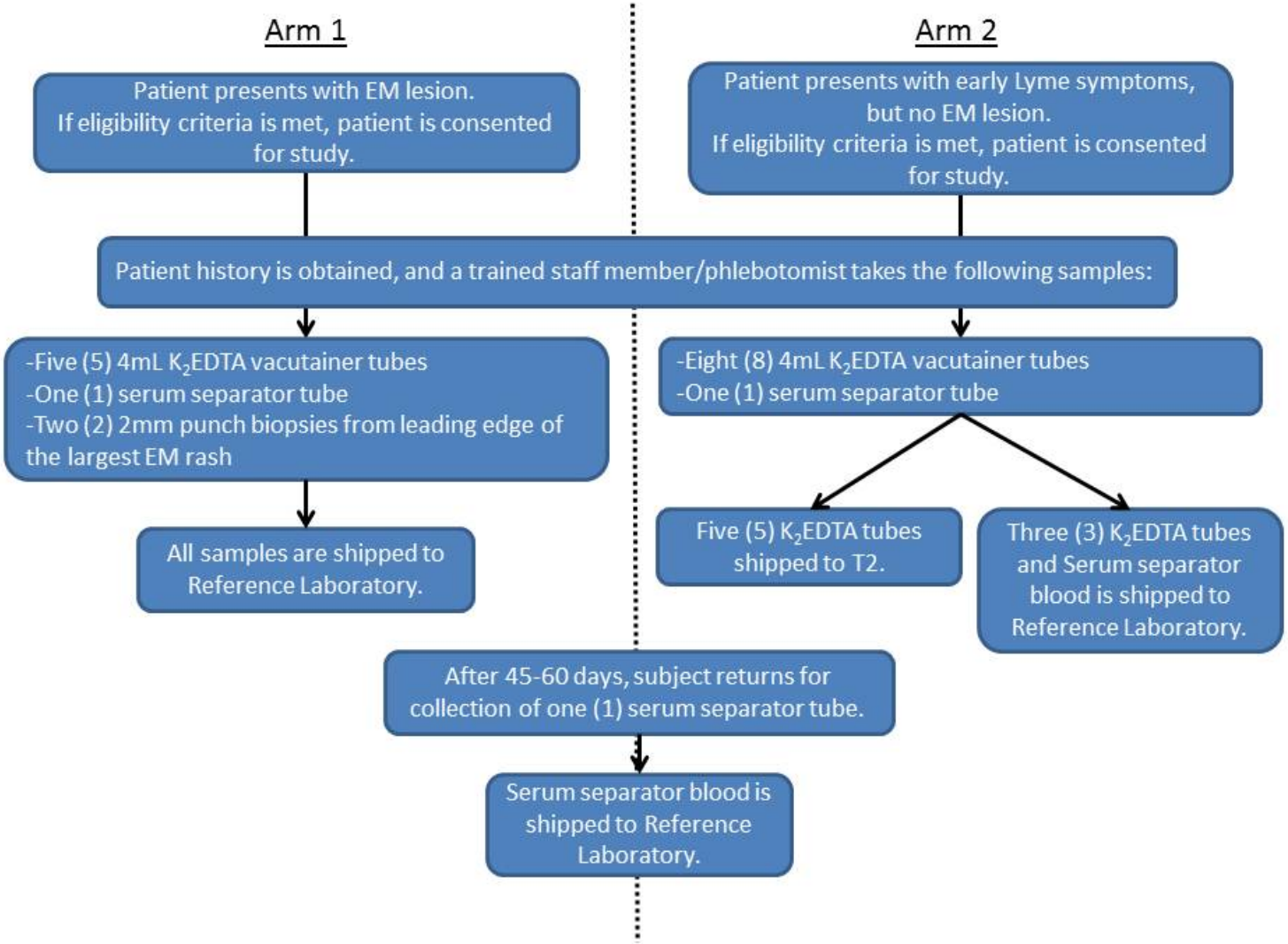
After approximately 45-60 days, patients will return to the site to donate a convalescent serum sample. Blood will be collected in one (1) 5 mL serum separator tube. The blood sample will then be forwarded to the reference laboratory for serology testing. Appointment for this follow-up visit will be set and documented during the initial visit.

All samples will be shipped in accordance with provided instructions to a single, defined reference laboratory for T2Lyme Panel and reference testing, with the exception of samples for blood culture, which will be forwarded to T2Biosystems. Sponsor may add additional sites as needed.

An estimated 300 patients are expected to be enrolled with the goal of obtaining at least 40 samples from early Lyme disease patients with a culture-positive tissue biopsy sample or without EM that seroconvert appropriately.

Each sample will be de-identified and assigned a specific study number that will designate which arm of the study they are enrolled in. Study numbers and labels will be provided to collection site(s) by the sponsor/study monitor. Study-associated test results will not be made available to patients.

See workflow below.



VI. Institutional Review Board (IRB) Approval

The Principal Investigator (PI) at each study site will obtain IRB approval for the study protocol, consent form, and study-related documents. IRB approval will be received prior to beginning the study.

VII. Sample Collection Protocol

The collection site technician must review all eligibility criteria prior to enrolling subjects. The screening process will be documented. The technician will review all relevant study information with the patient and answer questions. Informed consent (either by the subject or the subject's legal representative) will be documented for every study subject.

Arm 1:

Initial Visit

Subjects diagnosed with early Lyme disease by clinician documentation of an EM lesion. NOTE: Subjects must have the rash confirmed as EM by a clinician. Examples of EM presentation include:

- Red welts, sometimes with purple or blistered areas in the center
- Gradually expanding lesion
- Circular, sometimes with a bulls-eye shape
- Central clearing

Inclusion Criteria
Presence of a single or multiple erythematous skin lesion(s), consistent with EM (Erythema migrans) is required for all patients in Arm 1.
Subject is age 18 or older
Subject or subject's legal representative can read, comprehend and sign the study-specific informed consent form (ICF) after the specifics of the study have been explained.
Exclusion Criteria
Subject has a bleeding disorder
Subject is currently on antibiotics or has taken antibiotics in the past 30 days
Subject has an EM located on the face or neck
Subject is unable to provide informed consent

Collection of Punch Biopsies

NOTE: Clinicians must make every effort to obtain two (2) 2mm punch biopsies from the leading edge of the EM according to standard laboratory practices. *At least* one (1) 2mm punch biopsy must be provided for inclusion in the study.

1. Select the area to be biopsied. The punch biopsies must be taken from the leading edge of the EM, including both derma and sub-derma.
2. Take a focused, initial photograph of the EM. Any digital camera can be used, provided the photograph is clear and can be shared with the study monitor/sponsor. Include a subject ID label in the image.
3. Sterilize the area and anesthetize according to standard laboratory practices.
4. Stretch the skin surrounding the biopsy site with the thumb and index finger of the non-dominant hand.
5. Holding the punch biopsy instrument vertically over the skin with the dominant hand, rotate downwards using a twirling motion.
6. Once the instrument has penetrated the dermis, remove and transfer the biopsy sample and place in pre-labeled tube containing culture medium (provided).
7. Every effort must be made to collect a second 2mm punch biopsy. Identify a second site on the leading edge of the same EM, and follow steps 3-5.
8. Take a second photograph of the EM post-biopsy. Include a subject ID label in the frame.
9. Samples can be stored at room temperature, but must be shipped overnight to reference laboratory within 24 hours of collection.

Collection of Blood Samples

NOTE: Blood must be obtained by a trained phlebotomist.

1. Pre-label five (5) (Arm I) 4 mL K_2EDTA vacutainers and one (1) 5 mL serum separator tube with subject's de-identified study ID.
2. Disinfect the injection site and perform venous blood draw in accordance with institutional laboratory practices.
3. Fill every vacutainer to the recommended amount.
4. Invert the K_2EDTA tubes 8-10 times to fully mix the anticoagulant with the blood.

5. Store blood samples 2-8°C until shipment to reference laboratory. Samples must be shipped overnight, on ice, within 24 hours of collection.

Arm 2:

Initial visit

Subjects suspected with early Lyme disease by clinician documentation of appropriate epidemiologic history and clinical signs and symptoms (no EM) consistent with early Lyme disease.

Inclusion Criteria
Clinician documentation of appropriate epidemiologic history and clinical signs and symptoms (no EM) consistent with early Lyme disease.
Subject is age 18 or older
Subject or subject's legal representative is able to read, comprehend and sign the study-specific informed consent form (ICF) after the specifics of the study have been fully explained.
Exclusion Criteria
Subject has a bleeding disorder
Subject is currently on antibiotics or has taken antibiotics in the past 30 days
Subject is unable to provide informed consent

Collection of Blood Samples

NOTE: Blood must be obtained by a trained phlebotomist.

6. Pre-label eight (8) (Arm 2) 4 mL K_2EDTA vacutainers and one (1) 5 mL serum separator tube with subject's de-identified study ID.
7. Disinfect the injection site and perform venous blood draw in accordance with institutional laboratory practices.
8. Fill every vacutainer to the recommended amount.
9. Invert the K_2EDTA tubes 8-10 times to fully mix the anticoagulant with the blood.
10. Store blood samples 2-8°C until shipment to T2 Biosystems and reference laboratory. Samples must be shipped overnight, on ice, within 24 hours of collection.

Follow-Up Visit (Arms 1 and 2)

Collection of Blood Sample

NOTE: Blood must be obtained by a trained phlebotomist,

1. Pre-label one (1) 5 mL serum separator tube with the de-identified study ID.
2. Disinfect the site, and perform venous blood draw in accordance with institutional laboratory practices.
3. Fill vacutainer entirely.
4. Document date/time of sample collection on data form.
   Store at 2-8°C until preparation for shipment to reference laboratory.
   Sample must be shipped overnight, on ice, within 24 hours of collection.

VIII. Data Collection

NOTE: All documentation on provided data forms will be sent to the study monitor. Data will be submitted in accordance with an agreed upon schedule (between study site, study monitor, and sponsor) and instructions provided. Any shipments processed will be documented. Study monitor will provide sites with information specific to any errors or protocol deviations. These will be documented and corrected in a timely manner.

Initial Visit (Arm 1 and 2)

Prior to collecting specific case-related information or study samples, each subject will be assigned a subject ID number specific to the arm of the study. Collection site staff will maintain a patient log linking the patient's Medical Record Number (MRN) to the assigned study ID. This information will not be made available to the study monitor/sponsor.

The study monitor/sponsor will provide labels to affix to all patient-related documentation. Patient demographic information and medical history will be obtained. Information collected may include, but will not be limited to:

Demographic and epidemiologic data such as:

- Age/Date of Birth
- Gender
- Antibiotic history (within past 30 days)
- Previous Lyme diagnosis

- Was a tick observed at time of potential Lyme exposure?

Clinical signs and symptoms such as:

- ☐ Fever ≥ 100.4°F
- ☐ Chills
- ☐ Headache
- ☐ Fatigue
- ☐ Myalgia (Muscle aches)
- ☐ Anthralgia (Joint aches)
- ☐ Swollen lymph nodes
- ☐ EM lesion
- ☐ Neck Pain

Follow-up Visit (Arm 1 and 2)

After approximately 45-60 days, the subjects will return to the collection site, where the following information will be documented:

- Resolution or persistence of EM (if subject presented with an EM at initial visit)
- Any remaining Lyme disease-related symptoms/complaints
- Any ongoing constitutional symptoms (including, but not limited to: weight loss, fever, chills, dyspnea, pain, fatigue, decreased appetite)
- Adherence to prescribed treatment(s) related to Lyme disease

IX. Shipping

Samples will be shipping according to provided instructions (see Appendix 1).

X. Withdrawn Samples

Subjects may withdraw from participation at any point in the study. The principal investigator (or designee) may also determine that withdrawal from the study is in the best interest of the subject.

If a blood sample cannot be obtained from the patient's arm/hand after three (3) blood draw attempts at the initial visit, patient will be withdrawn from study. For subjects that present with an EM at the initial visit, if only the convalescent sample cannot be obtained, participant data will be maintained in the study. For subjects that do not present with an EM at the initial visit, subjects will be withdrawn if the convalescent sample cannot be collected. Subjects that do not provide two biopsies (or biopsy is unattainable) may also be withdrawn if an EM is present. Patients will be compensated by the sponsor for their involvement in the study.

A patient with an EM who consents to the initial visit but fails to present at follow-up visit will still be considered enrolled in the study, unless specifically requesting to withdraw from the study.

Samples may also be withdrawn from the study if sample volume is insufficient for testing (blood is clotted in all K_2EDTA tubes or tissue biopsy received is not of sufficient volume). In the event of shipment errors (leakage or delays that render samples unstable), samples will be evaluated and withdrawn, as necessary.

For every withdrawn subject, reason for withdrawal will be documented. However, based on subject agreement, any data or blood samples collected prior to study withdrawal will be analyzed and included in the study database. A patient who consents to the initial visit but fails to present at follow-up visit will still be considered as enrolled in the study, unless specifically requesting to withdraw from the study.

XI. Site File/Data Record Storage

The study site will maintain all study documentation for a minimum of three (3) years.

XII. Risk/Benefit Analysis

This method comparison presents no risk or benefits to patients. Results will not be used for diagnostic or clinical purposes. The benefit of this method comparison is to expand laboratory options for early Lyme disease diagnosis.

All patient identifiers will be removed. Results will be identified only by a unique study identification number.

XIII. Study Oversight

At the discretion of the Sponsor, this protocol may be prematurely terminated. Reasons may include but are not limited to: assay and/or instrument performance, new regulatory pathway information, business and/or market information.

This study may be subject to and will be made available for monitoring, auditing, IRB review and regulatory inspection as deemed necessary by providing direct access to study related source data.

XIV. Confidentiality

Samples enrolled in this study will be de-identified, therefore confidentiality of records identifying the subject is not a concern. Demographic information requested are not requirements and, if not readily known by the laboratory requisition form, do not need to be sought via a medical record review. Subjects will not be provided the study test results, nor will the results be included in their medical records.

The FDA may inspect all records related to this study. As this study does not require nor request a medical record review, the IRB and/or other regulatory authorities will not have access to study-related medical records.

XV. Protocol Departure

The protocol described for this study will only be changed or altered on the written approval of the PIs, study monitor, sponsor and IRBs. In instances of protocol deviation, notify the study monitor immediately.

XVI. Protocol Approval

I/We agree to perform this study according to the requirements listed in this revision of the protocol.

Sponsor Approval		
_____ Sponsor Printed Name	_____ Sponsor Signature	____ / ____ / _____ Date Signed
Principal Investigator (PI) Approval		
_____ PI Printed Name	_____ PI Signature	____ / ____ / _____ Date Signed
Study Monitor Approval		
_____ Study Monitor Printed Name	_____ Study Monitor Signature	____ / ____ / _____ Date Signed

XVII. Protocol Revision History

Revision Number	*Revision Date*	*Description of Changes*
1	March 27, 2018	Initial Release
2	March 30, 2018	Clarification of convalescent serum sample collection protocol.
3	June 8, 2018	Added procedures for Arm 2 of sample collection without presence of EM.
4	September 6, 2018	Updates for clarification of procedure and alignment with testing protocol.
4.1	October 1, 2018	Minor updates to language.

APPENDIX I

Sample Shipping Procedure

Samples that need to be shipped to Gundersen should be packaged using the following procedures:

1. Obtain the following material
    a. T2Biosystems insulated shipping box provided by T2 Biosystems (Image 1)
    b. One (1) frozen gel pack provided by T2Biosystems (Image 2)
    c. Two (2) tri-fold cardboard inserts provided by T2Biosystems (Image 3)
    d. Two (2) strips of bubble wrap
    e. Two Biohazard Transport bags (Image 4)
2. Place the frozen gel pack at the bottom of the Styrofoam box
3. Take one of the tri-fold cardboard inserts and fold the flaps over so they are facing the same direction.
4. Place the divider over the frozen gel pack so that the flat side sits atop the gel pack and the flaps are directed towards the bottom of the Styrofoam box.
5. Place the blood samples into the biohazard transport bags and seal.
6. Wrap the blood samples in bubble wrap.
7. Using a piece of tape, tape the wrapped samples to the cardboard divider.
8. Fold the second tri-fold cardboard insert and place this atop of the blood samples so that the flaps are directed towards top of the Styrofoam box.
9. Place the tissue samples (if collected) into a biohazard transport bag and seal.
10. Wrap the tissue sample in bubble wrap.
11. Using a piece of tape secure the wrapped tissue sample to the flat side of the cardboard insert.
12. Close the Styrofoam container.
13. Seal the outer cardboard box closed using packing tape.
14. Ensure that the UN3373, Biological Substance Category B label is on package.
15. Affix shipping label with the following address:

    Gundersen Health System
    Microbiology Research Laboratory
    Health Science Center
    1300 Badger Street
    Room 5032
    La Crosse, WI 54601

Samples that need to be shipped to T2 Biosystems should be packaged using the following procedures:

1. Obtain the following material
    a. T2Biosystems insulated shipping box provided by T2 Biosystems (Image 1)
    b. A tri-fold cardboard insert provided by T2Biosystems (Image 3)
    c. Strip of bubble wrap
    d. Biohazard Transport bag (Image 4)
2. Place the blood samples into the biohazard transport bag and seal.
3. Wrap the blood samples in bubble wrap.
4. Using a piece of tape, tape the wrapped samples to the bottom of the container.

5. Fold a tri-fold cardboard insert and place this atop of the blood samples so that the flaps are directed towards bottom of the Styrofoam box.
6. Close the Styrofoam container.
7. Seal the outer cardboard box closed using packing tape.
8. Ensure that the UN3373, Biological Substance Category B label is on package.
9. Affix shipping label with the following address:

T2 Biosystems
Attention: Roger Smith
101 Hartwell Ave.
Lexington, MA 02421

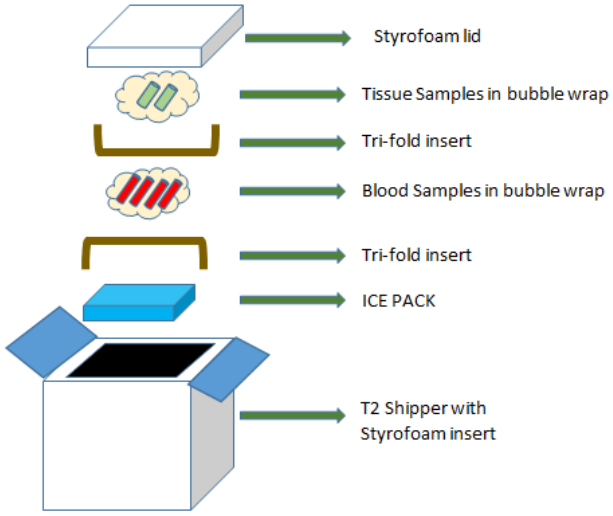


Figure 1: Diagram of sample packing

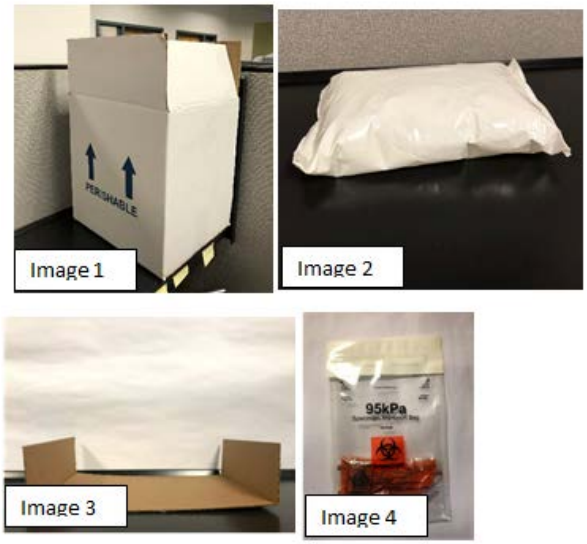


Figure 2: Images of shipping materials.