1. Introduction

1.A. Nephrotic Syndrome Study Network Overview

The Nephrotic Syndrome Rare Diseases Clinical Research Study Network (NEPTUNE) is a landmark research project aimed to establish concerted and innovative investigational strategies combining basic science, translational, and clinical methods to study both a biopsy-proven cohort of patients with FSGS, MCD, and MN (cohort A) as well as a cohort of children with incident nephrotic syndrome at the time of diagnosis (cohort B).

At the initiation of NEPTUNE II, 23 sites will collectively enroll two cohorts of participants: Cohort A – 150 FSGS/MCD/MN and Cohort B – 120 non-biopsy pediatric participants with incident nephrotic syndrome (NS). Collectively, these cohorts will continue to be followed for 36 months. Participants previously enrolled in NEPTUNE, under prior protocol versions, will continue to be followed for up to five years. Consented participants who subsequently do not qualify histologically will no longer be followed for the duration of the study.

1.B. Study Organization

Participants will be recruited from sites around the United States and Canada. A full list of sites participating in NEPTUNE can be found online:

http://rarediseasesnetwork.epi.usf.edu/NEPTUNE/centers/index.htm.

1.B.1. Data Analysis and Coordinating Center

The University of Michigan with Arbor Research Collaborative for Health (Arbor Research) will serve as the primary Data Analysis and Coordinating Center (DACC). As a cost-share funded study partially funded through the Office of Rare Diseases Research (ORDR), a Rare Disease Clinical Research Network exists through which limited study data will be deposited and backed up at regular intervals, with a final data transfer occurring at the end of the funding cycle.

1.B.1.a. *Data Management and Support*

NEPTUNE-Link will replace Velos as the system to be used for data capture. This data management platform has been expressly developed for NEPTUNE and is able to support large scale, international longitudinal cohort studies of renal disease.

Training materials are located in Appendix A. NEPTUNE-Link training and access will be regulated by the NEPTUNE Clinical Project Managers.

1.C. General Policy

1.C.1. General Protocol Policy

The objectives of the study are most likely to be achieved if the protocol does not require alteration. Any changes in the protocol will result in some degree of heterogeneity of the data, which complicates the analyses and may compromise the scientific integrity of the study. However, occasions may arise in which protocol changes are necessary. Therefore, changes in the protocol will be considered only if they are required to ensure participant safety or will significantly enhance the scientific validity of the study.

The Steering Committee must approve all protocol amendments or revisions. In turn, these amendments and/or revisions will require approval from the RDCRN as well as the NIDDK. Approved amendments must be submitted to the local site IRB's for approval and once approved, be incorporated into the protocol. IRB approval must occur prior to the implementation of an amendment. Amendments that include minor changes to the protocol may undergo expedited review if these changes fit into expedited approval criteria. All changes to the informed consent form must also be approved by the local site IRB.

1.C.2. Human Subjects Considerations

Regulatory Requirements for Informed Consent

Each Clinical Site is responsible for ensuring that informed consent is obtained from each participant using a current consent form according to the guidelines of its local Institutional Review Board (IRB) and in accordance with the Common Rule (45 CFR Part 46 subpart A, Protection of Human Subjects). The informed consent form must be obtained or on file (signed and dated by the participant) prior to initiation of any study related activity.

The Informed Consent form must provide the following information to each participant:

- A statement that the study involves research, an explanation of the purposes of the research and the expected duration of the participant's involvement, a description of the procedures to be followed, and identification of any procedures which are experimental.
- A description of any reasonably foreseeable risks or discomforts to the participant.
- A description of any benefits to the participant or to others which may reasonably be expected from the research.
- A disclosure of appropriate alternative procedures or courses of treatment, if any that might be advantageous to the participant.
- A statement describing the extent, if any, to which confidentiality of records identifying the participant will be maintained.
- For research involving more than minimal risk, an explanation as to whether any compensation and an explanation as to whether any medical treatments are available if injury occurs and, if so, what they consist of, or where further information may be obtained.
- An explanation of whom to contact for answers to pertinent questions about the research and research subjects' rights, and who to contact in the event of a research related injury to the participant.
- A statement that participation is voluntary, refusal to participate will involve no penalty or loss of benefits to which the participant is otherwise entitled and the participant may discontinue participation at any time without penalty or loss of benefits to which the participant is otherwise entitled.

Administration of Informed Consent

Each participant will be asked to sign one or more Informed Consent documents depending on the structure of the document at each Clinical Site to continue to be followed. These documents describe the Nephrotic Syndrome Study Network (NEPTUNE) as well as the genetic sample component of the study. These document(s) contain embedded YES/NO questions with signature requirements. Instruct the participant to read the Informed Consent(s) carefully and to raise any questions or concerns that he/she has and to sign ONLY after their questions or concerns have been answered fully. Participant questions should be answered using lay language.

Participants may decide to participate in the NEPTUNE Study but opt out of all, or parts, of the genetic data collection. Discuss the options and implications of participation in the genetics portion and explain the selections contained in the YES/NO questions about genetic samples. It is highly important to relay to potential participants that our study will be investigating genetics related to kidney disease.

As an added measure of protection, and as required by the Lead Site IRB, Genetic Information Nondiscrimination Act language has been added to the study template as follows:

> A new Federal law, called the Genetic Information Nondiscrimination Act (GINA), generally makes it illegal for health insurance companies, group health plans, and most

employers to discriminate against you based on your genetic information. This law generally will protect you in the following ways:

- Health insurance companies and group health plans may not request your genetic information that we get from this research.
- Health insurance companies and group health plans may not use your genetic information when making decisions regarding your eligibility or premiums.
- Employers with 15 or more employees may not use your genetic information that we get from this research when making a decision to hire, promote, or fire you or when settling the terms of your employment.

All health insurance companies and group health plans must follow this law by May 21, 2010. All employers with 15 more employees must follow this law as of November 21, 2009.

Be aware that this new Federal law does not protect you against genetic discrimination by companies that sell life insurance, disability insurance, or long-term care insurance.

Participants are also requested to consent to release their contact information to The NephCure Foundation. An embedded YES/NO question for the potential participants is a part of the informed consent template. Potential participants may continue in the NEPTUNE study without consenting to release their information to NephCure.

Consenting participants have the opportunity to be directly informed of studies for which they may potentially qualify based on their NEPTUNE study data when they consent to this study option.

Participants should be made aware of the possibility their diagnosis will not meet the eligibility criteria and that they may not be able to continue in the study. Participants may opt in/out of maintaining their collected samples as part of the NEPTUNE biorepository.

Provide a copy of the signed form to the participant before they leave the clinical center and instruct the participant that they should feel free to contact the clinical center should further questions occur to them after the visit.

It is possible that informed consents will be revised or amended throughout the course of the study. If so, a revised informed consent must be obtained from participants at follow-up prior to further collection of information or performing study procedures.

1.C.3. Participant Confidentiality

HIPAA

Participants are required to sign a Health Insurance Portability and Accountability Act (HIPAA) Authorization upon enrollment into the study. The HIPAA Authorization may or may not be incorporated into the Nephrotic Syndrome Study Network consent depending on the policy of the Clinical Center. This form describes both the kinds of health information collected in this study and also all of the disclosures of health information that will be made. The form must also list parties to whom disclosures of personal health information will be made.

Sites external to the Unites States must comply with equivalent national standards (Canadian sites are required to utilize the Personal Health Information Protection Act (PHIPA)) upon study enrollment.

Medical Record Release

This study may require the release of medical records from remote health care facilities. Each Clinical Center should obtain written authorization for the release of medical records from each study participant annually. The following procedures are recommended:

- During The Informed Consent/HIPAA Authorization process, each participant will be asked to sign and date three (3) copies of the Medical Release Form. It was communicated that these

will be issued as needed to obtain data for the research study. (The release of medical records should be listed in the HIPAA authorization form that they should have previously signed.)

- A copy of the signed release form can be obtained for the Clinical Site's records. Remote institutions may require the form with *original* signatures.
- Intermittently check with your institution to see if the Medical Release Form has been revised.
- Each study participant should sign three (3) currently dated Medical Release Forms during their yearly site visit(s) (Authorization to Request Patient Information, Authorization to Release Information, and each sites local Medical Release Form).

Additional Confidentiality Concerns

- Consent form(s), HIPAA authorizations and source documentation must be securely maintained in a participant research chart.
- Recruited study participants are assigned a Participant Study ID number, a unique study identification number. Creation of a unique Participant Study ID number is outlined in detail in Section 3.D.
- **All communication between the Data Analysis and Coordinating Center (DACC) Principal Investigators, personnel, and staff and the Clinical Centers Site-Investigators, personnel, and staff regarding participant data occurs via the Participant Study ID number only. Clinical Site personnel are responsible for maintaining a key code for all participants, and stored within an appropriate locked file system**.

1.C.3.a. *Local Versus Central Data Entry*

In response to research coordinator study burden concerns Central Data Entry is an option for all sites within NEPTUNE. Registration and initial study data must be entered locally to protect the participant's confidentiality.

All sites are responsible for registering their participants into NEPTUNELink and uploading the local pathology report.

2. General Policy

The NEPTUNE study and analysis will be performed under local ethics review board oversight (IRB (US)/Research Ethics Board (REB) (Canada), henceforth referred to as "IRB"). Each participating site, will obtain local IRB approval before initiation of study activities. IRB approval must be maintained throughout the conduct of the study and data analysis phase. Amendments to the study protocol must be submitted to the local IRB for approval prior to implementation at the study site. Sites should maintain current IRB approval until directed by the DACC to close the study. All IRB approval letters, initial and annual updates, must be forwarded to the DACC

2.A. Human Subjects Considerations

2.A.1. Regulatory Requirements for Informed Consent

Each Clinical Site is responsible for ensuring that informed consent is obtained from each participant using a current consent form according to the guidelines of its local Institutional Review Board (IRB) and in accordance with the Common Rule (45 CFR Part 46 subpart A, Protection of Human Subjects). The informed consent form must be obtained or on file (signed and dated by the participant) prior to initiation of any study related activity.

As a Rare Diseases Clinical Research Network protocol, the following elements will also be reviewed in informed consent documentation and must be represented in approved language at site audits:

- "The clinical information collected for this study will be stored at the Data Management and Coordinating Center at the University of South Florida in Tampa, FL and also sent to a Federal data repository. The data management center uses several layers of protection for the clinical data stored there. It meets all of the local and federal security requirements for research datacenters. Your information is stored only using a study ID."

- 21 CFR 50.25c FDA required ClinicalTrials.gov language

- A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by U.S. Law. This Web site will not include information that can identify you. At most, the Web site will include a summary of the results. You can search this Web site at any time.

Additionally, as an added measure of protection the Genetic Information Nondiscrimination Act language is included in the consent form.

A new Federal law, called the Genetic Information Nondiscrimination Act (GINA), generally makes it illegal for health insurance companies, group health plans, and most employers to discriminate against you based on your genetic information. This law generally will protect you in the following ways:

- Health insurance companies and group health plans may not request your genetic information that we get from this research.

- Health insurance companies and group health plans may not use your genetic information when making decisions regarding your eligibility or premiums.

- Employers with 15 or more employees may not use your genetic information that we get from this research when making a decision to hire, promote, or fire you or when settling the terms of your employment.

All health insurance companies and group health plans must follow this law by May 21, 2010. All employers with 15 more employees must follow this law as of November 21, 2009.

Be aware that this new Federal law does not protect you against genetic discrimination by companies that sell life insurance, disability insurance, or long-term care insurance.

2.A.2. Administration of Informed Consent

The informed consent form must be signed and dated by the patient or the legal guardian if applicable prior to initiation of any study-related activity. The guardian of a minor patient will be asked to sign the study-specific informed consent, and minor patients will sign the assent form in accordance with local IRB standard operating procedures. All participant questions should be fully answered before the informed consent/assent form is signed. Participants should be made aware that consent to participate in NEPTUNE indicates consent for biospecimen storage and de-identified data transfer to the NIH Repositories. A copy of the consent form should be provided to the participant.

Additionally, there are several optional components to the NEPTUNE study. Participants may participate in the main NEPTUNE study but opt in or out of the following parts:

- Collection of samples for genetic research
- Creation of cell lines for research
- Sharing contact information with NephCure Kidney International
- Contact for other studies
- Retaining specimens if the participant is a screen fail

During the consent process, the local study team will discuss the options and implications of participation in each of these study components and explain the selections contained in the optional consent sections.

If a participant decides to share their contact information with NephCure Kidney International, an email should be sent to PatientAdvocate@nephcure.org with the patient's contact information.

It is possible that informed consents will be revised or amended throughout the course of the study. If so, the participant may be asked to sign the revised informed consent in accordance with local IRB policy prior to further study participation.

Participants are also requested to consent to release their contact information to The NephCure Foundation. An embedded YES/NO question for the potential participants is a part of the informed consent template. Potential participants may continue in the NEPTUNE study without consenting to release their information to NephCure.

Consenting participants have the opportunity to be directly informed of studies for which they may potentially qualify based on their NEPTUNE study data when they consent to this study option.

Participants should be made aware of the possibility their diagnosis will not meet the eligibility criteria and that they may not be able to continue in the study. Participants may opt in/out of maintaining their collected samples as part of the NEPTUNE biorepository.

Provide a copy of the signed form to the participant before they leave the clinical center and instruct the participant that they should feel free to contact the clinical center should further questions occur to them after the visit.

It is possible that informed consents will be revised or amended throughout the course of the study. If so, a revised informed consent must be obtained from participants at follow-up prior to further collection of information or performing study procedures.

2.A.3. Participant Confidentiality

HIPAA

Participants are required to sign a Health Insurance Portability and Accountability Act (HIPAA) Authorization upon enrollment into the study. The HIPAA Authorization may or may not be incorporated into the Nephrotic Syndrome Study Network consent depending on the policy of the Clinical Center. This form describes both the kinds of health information collected in this study and also all of the disclosures of health information that will be made. The form must also list parties to whom disclosures of personal health information will be made.

Sites external to the Unites States must comply with equivalent national standards (Canadian sites are required to utilize the Personal Health Information Protection Act (PHIPA)) upon study enrollment.

Medical Record Release

This study may require the release of medical records from remote health care facilities. Each Clinical Center should obtain written authorization for the release of medical records from each study participant annually. The following procedures are recommended:

- During The Informed Consent/HIPAA Authorization process, each participant will be asked to sign and date three (3) copies of the Medical Release Form. It was communicated that these will be issued as needed to obtain data for the research study. (The release of medical records should be listed in the HIPAA authorization form that they should have previously signed.)
- A copy of the signed release form can be obtained for the Clinical Site's records. Remote institutions may require the form with *original* signatures.
- Intermittently check with your institution to see if the Medical Release Form has been revised.
- Each study participant should sign three (3) currently dated Medical Release Forms during their yearly site visit(s) (Authorization to Request Patient Information, Authorization to Release Information, and each sites local Medical Release Form).

Additional Confidentiality Concerns

- Consent form(s), HIPAA authorizations and source documentation must be securely maintained in a participant research chart.
- Recruited study participants are assigned a Participant Study ID number, a unique study identification number. Creation of a unique Participant Study ID number is outlined in detail in Section 3.D.
- **All communication between the Data Analysis and Coordinating Center (DACC) Principal Investigators, personnel, and staff and the Clinical Centers Site-Investigators, personnel, and staff regarding participant data occurs via the Participant Study ID number only. Clinical Site personnel are responsible for maintaining a key code for all participants, and stored within an appropriate locked file system**.

3. Participant Population

3.A. Recruitment Overview

Clinical centers are committed to recruiting a total of 450 active, study participants meeting the eligibility requirements for Cohort A, the Biopsy Cohort and a total of 120 participants meeting eligibility criteria for Cohort B, cNEPTUNE.

Overall recruitment may exceed this during ongoing enrollment in an effort to achieve an active population of participants.

3.B. Visit Schedule Information

Tables 1-4 below describe the visits used throughout this manual to describe interaction with NEPTUNE participants. All contacts and visits will have a permissible window (±30 days) of contact surrounding them that defines the period of time during which a visit is considered on time (i.e., visit window), with the exception of post-biopsy baseline visits. This window will remain open for 45 days post-biopsy and inability to complete the baseline study visit will terminate the participant for NEPTUNE II (enrolled under Protocol V4.0). Every effort should be made to conduct study visits at regular intervals.

Table 2A. NEPTUNE Visit Schedule for participants enrolled under Protocols 2.5, 3.0 and 3.5

Pre-Screen	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9	Visit 10	Visit 11	Visit 12	Visit 13
[V0]	[V1]	[V2]	[V3]	[V4]	[V5]	[V6]	[V7]	[V8]	[V9]	[V10]	[V11]	[V12]	[V13]
Chart Review or MD referral	Screen/ Eligibility	Baseline	Biopsy	4 months Follow-up	8 months Follow-up	12 months Follow-up	18 months Follow-up	24 months Follow-up	30 months Follow-up	36 months Follow-up	42 months Follow-up	48 months Follow-up	54-60 months Follow-up

Table 2B. NEPTUNE Visit Schedule for Cohort A (Biopsy Cohort) enrolled under Protocol V4.0

Year	Year 1*						Year 2*		Year 3*	
Screen/ Eligibility	Visit Biopsy	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9
[VSE]	[VBX]	[V1]	[V2]	[V3]	[V4]	[V5]	[V6]	[V7]	[V8]	[V9-]
Chart Review or MD referral	Biopsy	Baseline	4 months Follow-up	8 months Follow-up	12 months Follow-up	18 months Follow-up	24 months Follow-up	30 months Follow-up	36 months Follow-up	Annual Chart Extraction

Table 2C. Cohort B - cNEPTUNE

Year 1*					Year 2*		Year 3*	
Enrollment/ Baseline	Week 6	Month 4	Month 8 Phone Visit	Month 12	Month 18	Month 24	Month 30	Month 36

Table 2D. Cohort B – cNEPTUNE patients who receive a biopsy

	Time since Biopsy*			
Biopsy	4 Months	8 Months	12 months	Every 6 months

* One unscheduled relapse visit per year may occur

3.C. Cohort Selection

Two prospective cohort studies will be performed, namely: (1) The FSGS/MCD Cohort Study and (2) the MN Cohort Study. Each cohort study is a prospective incident cohort study (n=250 for the FSGS/MCD study and n=200 for the MN study) using a consecutive sampling frame. Each cohort study is population-based, that is, all subjects who meet the inclusion criteria at the participating centers will be enrolled if the subjects or their legal guardians provide comprehensive written informed consent. Each study will be performed simultaneously at the participating clinical centers listed in Section 1.B.1. A recruit-to-replace strategy will be employed throughout the enrollment phase until the end of the funding cycle and an active, targeted population reaches enrollment goal.

3.C.1. Inclusion Criteria

Cohort A:

Adult and pediatric participants

- A new diagnosis of FSGS or MCD or MN according to characteristic light, electron (EM), and immunofluorescence microscopy (IM), with presence of at least five glomeruli per biopsy available for analysis. Biopsy slides will be reviewed and diagnosis confirmed by 2 study pathologists according to standardized criteria developed by the pathology committee (see Appendices C and D);
- Documented urinary protein excretion ≥1500 mg/24 hours or spot protein: creatinine ratio equivalent at the time of diagnosis or within 3 months of the screening/eligibility visit. Alternatively, an albuminuria equivalency determined by the local site PI;
- < 80 years of age
- Completion of V1 (baseline visit) within 45 days of V_{BX} (biopsy visit)
- Informed Consent

Consented individuals determined to be ineligible post-biopsy procedure will be requested to consent for their tissue and samples to remain part of the NEPTUNE biorepository. These samples may serve as non-nephrotic control samples for future studies and consenting participants will be compensated up to $50 or as allowed by local IRBs. These participants will not be followed and will be categorized as screen failures.

Cohort B:

- Age < 19 years old
- < 30 days of treatment for Nephrotic Syndrome
- Proteinuria:
  - Urinalysis > 2+ and edema
  - Urinalysis > 2+ and serum albumin < 3
  - Urine protein:creatinine ratio > 2 and serum albumin < 3
  - Urine protein:creatinine ratio > 2 and edema
- Willingness to comply with study requirements
- Informed consent/assent

3.C.2. Exclusion Criteria – Cohort A

- Prior solid organ transplant
- A clinical diagnosis of glomerulopathy without diagnostic renal biopsy

- Clinical, serological or histological evidence of systemic lupus erythematosus (SLE) as defined by the ARA criteria. Patients with membranous in combination with SLE will be excluded because this entity is well defined within the International Society of Nephrology/Renal Pathology Society categories of lupus nephritis, and frequently overlaps with other classification categories of SLE nephritis (68)
- Clinical or histological evidence of other renal diseases (Alport, Nail Patella, Diabetic Nephropathy, monoclonal gammopathy (multiple myelomas), genito-urinary malformations with vesico-uretheral reflux or renal dysplasia)
- Known systemic disease diagnosis at time of enrollment with a life expectancy less than 6 months
- Unwillingness or inability to give a comprehensive informed consent
- Unwillingness to comply with study procedures and visit schedule
- Institutionalized individuals (e.g., prisoners)
- Laboratory information unavailable prior to consent and biopsy procedure subsequently supporting exclusion criteria will deem a participant ineligible

3.C.3. **Exclusion Criteria – Cohort B**

- End Stage Kidney Disease (ESKD) defined as the need for chronic dialysis or kidney transplant
- Prior solid organ or bone marrow transplant
- Secondary NS (systemic lupus erythematosus (SLE), vasculitis, Henoch Schonlein Purpura, Hepatitis B, C or HIV nephropathy)
- Clinical or histological evidence of other renal diseases (Alport syndrome, Nail Patella syndrome, Diabetic Nephropathy, monoclonal gammopathy (multiple myelomas), genito-urinary malformations with vesico-uretheral reflux or renal dysplasia)
- Known systemic disease diagnosis at time of enrollment with life expectancy less than 6 months
- Unwillingness or inability to give a comprehensive informed consent
- Unwillingness to comply with study procedures and visit schedule
- Institutionalized individuals (e.g., prisoners)

3.C.4. **Eligibility Verification**

Consented individuals determined to be ineligible post-procedure will be requested to consent for their tissue to remain part of the NEPTUNE biorepository. These samples may serve as non-nephrotic control samples for future studies.

Study ID Numbers

3.C.5. **Participant Study ID Number Assignment**

Once eligibility has been determined, and the individual has signed the Informed Consent Document, a unique Study ID can be assigned. The Study ID will be assigned by the institution recruiting the participant and will contain the following components:

- Year of Enrollment (10, 11, 12 to indicate 2010, 2011, 2012, respectively)
- Study Number (the Study Number is 26609 and represents the lead site (University of Michigan) IRB Approval number providing study identification to all samples and data)
- Clinical Site ID (designated below in Table 2)
- Participant Number (this is the chronological participant number beginning with 1 and incrementing by 1's with leading zeroes for adult participants; beginning with 501 and incrementing by 1's for pediatric participants. Please note this distinction is strictly for identifying adult versus pediatric specimen kits at sites recruiting for both patient populations).

Table 2. Clinical Site ID's

Participant ID			
Year	StudyID	Site ID	Participant #
10 11 12 13 14 15	26609	000 = U Michigan 001 = NYUMC 002 = Johns-Hopkins 007 = U Illinois at Chicago 009 = Toronto 096 = Credit Valley 097 = York Central 098 = Sunnybrook 099 = Scarborough 020 = Case Western Res Univ 038 = Rainbow Babies' 021 = Children's Hosp - LA 022 = Harbor - UCLA 023 = Long Island Jewish 024 = Mayo 025 = Montefiore 026 = Univ Miami 027 = Univ N Carolina 028 = U Penn 029 = U Wash 030 = NIDDK 031 = Columbia 032 = Temple 033 = Emory 034 = Wake Forest 035 = Children's Mercy 036 = Stanford 040 = UTSW 095 = SickKids	001 002 003 … … … … 011 012 … … … … … 501 502 …

Light text indicates a sub-site of a NEPTUNE Site

Example Participant Study ID:

08-26609-000-001 = **Adult** participant enrolled in **2008** for the **NEPTUNE Study** at the **University of Michigan**, **participant #1**.

08-26609-000-501 = **Pediatric** participant enrolled in **2008** for the **NEPTUNE Study** at the **University of Michigan**, **participant #1**.

4. Study Visits

4.A. Screening/Eligibility Overview [V_{SE}]

All potential participants will be identified and recorded in NEPTUNE-Link. As necessary, a partial waiver of informed consent should be obtained according to local IRB guidelines to screen these potential participants.

Prior to meeting with the participant, the RC will complete the first portion of the Add Patient Details worksheet. This is a local use only worksheet to aid research coordinators in data entry.

4.A.1. Add Patient Detail Worksheet

Prior to [V_{SE}] the RC will complete the first portion of the Add Patient Detail worksheet. The RC will bring this form with them to the Screening/Eligibility Visit [V_{SE}] and complete the remainder of the form with the potential participant if the individual is interested in learning more about the study.

Information collected from the potential participant's chart and with the potential participant at the screening visit includes the following:

- First and Last Name and Patient Identifier (local MRN)
- Primary Treating Nephrologist
- Date of birth, sex, ethnicity, race, height and weight
- Inclusion Criteria and qualifying laboratory values
  - Qualifying laboratory values must be documented in the medical chart
  - Qualifying values must be from within 90 days of the V_{SE}
- Exclusion Criteria
- Consents
- Diagnosis
  - To be completed after a local diagnosis is given
- Contact Details
- Census Tract Details (see Census Tract Appendix for instructions)

4.A.2. NEPTUNE-Link Census/Screening Log

A log will be kept of each potentially eligible subject for NEPTUNE utilizing the Census tab in NEPTUNE-link. Information contained in the census will include:

- Screening ID: Sequential numbering of individuals entered into the census/screening log
- NEPTUNE StudyID: See MOP Section 2.D.
- Action: If an element of enrollment is missing information a boomerang symbol will appear here, see the NEPTUNE-Link MOP for specific details
- Last Name: (Optional) encrypted information for local site use only
- First Name: (Optional) encrypted information for local site use only
- Participant Identifier: (Optional) encrypted information for local site use only
- Primary Treating Nephrologist: (Optional) encrypted information for local site use only
- Eligible: Algorithm generated based on Patient Detail entry into NEPTUNE-Link
- Consent: System decision based on answer to subject consent information in Patient Detail
- Local Diagnosis: Based on local pathology report, indicates ability for Cohort A participant to proceed to Baseline V1
- Biopsy Report: Link to upload or replace the de-identified local biopsy report for NEPTUNE pathology review

- Cohort: Algorithm generated based on Patient Detail entry into NEPTUNE-Link
- iTask: Link to the participant's subject-level task page
- SMS: for Cohort B (cNEPTUNE) only, if populated link to participant SMS texting preferences
- Study End: Date of study termination

This log will serve as a basis to ascertain the bias of potential subjects against those who are enrolled. No identifiable information will be visible without the NEPTUNE-link namekey.

Cohort A Screening/Consent Contact and Biopsy Visit [V_{SE}/V_{BX}]

4.B. Screening Visit Overview

This visit serves as an introductory visit for pre-screened individuals who appear to be potential candidates for the NEPTUNE Study. Ideally, this visit will occur at the time of a nephrology clinic visit or consultation, though it may also occur at the time of biopsy (Cohort A); or as an emergency room consult (cNEPTUNE). During this visit, eligibility will be reviewed with the potential candidate and the study team will assess the potential participant's level of interest.

All pre-screened individuals who appear to be potential candidates for the NEPTUNE Study should receive the NEPTUNE Registry brochure, as well as The NephCure Kidney International brochure (IRB approval required).

Suggested language to approach a potential participant for Cohort A is included in Appendix D. Informed consent should be obtained at this time in accordance with the protocol and MOP 1.C.2. Once informed consent has been obtained, a participant ID should be assigned (MOP 2.D.) and case report forms and biological samples should be collected according to Tables 3A and 3B.

4.B.1. Approaching Potential Participants

In Clinic or Outpatient Procedures Area (Conventional Approach):

Once approval has been obtained to contact the potential participant, he/she will be approached in the appropriate clinic or procedure area. A RC will inform the individual of the study and ask if they are willing to hear more about the study (see suggested screening language).

If the Potential Participant Agrees:

1. Review the informed consent with the individual/family and answer any questions that arise.
    a. After the informed consent has been reviewed and signed, complete the Add Patient Details worksheet, assign the participant a study ID number and enroll him/her into the NEPTUNE study.
    b. As time permits, begin the Pre-Enrollment Medication Log. Please note a medication log must be started prior to the biopsy.
    c. Schedule or complete the limited elements of the Baseline Visit [V1], time permitting.
2. If the individual is not interested or not eligible based on the completed Add Patient Detail Worksheet, thank them for considering the study and for their time.
    a. Provide the patient information about the NEPTUNE Registry and NephCure Kidney International and answer any questions they may have about the registry study.
    b. Provide a brief follow-up so they know they are valued as patients and have a conclusive response from the study.

3. PLEASE REMEMBER TO RECORD THIS INFORMATION ON THE CENSUS SCREEN OF NEPTUNE-LINK.

Informed consent may be obtained at this visit or during the Biopsy Visit [V_{BX}]; depending on the recruitment strategy at a site. If the individual is prepared to consent and meets all study criteria, [V_{SE}] and [V_{BX}] can be in succession on the same day.

4.C. Biopsy Visit [V_{BX}]

This visit is concurrent with the kidney biopsy procedure. It may also occur at the same time as V_{SE}.

IT IS ABSOLUTELY PERTINENT THE SITE RC ARRANGE STUDY SPECIFIC DETAILS WITH THE PHYSICIAN PERFORMING THE RENAL BIOPSY

4.C.1. Biopsy Biospecimens

Prior to the biopsy procedure (day of, or up to 7 days preceding), it is essential the RC obtain a small blood sample (according to Table A above) and a clean catch spot/random urine sample.

It is essential the urine sample be obtained *prior* to the biopsy, as any traces of hematuria would be non-interpretable due to the procedure. When necessary, the blood specimens can be obtained same-day, post-procedure. Sample procurement details should be recorded in the Biopsy Specimens worksheet and Biosample CRF in NEPTUNE-Link.

During the clinically indicated renal biopsy procedure, an additional renal biopsy core will be obtained for research purposes. This tissue must immediately be submersed in the NEPTUNE study cryovial with the green-cap, containing RNA-Later. The additional core will be stored with the NEPTUNE RC until it is confirmed that adequate tissue necessary for histologic diagnosis of renal disease has been received, processed, and the treating physician, in consultation with the renal pathologist, has determined there is no longer clinical need for this sample, at which time it will be released for research use in the NEPTUNE Study. The procurement date should be recorded in the Biopsy Procedure Details and Biopsy CRF**.**

Based on the local histological report thereafter, NEPTUNE participants will be assigned into an initial study cohort (FSGS, MCD or MN). This information will be entered into the Pathology Diagnosis, Section F of Add Patient Details in NEPTUNE-Link. Participants who have a diagnosis that is not consistent with the Pathology definitions (see protocol appendices) should be withdrawn from the study as they do not meet inclusion/exclusion criteria. Be sure to fill out the withdrawal form in NEPTUNE-Link, specifying the reason for withdrawal as screen failure.

4.C.2. Biopsy Occurs Prior to Potential Participant Screening/Consent:

At some institutions an additional biopsy core will be obtained in RNA-Later as part of routine clinical procedure. If this is the case, and approval has been obtained to approach the potential participant, the participant may be contacted about the study following the collection of the biopsy tissue.

Complete Screening/Consent (V_{SE}) with the participant at this time. Every effort should continue to be made to collect V_{BX} biospecimens within the +/- 7 day window.

4.C.3. Biopsy Biosample Processing/Shipment

The additional renal core should be shipped with the biopsy visit [V_{BX}] samples on dry ice to the NEPTUNE Biobank Core. No processing is necessary for the biopsy core after the tissue has been released for research. Blood and urine biospecimens should be recorded in the V_{BX} Biopsy Specimens document and processed according to the blood and spot/random urine protocols. (Please see Appendix L, Biospecimen processing for further details).

4.C.4. Biopsy CRF

The Biopsy Procedure Details worksheet, Section A should be completed at time of biopsy. Included in the data collection are: date of biopsy, procurement of tissue for the NEPTUNE study, and total number of passes for the clinically indicated renal biopsy procedure (including the research core).

Section B should be completed when preparing stained and unstained slides for shipment to the NIDDK Histopathogical Archive.

4.C.5. Current Medications Log (Biopsy CRF Section D)

All *current medications taken at time of biopsy* should be documented in the medication log information including dates of start/stop recall, dosage, reason for stopping and route of administration.

4.D. Re-Screening Potential Participants

If a Cohort A participant's biopsy has been postponed for greater than the 90 day window of eligibility, the participant should be re-screened when the biopsy is re-scheduled. If this occurs, utilize the information already captured within NEPTUNE-link. Additionally, local lab results nearest to the date of the biopsy procedure should be recorded.

4.E. Baseline Visit [V1] / cNEPTUNE Enrollment

Participants in Cohort B (cNEPTUNE) should have their baseline visit done at the time of Screening/Consent (V_{SE}). Cohort A – Biopsy participants must complete the baseline visit within 45 days of the biopsy visit, per eligibility. If this is not able to be achieved, please withdraw the participant and indicate Ineligible as the reason.

Cohort A participants should complete the baseline visit after their biopsy to ensure a local diagnosis of a target population (FSGS/MCD or MN). A 24 hour urine sample should be obtained along with fasting blood samples. Participants in Cohort B will have non-fasting bloods obtained along with a spot urine sample and a first morning urine sample (if available).

One week prior to the study visit the RC will mail the participant a visit reminder card that will prompt the patient to come fasted and bring their Medication Diary and Birth History Handout to the visit. Additionally, the RC will call the participant the day before their appointment and remind them to come fasted and bring their Medication Diary and Birth History Handout (See Appendices H & I).

Activities and information that will be acquired from the participants that are scheduled to occur at a complete visit include all items designated below with the fasting blood draw and 24 hour and spot urine samples.

4.E.1. Visit Details

Table 3A. Case Report Form Elements Frequency

Form	Enrollment/Baseline	Follow Up/ Relapse Visit
Eligibility	X	
Demographics	X	X
Health Insurance and Utilization	X	X
Family Information/History	X	X

Birth History	X	
Comorbidities	X	X
Nephrotic Syndrome Information	X	X
Physical Exam	X	X
Clinical Information Updates	X	X
Labs	X	X
Pre-enrollment Medications	X	
Current Medications	X	X
PROMIS assessment	X	X
Medication Adherence assessment	X	X

4.E.2. **Baseline Case Report Form (CRF – NEPTUNELink)**

At this visit the RC will obtain documentation for permission to obtain medical records with appropriate signature(s). Please have participants sign the local Authorization to Release Patient Information Form, the Authorization to Request Patient Information Form, as well as your institution's required medical release form.

This information will be updated at annual visits.

Baseline information will be obtained from the participant and recorded on the respective study visit worksheet. Additionally, the treating physician or RC will conduct a limited physical examination to be recorded on the outlined worksheets. (Please see Appendix E for Physical Measurement Instructions).

The NEPTUNELink Baseline CRF contains the following sections, identified as follows by letter in NEPTUNELink and the study worksheets:

4.E.2.a. ***Section A: Demographics***

- Participant's language and proficiency
- Race and Ethnicity
- Racial Background
- Employment and Education
- Tobacco, Drugs, and Alcohol use

4.E.2.b. ***Section B: Healthcare Insurance & Utilization***

- Accessing healthcare resources
- Type of healthcare insurance(s)

4.E.2.c. ***Section C: Family Information***

- Family history of kidney disease
- Siblings with one common parent
- Biological children
- Family history of diabetes
- Family history of hypertension

4.E.2.d. ***Section D: Birth History***

- Birth weight, gestational age, multiple birth status
- Kidney complications at birth
- Parental age, race and ethnicity

- Maternal and paternal grandparent race and ethnicity

4.E.2.e. *Section E: Comorbidities*

Other medical history information is collected in this section. Please record the approximate month and year for each diagnosis. If unknown please collect year and if prior to 2005 please indicate.

- Thromboembolic events
- Diabetes
- Infection: HIV, Hepatitis, EBV, CMV, other viral infection
- Cancer
- Cardiovascular disease
- Peripheral vascular disease
- Gastrointestinal and liver disease
- Lung diseases
- Neurologic or psychiatric disease
- Solitary Kidney
- Sickle cell (disease or trait)
- Rheumatologic disease
- Congenital diseases
- Allergies

4.E.2.f. *Section F: Nephrotic Syndrome Presentation*

This section aims to collect information regarding the initial presentation of the disease, when participant was initially seen by a physician for possible kidney disease.

- Date of onset
- Height/weight prior to onset
- Date diagnosis
- Symptoms
- Disease classification (see Section 4.G.3)
- School/work absences due to kidney disease
- Diet
- Remission(s) between disease onset and study enrollment
- Remission(s)/relapse(s) in past 12 months

4.E.2.g. *Section G: Physical Exam, Vital Signs*

- Symptoms within the past 7 days
- Clinical Exam:
  - Weight, height
  - BP: Seated and standing
  - Heart rate
  - Edema status: present vs absent

    Periorbital

    Lower extremity

    Sacral

    Anasarca

    Genital

    Ascites
  - Pubertal status and age of first menses
  - Menopausal status and age of last menses

4.E.2.h. *Section H: Labs*

Results from local laboratory testing done for standard clinical care will be captured at all NEPTUNE visits. At the baseline visit, historical values will be collected from each of the following time points:

- Presentation: lab values from participant's initial disease presentation
- Worst case: Worst disease activity between presentation and biopsy (both highest serum creatinine and highest urine protein level, which can be independent results or from the same date)
- Most Recent: All labs performed between the time of biopsy and enrollment, including most recent lab values

A value of "0" should only indicate a numerical value of "0". If there are no results in the medical record, please leave the field blank. Labs to be collected include:

- Urine: Urinalysis, Spot urine, Timed Urine
- CBC, AIC, Metabolic Panel, Liver Function Panel, Cystatin C
- Mineral metabolism, Lipid Panel, Rheumatologic Serologies, Parvovirus

4.E.2.i. *Section I: Pre-Enrollment Medications*

Pre-enrollment medication log information should include all prescription immunosuppressive and RAAS blockade medications received prior to enrollment including dates of start/stop recall, dosage, reason for stopping and route of administration.

4.E.2.j. *Section J: Current Medications*

Current medication log information will pull data from the biopsy current medications log and should be updated at every visit to include all medications, vitamins and herbal supplements including dates of start/stop recall, dosage, reason for stopping and route of administration.

- Corticosteroids
- Non-infusion immunosuppression medications
- Infusion immunosuppression medications
- Vaccinations
- Other medications

4.E.2.k. *Section K: Clinical Information Updates*

Please record any episodes of acute dialysis since the onset of nephrotic syndrome. Acute dialysis is defined as any dialysis treatments that were temporary (the participant was able to recove sufficient renal function to stop dialysis), or any ongoing dialysis sessions where the participant is expected to have a reasonable chance of recovery.

- Dialysis history

4.E.2.l. *Section L: Questionnaire Completion*

Checking yes in this section indicates that the current CRF has been completed with all available information. It will be removed from the Task list in NEPTUNELink but remain available from the iTask link on the Census tab.

4.E.3. PROMIS and Medication Adherence Questionnaires

The PROMIS questionnaire and medication adherence should be administered at each in-person study visit. The Parent-Proxy form is filled out by parents of children 0-10 years old; the Child form is filled out by children 8-17 years old and the Adult form is filled out by adults 18 years and older.

The "reasons for medication non-adherence" form should be filled out on an annual basis in addition to the PROMIS and medication adherence forms.

4.E.3.a. ***PROMIS – age specific***

- Participants > 18 years
- All Peds: 8 – 17 years
- Parent proxy: children ≤ 10 years

4.E.3.b. ***Medication Adherence***

- Participants > 18 years
- All Peds: 8 – 17 years
- Parent proxy: children ≤ 10 years

4.E.4. Biobank Specimens and CRF

Study Visits will include the following biospecimens procurement, to be recorded on the corresponding study visit Biobank Specimens Worksheet and entered into NEPTUNELink:

- Fasting blood draw by weight according to Table 3A. Blood volumes will be reduced in pediatric participants and in all participants, adult and pediatric, with a hematocrit below 28%.
- 24-hour urine for central measures of Protein, Albumin, and Creatinine. A timed urine collection will be substituted for 24-hour specimen when subject is unable to collect 24 hour specimen.
- A "clean catch" research urine sample will be collected from all participants. Approximately 60 cc of the "clean catch" urine will be processed and transferred to the NEPTUNE Biorepository.

Blood

There will be a 100 cc fasting blood draw for adults at the Baseline Visit (See Appendix J for the blood collection procedures and the Biobank Baseline Blood MOP for blood processing/shipping procedures) and 65 cc fasting blood draw for follow-up visits. Pediatric blood draws will be according to weight as follows in Table 3B:

Table 3B. Blood volume draw by age/weight

Age	<18 years			≥18 years
Weight	< 20 pounds	21-51 pounds	> 52 pounds	> 52 pounds
Biopsy [V_{BX}]	10 cc	20 cc	40 cc	40 mL
Baseline	20 cc	50 cc	100 cc	100 mL
Follow-up	20 cc	50 cc	65 cc	65 mL
Relapse Visit (V_R)	10 cc	20 cc	40 cc	40 mL
Adult participants with a hematocrit below 28% and above 25% will have a 50cc fasting blood draw. Adult participants with a clinically measured hematocrit below 25% will not be drawn at a visit ± 30 days from documented hematocrit value.				
Pediatric participants > 52 pounds, with a hematocrit below 28% and above 25%, will have a 20cc blood draw. Pediatric participants < 52 pounds with a hematocrit below 28% and above 25%, as well as all pediatric participants with a hematocrit below 15%, will not be drawn				

Clean Catch Urine

A "clean catch" research urine sample will be collected from all participants. Approximately 60 cc of the "clean catch" urine will be processed and transferred to the NEPTUNE Biorepository (See Appendix K for urine collection procedures and the Biobank-Urine Spot MOP for processing/shipping procedures).

24 Hour Urine/Timed Urine

A 24 hour urine sample will be collected from adult and continent child participants for Protein, Albumin, Creatinine, Urea Nitrogen and Sodium. In children below five years of age, a timed urine will be used and for pediatric participants unable to produce a timed urine, a urine capture will be attempted (See Appendix K for urine collection procedures and the Biobank-Urine 24 hour MOP for processing/shipping procedures).

4.F. Follow-Up and Relapse Visits

Follow-Up Visits for Cohort A (Biopsy Cohort) in year one will occur in person at four-month intervals (months 4 and 8) based on the Baseline Visit according to Tables 2A-D. A Relapse visit may occur once per year if the participant is scheduled to see their nephrologist because of a relapse and this visit does NOT fall within a scheduled study window.

An additional visit, by phone, will occur in year 1 for Cohort B (cNEPTUNE) as shown in Table 2C.

Thereafter, Follow-Up Visits will occur at six-month intervals for the remainder of the study. Participants may be contacted by phone as necessary between visits to update contact information, ascertain interim medical history and potential outcomes of events, and assess health resource utilization. Follow-Up Visits are expected to occur within a range of three months before and up to three month after the visit timeline according to the visit calendar as they are meant to be contiguous.

If a scheduled visit or procedure is unable to be performed within the range of the study visit prior to the following visit's window, it will be documented as "missed". Study data that is able to be obtained (e.g., local labs, updates from the electronic medical record, etc), should be documented in the missed visit's case report forms.

The follow-up/relapse visits will include updates to the participant's medical history, concomitant medications, pregnancy history, healthcare utilization assessment, quality of life questionnaire(s), and a limited physical examination to include blood pressure, edema, weight and height. Quality of life should also be measured using the PROMIS instrument, and once per year, the medication adherence questionnaire should be administered.

4.F.1. Local Lab Tests

The following local lab results, from within the study visit window, should be obtained from the participant's medical chart and recorded on the Follow-Up Labs Worksheet and transferred to NEPTUNE-Link at each Follow-Up Visit.

- Urine: Urinalysis, Spot urine, Timed Urine
- CBC, AIC, Metabolic Panel, Liver Function Panel, Cystatin C
- Mineral metabolism, Lipid Panel, Rheumatologic Serologies, Parvovirus

4.F.2. Follow-Up Biospecimens

The Follow-Up and Relapse Visits will include the biospecimen procurement as outlined in table 3B above, to be recorded on the Follow-Up Biobank Specimens Worksheet and transferred to NEPTUNELink.

Blood

Blood draws at follow up visits will be done according to Table 3A.

See Appendix J for the blood collection procedures and the Biobank Follow-Up Blood MOP for blood processing/shipping procedures.

Clean Catch Urine

A "clean catch" research urine sample will be collected from all participants at all Follow-Up Visits. Approximately 60 cc of the "clean catch" urine will be processed and transferred to the NEPTUNE Biorepository (See Appendix M for urine processing procedures).

24 Hour Urine/Timed Urine

A 24 hour urine sample will be collected from adult and continent child participants when possible. This sample will be assessed centrally for Protein, Albumin, Creatinine, Urea Nitrogen and Sodium. In children below five years of age, a timed urine will be used and for pediatric participants unable to produce a timed urine, a urine capture will be attempted (See Appendix K for urine collection procedures and the Biobank-Urine 24 MOP for processing/shipping procedures).

4.G. Cohort B Specific Procedures:

4.G.1. Text Messaging (SMS):

Cohort B (cNEPTUNE) participants will take part in the SMS text messaging system for the initial 12 months of NEPTUNE participation. They will be enrolled at the time of consent. Information gathered by the research coordinator at the time of enrollment will include:

- Phone number
- Text message recipient (patient or parent/guardian)
- Text language
- Preferred day of week for weekly texts
- Time zone for participant
- Local start and end time for texts
- Vacation start and stop (for intermittent use)
- Local healthcare team contact email for alert notifications
- Local study contact email for alert notifications

This information must be entered into NEPTUNE-Link the **day of consent** and will be automatically transferred to the SMS system. Participant responses to text messages will be returned to NEPTUNE-Link for the local study team to review.

Participants will receive text messages asking about home urine protein monitoring results, relapse triggers (infections, allergies, stressors), edema status and location, consumption of prescribed immunosuppressive medications and nephrotic syndrome related absences from school/work.

Parent or child (≥ 12yrs) can respond to text messages at the discretion of the parent/child dyad (with approval from coordinator/local PI). The SMS alert system may be paused for planned vacations or during hospitalizations by entering start and end dates into the NEPTUNE-Link SMS administration link found on the Census page.

Alerts will be sent to the email addresses designated in NEPTUNE-Link for items such as dipstick ≤ 2+ for 3 days after being trace/negative, abdominal or genital edema, anasarca, if the patient reports not taking immunosuppressive medications, or if there is no response for one week without prior notification to the study team to "pause" the system.

Participants will receive a stipend of $15/month to cover the cost of an unlimited text message plan if they use their own cell phones. Participants without a cell phone may receive a text enabled cell phone from NEPTUNE for the first year in lieu of the stipend.

Table 3C. SMS Message Frequencies and Alerts

Item	SMS Frequency	Alert*
Home Urine Protein Monitoring	Daily x3 mo then Weekly	≥ 2+ x3 days after neg/trace
Triggers: infections, allergies, stressors	Weekly	
Edema Status	Daily x2 wk then Weekly	New onset abdominal or genital swelling, or anasarca
Consumption of immunosuppressive medications	Weekly	If not taking medications
NS Related Absences from School/Work	Weekly	
*Alert will also be generated if there is no response x3 days (for daily messages) or 1 week (weekly messages)		

4.G.2. Home Urine Protein Monitoring

Home urine protein testing using Chemstix™ will be performed daily for the first 90 days, weekly for the next 9 months (total 1 year). Results of this testing will be reported with SMS text messaging system and confirmed at study visits. Study participants will be supplied Chemstix™ as part of the cNEPTUNE cohort study. These will come in each cNEPTUNE baseline blood draw kit from the biobank.

4.G.3. Disease Classification

Disease classification refers to response patterns by patients to steroid treatment. As such, please use the following categorizations to respond to the relevant data point at study visits:

Steroid Response Pattern:

- Unknown/Untreated:
  Participants have never been treated with steroids and/or have an unknown response pattern
- Infrequently relapsing (IRNS):
  Maximum of 1 relapse within 6 months of initial remission OR
  <3 Relapses within any 12-month period

- Frequently relapsing/steroid dependent (FRNS/SDNS):
  >2 Relapses within 6 months of initial remission OR
  >4 Relapses within any 12-month period, OR
  Two consecutive relapses during steroid therapy OR
  Relapse within 14 days of cessation of steroids

- Steroid resistant:
  Failure to achieve remission after 8 weeks of corticosteroid therapy

- Multi Drug Dependent:
  Requires >1 immunosuppressive therapy to maintain remission or minimize the frequency of relapses

- Multi Drug Resistant:
  Failure to achieve remission on >1 immunosuppressive therapy (including corticosteroids)

- Not Applicable:
  Steroids are not indicated (based on physician's determination)

5. Withdrawal and Transfer

As a longitudinal, observational cohort study, the goal of NEPTUNE is to follow participants over the course of the study, recording clinically indicated laboratory data, obtaining study biospecimens, and accumulating phenotypic data through direct input from the participants.

5.A. Participant Withdrawal

If a participant officially withdraws their consent from the study, record this in NEPTUNE-Link by changing “Study Status” for that participant.

Participant withdrawal should also be noted in the participant’s research chart in a Note to File. When possible, retain permission to use already collected data and samples.

5.B. Participant Transfer

5.B.1. Participants Moving to a New NEPTUNE Site

Participants who are moving away, permanently or temporarily (e.g., for college), can continue to be followed at another NEPTUNE site. See the NEPTUNE website for a complete list of participating sites.

With patient approval, the research coordinator should contact the new NEPTUNE study site. The study team at the new study site should consent the patient, and once completed, the original study site will copy and share study documents. The new study site should schedule the first visit with the patient and once the first appointment is completed, should communicate with the original site that the transfer has been completed. A NEPTUNE participant transfer CRF should be completed.

The study ID will remain the same. Both sites will work together to ensure all necessary data is shared and the new site will then be responsible for participant retention.

5.B.2. Participants Moving to a New Non-NEPTUNE Site

Participants who are moving away, permanently or temporarily (e.g., for college), can continue to be followed by the NEPTUNE study through remote interactions and transfer of medical records on a regular basis to the original NEPTUNE site. Biosample collection should be collected if a subsequent in-person visit occurs at any NEPTUNE study site.

15. Appendix B. Pathology Manual of Procedures

Scientific information compiled by Dr. L. Barisoni

Research Coordinator questions can be addressed by the NEPTUNE Program Manager at 1-877-9-NEPTUNE (734-615-5021)

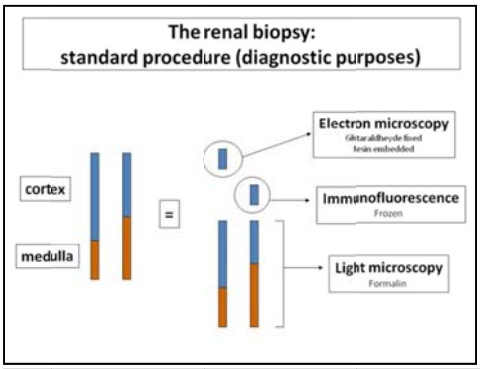
Renal biopsy procedure

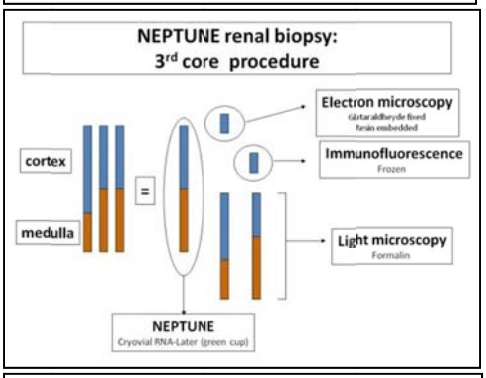
Goals:

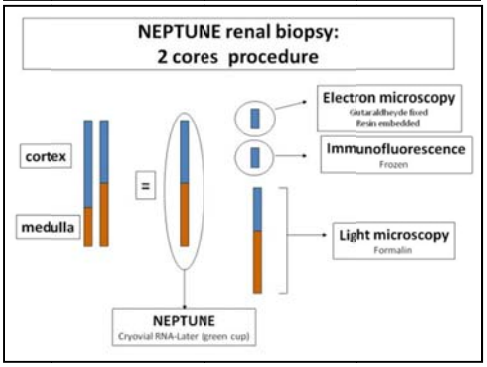
1. Obtain diagnostic material, including tissue for Light Microscopy (LM), Immunofluorescence (IF) & Electron Microscopy (EM).

2. Preserve integrity of diagnostic material to fulfill adequacy criteria for NEPTUNE pathology evaluation (LM, IF & EM).

3. Obtain research core without compromising diagnosis. Research core should immediately be placed in RNA later. Possibly within 30 seconds.







NOTICE OF RESEARCH PARTICIPATION
KIDNEY SPECIMENS

LOCAL FORM ONLY

INCLUDE WITH CLINICAL SPECIMENS FOR PATHOLOGY

Patient Name:_____ **Date:**_____
(Last) (First) (MI) (MM/DD/YYYY)

Patient ID:_____ **NEPTUNE Study ID:** ____ **- 26609 -** _____ **-** _____
(Indicate YOUR Medical Center's Medical Registration Identifier)

The above-named patient has consented to participation in NEPTUNE (**NEP**hrotic Syndrome S**tu**dy **Ne**twork). Participation in this study requires an additional renal core to be obtained during the clinically-indicated biopsy procedure. The additional core will remain with the research coordinator named below, at -80 C.

This additional core will not be released for research use prior to the renal pathologist's preparation, review, and histopathological diagnosis and if necessary, the core will be returned for diagnostic use (sample immersed in RNA-Later, valid for light microscopy only) by contacting the research coordinator named below. Please contact your site research coordinator (identified below) when you have completed your diagnosis and slides are available for pick-up.

Research Coordinator: *please print legibly*

First Name:		
Last Name:		
Work / cell phone:	Work:	Cell:
Pager: (if applicable)		
Email:		

Sample available for research: ☐ **Yes** ☐ **No**

Pathology release signature:	
Pathologist name printed:	
Treating Physician:	
Name printed:	

15.A. *Requested Pathology Materials*

In addition, NEPTUNE is requesting the following processed and unprocessed slides for research indications only:

	Glass Slides	EM Material	IF Material
To be returned within 2 weeks	• All LM glass slides available processed for LM • Thick sections of tissue processed for EM • H&E from frozen sections of tissue processed for IF		
	• Minimum of 4 unstained slides	CD with copy of digital EM images (preferred) *or* EM prints	CD with IF images if available and with pathology report

Fee to be paid to local/center pathology department for each case = $25

Compliance with HIPAA regulations:

Goal: Replace personal identifiers from all pathology material and use Neptune Study ID.

Strategy: All glass slides require NEPTUNE pre-printed labels to cover original label on the glass slide (please see instructions pps. 5-7).

All patient identifiers need to be deleted from each EM image or print before scanning or shipping and NEPTUNE Study ID placed on each print or image. All patient identifiers must be deleted from digital images before shipment (please see instructions pps 8-12).

Patient identifiers should be deleted from scanned pathology report and replaced with NEPTUNE Study ID (study ID is located on Form 11A-1 at (A) page 2).

Caution: cropping portions of digital images may be reversible, please ensure that the information is truly deleted.

In addition, NEPTUNE is requesting the following processed and unprocessed slides:

- Glass slides
  - All LM glass slides available processed for LM
  - Thick sections of tissue processed for EM
  - H&E from frozen sections of tissue processed for IF
  - Minimum of 4 unstained slides
- EM material
  - CD with copy of digital EM images (preferred) or CD with scanned EM images
  - or EM prints
- IF material
  - CD with IF images if available and with pathology report (can be same CD containing EM)
  - or copy of de-identified pathology report

Fee to be paid to local/center pathology department for each case = $25

Compliance with HIPAA regulations:

Goal: Replace personal identifiers from all pathology material and use Neptune Study ID.

Strategy: All glass slides require label to cover original label on the glass slide. New label from participant kit includes the following:

- Neptune Study ID
- Blank level indicator (to be completed on site)

All patient identifiers need to be deleted from all EM prints before scanning or shipping and NEPTUNE Study ID placed on each print or image. All patient identifiers must be deleted from digital images before shipment (please see (A) page 2).

Patient identifiers should be deleted from scanned pathology report and replaced with NEPTUNE Study ID (please see (A) page 2).

Caution: cropping portions of digital images may be reversible, please ensure that the information is truly deleted.

Note: Any biopsy with one or more slides, prints, reports, and images with personal identifiers will be returned to the originating site (including portions that have been de-identified) for corrections.

Shipment of pathology material to NIH – please send all materials in one shipment (items 1-3, Form [11B]). Please include the self-addressed Fed-ex label for the return shipment from the participant kit.

Mailing address for pathology materials:

Ms. Lisa Swearinger
Kidney Disease Section
10 Center Dr, Room 3N116 NIH
Bethesda, MD 20892-1268

Tel: 301-496-3092 lisas@intra.niddk.nih.gov

NIH protocol: scanning and storage of image files

When kidney biopsy material arrives at NIH, Ms. Swearinger will receive and record what is received in a dedicated logbook. **Any discrepancies between the package contents and the manifest will be reported by Lisa Swearinger to the site research coordinator within 24 hours.**

- LM: Kidney biopsy glass slides will be scanned on Aperio image system at 40X. Order will be according to level. Slide label should include Neptune Study ID and level. Files will be uploaded into the database.
- EM: Digital files will be uploaded directly. Electron microscopy prints will be scanned on flat bed scanner. Prints or digital files should include Neptune Study ID.
- IF: Images will be uploaded directly. Files should include antibody used and Neptune Study ID.
- Biopsy report including immunofluorescence description will be uploaded (scanned if necessary). Neptune Study ID should be included.
- NCI Slide Path database: Folders for each study site, and folders within for each Study ID.
- NCI server: uses RAID 5 back-up (redundant array of inexpensive disks) – 4 copies – as well as tape backup.
- **Kidney materials will be returned to the originating institution via UPS within 2 weeks of arrival**; if for some reason this cannot be achieved, an email will be sent to the originating PI with an explanation and anticipated send date.

- Images can be accessioned by the NIH approved staff and NEPTUNE Pathology Committee members. External access will be available via LDAP (Lightweight Directory Access Protocol - same pathway as eRA commons) – including password support. Each approved pathologist user will have a personal password.

RESEARCH COORDINATORS

SLIDE HANDLING & PREPARATION

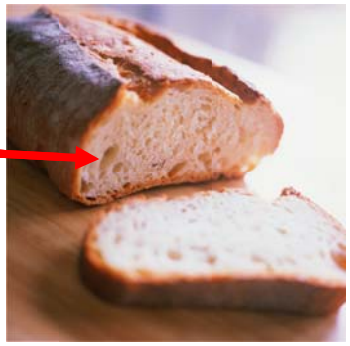
The NEPTUNE Site Research Coordinator should make arrangements with the local pathology team to be alerted when the NEPTUNE Study Participant's slide reading is complete. Local details may supercede the following description, please work with the NEPTUNE Clinical Program Manager and your site as necessary.

NOTE: Glass slides are fragile! Please take precaution when transporting and preparing for shipment.

The slides will likely be received on a tray. For the purposes of "re-labeling" there will be various slides; however, the information most necessary to transfer when each slide is de-identified is the NEPTUNE participant study ID, and the slide LEVEL (sequential series). Research Coordinators will need to be able to identify stained versus non-stained slides (BLANKS), as well as decipher the local pathology site's level-based ordering rubric.

If considering the slides as a loaf of bread,  each slice represents a slide "level". In an effort for the NEPTUNE Pathology Committee to re-construct 3-D images from the scanned slides, it is necessary to label each "slice" of bread sequentially to line up physiological cell markers and disease anomalies.

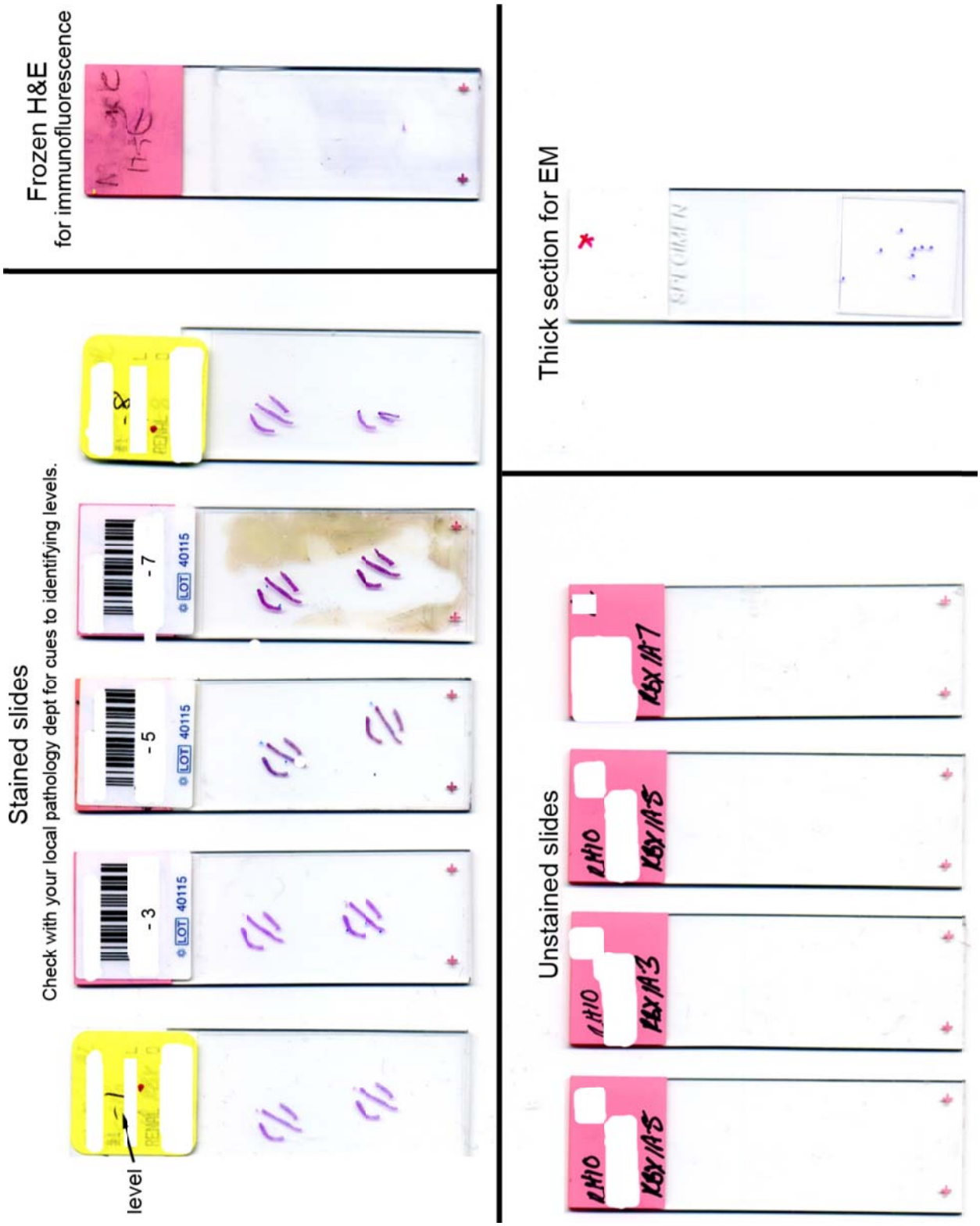
Analogy: an air bubble in the bread represents a glomerulus



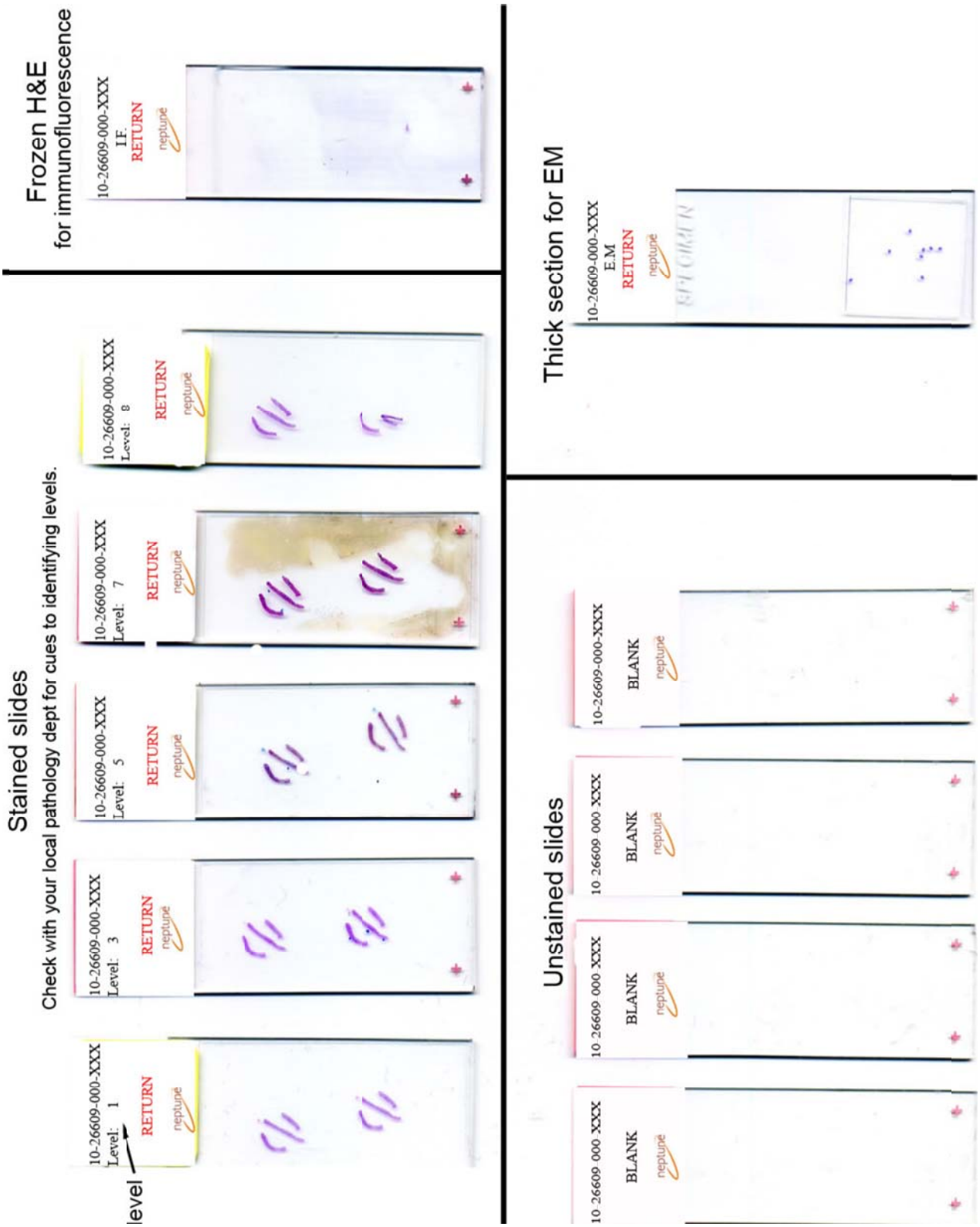
Place the study ID label *over* your local site's patient identifier taking note of the slide "level". If possible, it is recommended Research Coordinators meet initially with the site renal pathologist or a pathology technician to discuss identifying this essential detail. Please refer to slide set on the following page for reference.

In the stained slides area note the number details "1" - "8" indicating corresponding levels, respectively. When applying the NEPTUNE study participant label, be sure to write (with permanent pen, prior to transferring labels) the numbers identified for the NEPTUNE Participant's slides, in the respective blank Level: ____ area.

"Identified" slides to be re-labeled for NEPTUNE use.



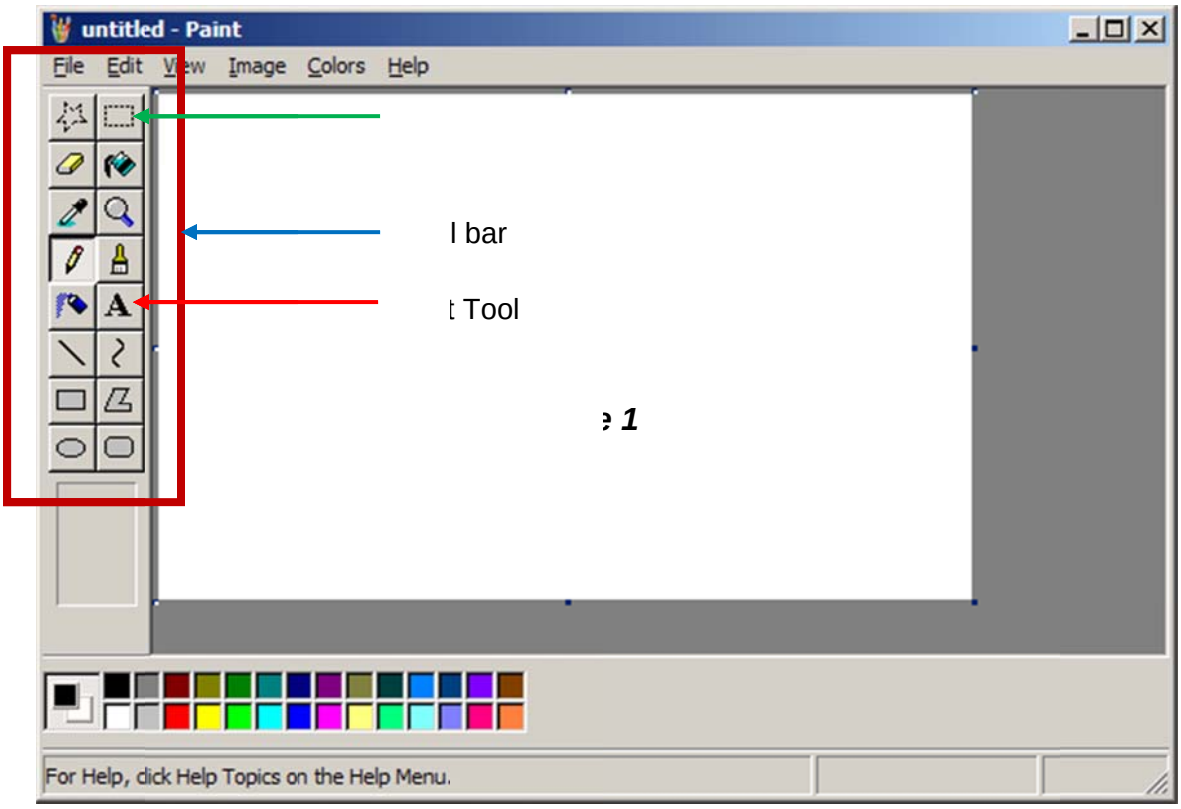
"De-identified" slides prepared for shipment to NIDDK Histopathological Archive for scanning and NEPTUNE study use.



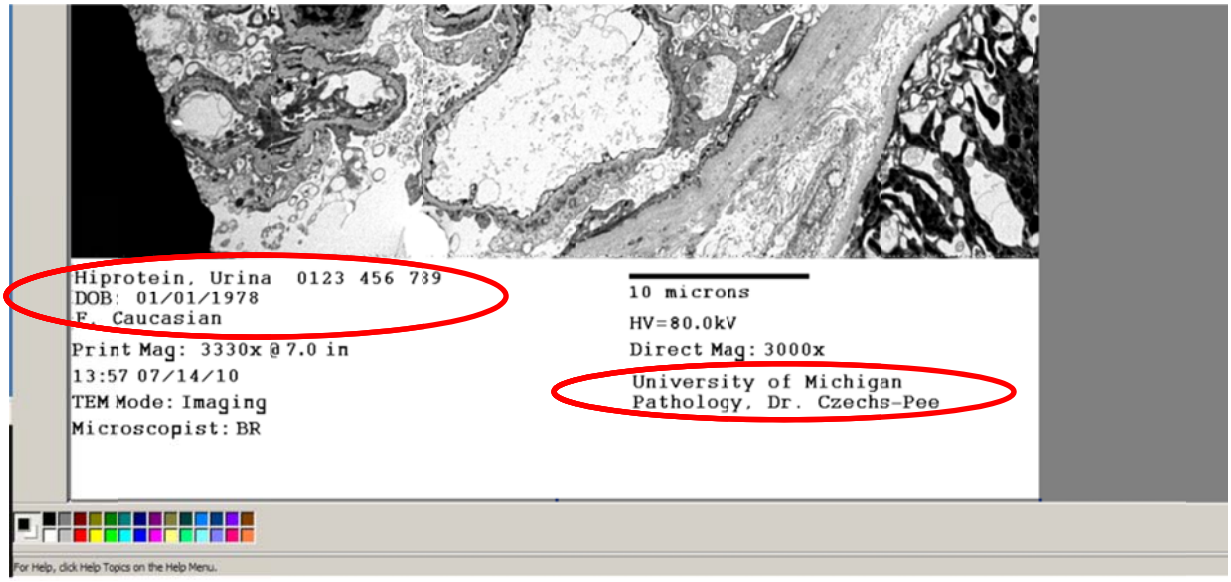
ELECTRON MICROSCOPY RE-LABELING INSTRUCTIONS

1. Digital Images

a. Open “PAINT” or a similar free image-adjusting software.



b. Open the EM document and review the information, noting any details which are “Identifying”:



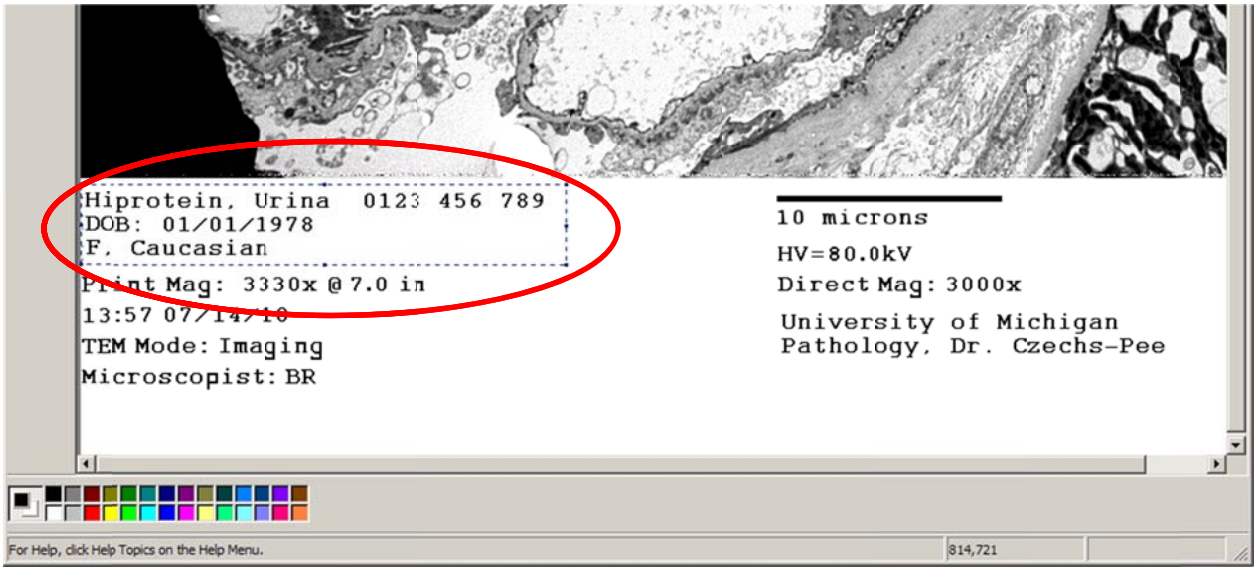
c. Review the image:

In the above image, Ms. Urina Hiprotein and her medical registration number 0123 456 789 must be removed. Other information which must be removed includes:

- Date of birth
- Gender identification (F)
- Racial identification (Caucasian)
- Site Identification by name (University of Michigan)
- Pathologist's name (Dr. Czechs-Pee)

d. Selecting/Deleting identifiable information: From the tool bar on the left of image 1 (this may vary at your site, depending on default settings), click on the "Select Tool". When over the image, the arrow becomes a cross-hair.

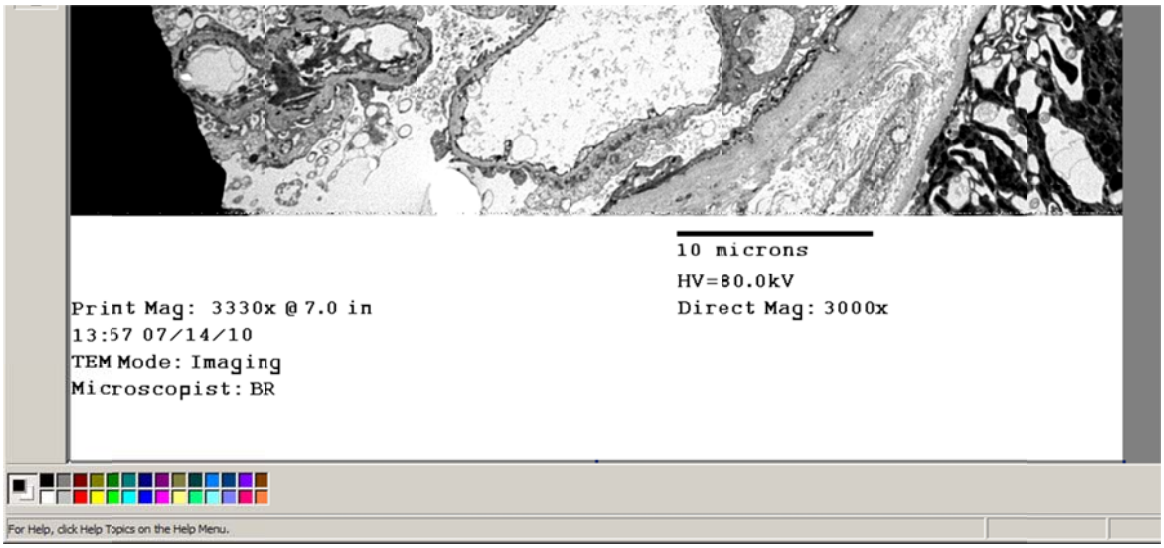
Move your mouse over the beginning of the text to be removed and click. Hold the click key on the mouse while dragging to select all text you would like to delete (see dashed box below).



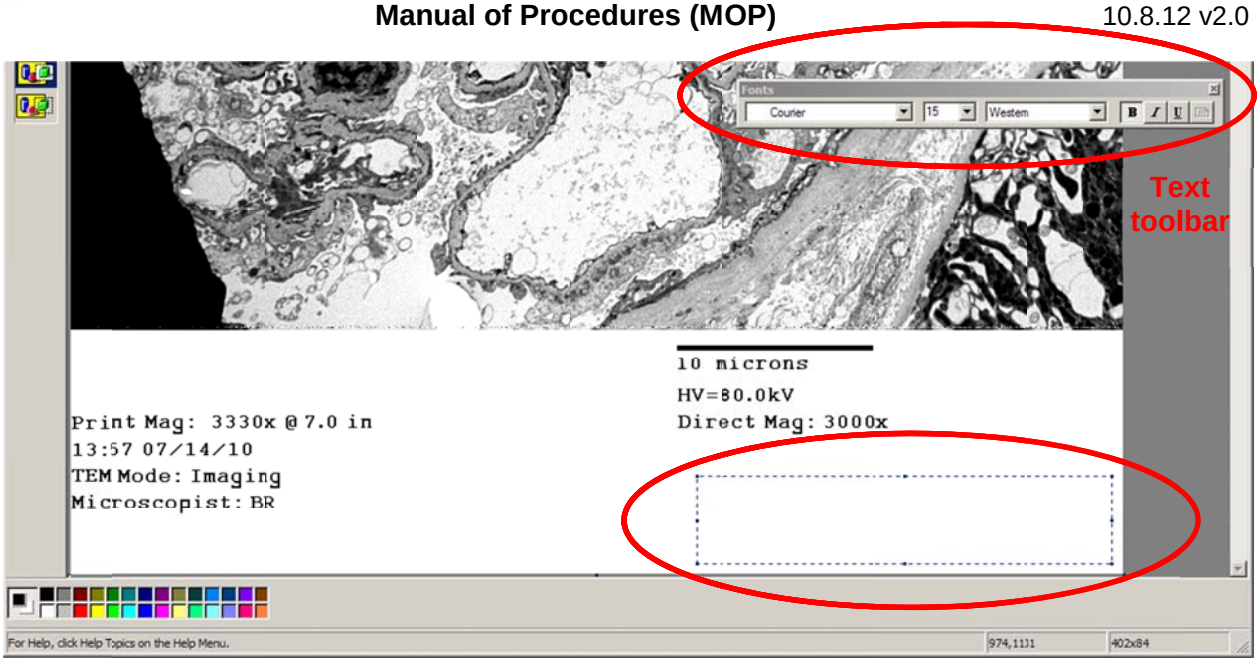
Release the mouse and the selected area will remain. The selection can be deleted by:

- Right clicking on the mouse and selecting “Cut”; or
- From the top menu bar, selecting “Edit” and choosing “Cut”; or
- Pressing “Ctrl-X” simultaneously

Repeat step d. above as necessary to remove all identifying information until the image resembles:



e. Re-labeling EM images with NEPTUNE Study ID: From image 1 above, locate the “Text Tool”. Click on the tool and then move the mouse to the LOWER RIGHT CORNER of the image. Drag a rectangle by clicking on the mouse and enlarging the box (see below):



Once the box is drawn, release the mouse key and type the following details:

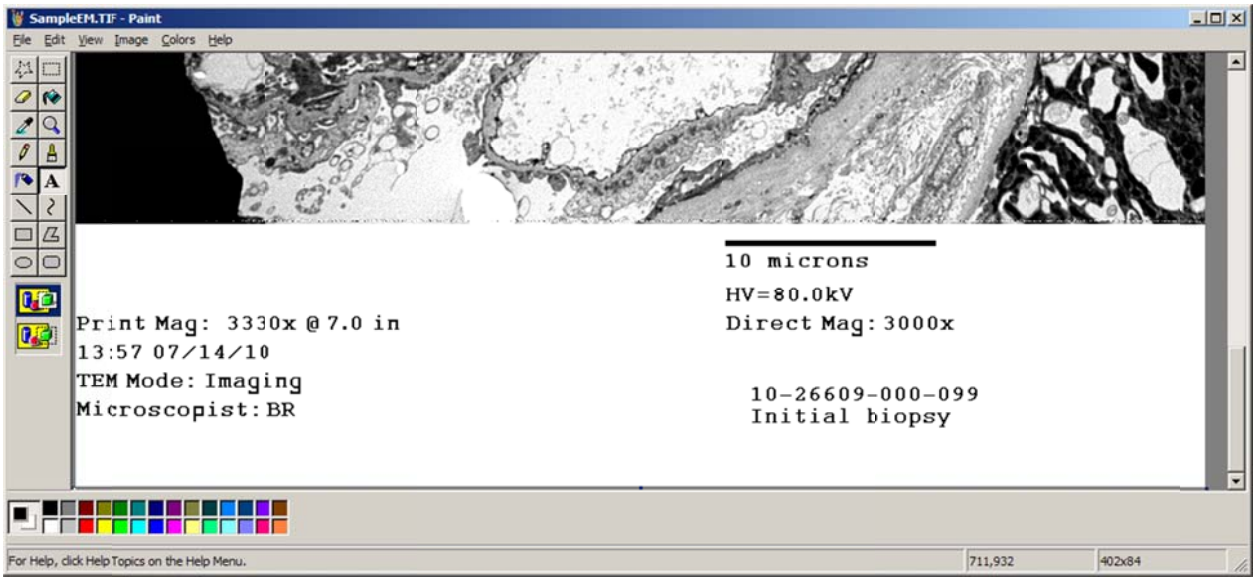
- Participant Study ID (10-26609-Site-StudyPt)
- Initial Biopsy *

***If this is a follow-up biopsy at a later time in the study (not the enrollment or initial biopsy visit), please indicate as "Post-time biopsy" with time in months, e.g., "Post 24-mos biopsy".**

If you are unable to see the Text toolbar above, you may need to click the "View" dropdown menu and select "Text Toolbar". Please use the following:

- Courier
- 15
- Western
- Bold

The final study image should resemble as follows:



f. Taking care to **not overwrite the original** image (do not just click “save”), please save the modified image by selecting:

File >
Save as>

The file name should be the Participant Study ID appended with the image number of the total quantity of images as follows:

- 10-26609-000-099_1of4
- 10-26609-000-099_2of4
- 10-26609-000-099_3of4
- 10-26609-000-099_4of4

2. **EM Prints**

a. Sites without digital images of the EM pathology:

1. Please obtain the prints from your site pathologist and enclose in the 9 x 12 inch envelope included in your kit (only sites with prints will have this envelope).
2. Place all images between the 2 cardboard pieces to protect the images from being bent in transit.

b. De-identification will take place at the NIDDK histopathology archive (a third party will be scanning and immediately de-identifying via the above-described method) and prints will be returned to your site.

PATHOLOGY REPORT DE-IDENTIFICATION INSTRUCTIONS

3. **Electronic Pathology Reports**

a. If your site has an electronic record of the kidney biopsy report:

1. Open the pathology report on your desk top;
2. Open a blank Microsoft office document, or similar text editing software;
3. In the pathology report, select ALL text, copy (usually <CTRL+C> on most PC systems) and return your cursor to the text editing software
4. Paste all <CTRL+V>
5. Remove all identifying information from the report, including:

- Patient hospital identifier
- Reference to age, gender (excluding “pediatric” or “adult”)
- Race
- Ordering physician’s name/initials
- Pathologist name/initials
- Other information deemed identifiable
- Any details identifying site (name(s) of hospital(s), addresses of healthcare institutions, phone numbers, non-treating physicians names, etc)

Information can be de-identified by completing deleting the text (Please insert <DELETED TEXT> when you do this, so it is clearly understood details are missing).

b. If your site has a printed record of the kidney biopsy report:

1. Obtain a legible copy of the report;
2. Copy and complete original for the participant’s study chart (or shadow chart);
3. You should have 2 copies: one for the NIDDK, one for the study chart
4. On the NIDDK copy, using a black permanent marker, draw a line through all identifying details (listed above). Please be sure this de-identifying includes removing details from institutional letterhead if applicable).

Once all images and the local pathology report has been de-identified, please burn these documents and the de-identified pathology report to the enclosed, pre-labeled NEPTUNE CD. This should be sent to the NIDDK with the pathology slides in the pre-addressed. The pathology report can be copied and pasted from an electronic file into a word file, please see appendix C to review an example of details removed from the report.

De-identified Pathology Report Example

HISTORY:

The patient is a [redacted] year-old <DELETED TEXT> with a several year history of proteinuria and nondysmorphic hematuria. [redacted] [redacted] sister has similar signs and symptoms.
Urinary protein:creatinine ratio has ranged from 5 to 6, serum albumin has been 2.5 to 3.5 gm/dL, serum creatinine to 0.7 mg/dL. [redacted] has edema. ANA is positive, otherwise all serologies negative.

GROSS:
------
1. Labeled with patient's name and hospital registration number. Received in formalin are three tan cores ranging from 0.5 cm to 1.0 cm. (3ns)
2. Labeled with patient's name and hospital registration number. Received in Michel's fixative are four, 0.1 cm, tan-gray, soft tissue bits submitted for immunofluorescence. (4ns)
3. Labeled with patient's name and hospital registration number. Received in glutaraldehyde are four tan cores 0.1 cm each; held for processing in the EM lab. (4ns)[redacted]

[redacted] mm/dd/yyyy

MICROSCOPIC:
LIGHT MICROSCOPY:

Sections are stained with H&E, PAS, trichrome and Jones-Silver. There are three cores of renal cortex that contain thirty glomeruli, eight of which are globally sclerotic. An additional six glomeruli display focal segmental sclerosis with obliteration of capillary lumina, excess matrix and adhesion to Bowman's capsule. The remaining glomeruli appear mildly enlarged with patent capillary lumina and glomerular basement membranes of normal thickness and contour. Tubular atrophy and interstitial fibrosis involves approximately 10% of the renal cortex. Collections of foam cells can be seen in the interstitium in a multifocal pattern throughout the tissue. Mild tubular injury can be identified in patchy locations. A few small arteries are sampled and appear normal.

IMMUNOFLUORESCENCE MICROSCOPY:

Direct immunofluorescence examination of the renal biopsy specimen (two glomeruli) with FITC-conjugated antisera for IgG, IgA, IgM, C3, C4, C1q,albumin and fibrinogen revealed: Controls for IgG, IgA, IgM, and C3 are positive.

IgG: Negative.
IgA: Negative.
IgM: Negative.
C3: Focal glomerular positivity (nonspecific).
C1q: Negative.
C4: Negative.
Albumin: Negative.
Fibrinogen: Negative.
Kappa: Negative.

Lambda: Negative.

ELECTRON MICROSCOPY:

All of the tissue submitted for electron microscopy was embedded and examined at the thick section level. One available glomerulus is selected for ultrastructural evaluation.

The ultrastructural preservation of the tissue is adequate. Glomerular capillary lumina are patent and mesangial areas are normal in size and cellularity. The glomerular basement membranes are diffusely thickened with an average thickness of 116 nm (range 79 to 142 nm). A few portions of the basement membrane display mild textural irregularities including internal lucent rarefactions and slight lamellation. Some portions of the basement membrane are focally thickened and wrinkled. Podocyte foot process effacement involves approximately 70% of the glomerular capillary surface. Podocytes appear hypertrophic and reactive with lipid and protein resorption droplets and focal microvillous transformation.

MICROSCOPIC DIAGNOSIS:

Renal biopsy, light, fluorescence and electron microscopy:

1. Focal segmental and global sclerosing glomerulopathy, moderate, with diffuse thinning and segmental lamellation and thickening, consistent with hereditary nephritis.
2. Tubular atrophy and interstitial fibrosis, mild.

COMMENT:

The immunofluorescence findings provide evidence against glomerular disease of the immune complex type. Electron microscopy reveals predominant thinning and focal thickening of the basement membranes with some irregularities. This, in conjunction with the light microscopic findings of focal and global glomerular sclerosis and multifocal collections of interstitial foam cells, and a XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX hereditary nephritis often have a predominantly thin basement membrane phenotype, as appears in this case.

[redacted]

I, [redacted] the signing staff pathologist, have personally examined and interpreted the slides from this case.

17. Appendix D. Suggested Screening Language

*** RC should have screened for items on PART I of Eligibility WORKSHEET in advance of eligibility questions for potential participant ***

Suggested Screening Language:

This is a study for Nephrotic Syndrome which includes FSGS, MCD, and MN. You qualify as a potential participant because you are *already* getting a renal biopsy. We would ask for your permission to obtain some additional renal tissue from that procedure, in addition to some blood and urine. We would like to collect blood and urine over the course of the study.

This study may require additional time or visits to [Healthcare facility]. Initially, there will be 13 total visits (year 1: every 4 months, years 2-5: every 6 months).

We would also ask for you to complete some questionnaires during some of the study visits and provide nail clippings.

Would you be interested to see if you are eligible to participate?

Would you be willing to sign an informed consent agreeing to be in the study after learning more about the study details?

18. Appendix E. Physical Measurements

18.A. Height

Height Measurement

Using a clinic stadiometer or infantometer, usually attached to the clinic scale, obtain participant's height, without shoes. Record the height in centimeters (cm) on the worksheet.

1 inch = 2.54 cm

18.B. Weight

Weight Measurement

Using a clinic scale, obtain the participant weight (without shoes) in pounds or kilograms and record the result on the worksheet. If the weight is obtained in lbs, convert the result to kg and record this on the worksheet as well.

1 lb = 2.2. kg

18.C. Blood Pressure

18.C.1. General Information

In this section, step-by-step procedures for blood pressure measurement will be presented. Please be sure to follow the procedures outlined below unless exceptional circumstances arise. If, and when, these circumstances arise please seek a Blood Pressure Consultant, so that participants will be appropriately evaluated. Blood pressure will be measured at the Baseline Visit, and at every Follow Up Visit. You will record blood pressure results on the Baseline Clinical Information and Follow Up Clinical Information Worksheets, as well as on the coordinating Velos Case Report Forms.

Preparation for Blood Pressure Measurement

Participants should abstain from caffeine, smoking, and exercise at least one-half hour prior to and until completion of the blood pressure measurement. Currently prescribed medications, including those affecting blood pressure and non-prescription drugs, should be recorded on the Concomitant Drug Log (Form 5) and in Velos.

Participants should be given full explanation and instructions about the preparation for the blood pressure examination and an opportunity for brief questions. Blood pressure measurement should take place in a separate, quiet room where no other activity is taking place, and where temperature fluctuations are minimal. Scheduling procedures should try to establish consistent appointment times to minimize as much as possible the impact of daily blood pressure variation. Equipment being used should be calibrated by local institutional standards and made ready for use prior to the appointment. Arm measurement, cuff selection and placement should be completed prior to a five minute rest period in this quiet room. Explain to the participant that the five-minute rest period will provide for more valid blood pressure measurements. Preferably, at this time, the

observer should leave the room. The participant should be relaxed, seated with back supported with legs uncrossed and feet comfortably flat on the floor, not dangling. The participant should be instructed to refrain from using a cell phone. An aneroid sphygmomanometer is the preferred standard equipment for all blood pressure measurements at each NEPTUNE study visit. We recommend that this device is calibrated at the initiation of the study and annually according to sites' local institutional policies.

An appropriate electronic blood pressure device may be used in place of the aneroid sphygmomanometer in the place of untrained clinical staff. Please use the device according to the manufacturer's instructions.

Arm Measurement and Cuff Sizes

The proper cuff size should be used to avoid under- or over-estimating the correct blood pressure. To determine the proper cuff size, please follow the procedures outlined below:

- Measure the arm circumference at the midpoint of the arm at each visit.
- Take the measurement on the right arm which has been bared from the shoulder.
- With the participant standing, holding the forearm horizontal (90 degree angle), measure the arm from the acromion (or boney extremity of the shoulder girdle) to the olecranon (or tip of the elbow) with a plastic coated metric tape.
- Mark the midpoint on the dorsal surface of the skin.
- Ask the participant to relax their arm straight down along the side of their body.
- Measure the circumference of the arm by drawing the metric tape snugly around the arm at the level of the midpoint marking. **Make sure the tape is kept horizontal and do not wrap it so snugly that it indents the skin.**
- Using the measurement obtained, consult the chart below to determine cuff size. Try to use the same cuff size for every measurement taken from that particular participant.
- Do not use the cuff itself as a measurement device and refer to the chart below for every cuff size determination.

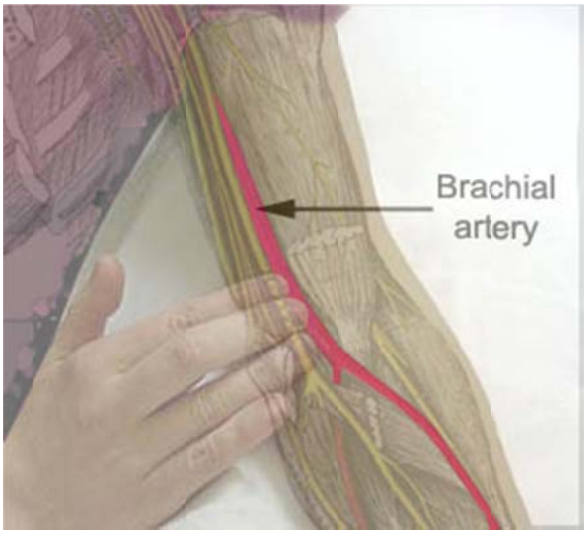
Arm Circumference	**Cuff Size (cm)**
< 24 cm	Child, Small Adult
24.0 to < 33.0 cm	Adult, Regular
33.0 to 41.0 cm	Large Adult
< 41.0 cm	Thigh, Extra Large

Applying the Blood Pressure Cuff

To ensure correct placement of the blood pressure cuff, please follow the steps below:

- Ask the participant to remove any outer clothing and roll up their sleeve so that the area where the cuff will be applied is bare.

- Seat the participant with their elbow and forearm resting comfortably on a table with the palm of their hand turned upward.
- Fold the cuff bladder (the inflatable portion of the blood pressure cuff) in half mating each corner of the bladder to find and mark the midpoint on the cuff cover with a piece of tape or pencil.
- Locate the brachial artery is located by palpation and place the cuff around the upper right arm so that the piece of tape (on the cuff) lies over the brachial artery and the mid-height of the cuff is at heart level. The lower edge of the cuff should lie about 1 inch above the natural crease in the center of the arm.

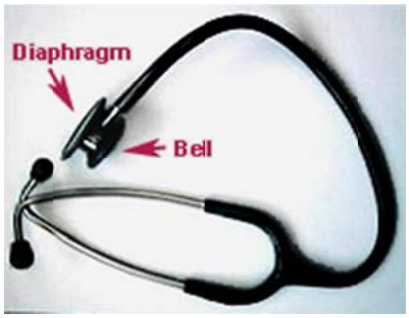


http://www.medicine.mcgill.ca/physio/vlab/cardio/back.htm

The brachial artery is usually located above the crease of the arm, slightly toward the body.

- Wrap the cuff snugly around the arm, with the palm of the participant's hand turned upward, and lock the fabric fastener.

Stethoscope



http://www.medicine.mcgill.ca/physio/vlab/cardio/back.htm

A standard Littman stethoscope (or other comparable stethoscope) with a bell should be used. Stethoscope tubing should be about 12 to 15 inches from the bell piece to "Y"

branching. This length provides optimal acoustical properties and allows the observer to easily read the sphygmomanometer. Please refer to the following when using the stethoscope:

- Place the ear pieces into the external ear canal. The ear pieces should be tight enough to exclude outside sound but not so tight that they cause discomfort.
- Turn the valve, between the bell and the diaphragm, in the direction of the bell.
- Applying light pressure, place the bell of the stethoscope on the skin over the brachial artery, immediately below, but not touching, the cuff.

18.C.2. Blood Pressure Measurement Step By Step

If a trained clinician is unavailable to perform the blood pressure measurement, sites should follow the local institutional policy for obtaining blood pressure with either an aneroid sphygmomanometer or electronic blood pressure measurement equipment locally available.

Provided below are suggested guidelines to determine blood pressure measurement:

Determining the Peak Inflation Level

Determine the Peak Inflation Level for the participant:

- Attach the cuff tubing to the aneroid sphygmomanometer.

http://www.mystethoscope.com/imagemagic.php?img=3Nra2tLsorm%2Fxt3h2rXL0p6n3d3g&w=300&h=368&page=prod_info

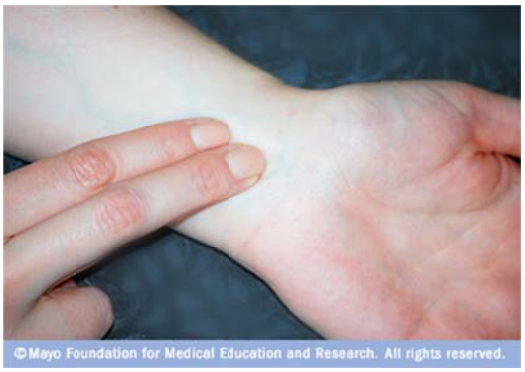
- While palpating the pulse, observe the sphygmomanometer and inflate the cuff rapidly to 60 mmHg and then slowly inflate in increments of 10 mmHg until the pulse is no longer felt. If the pulse is still felt, the cuff pressure should be increased until the pulse disappears. The pressure at which the pulse is no longer heard is called the Observed Pulse Obliteration Pressure. Record this value on the worksheet.
- After the Pulse Obliteration Pressure has been determined, quickly and completely deflate the cuff.
- Add 30 mmHg to the Pulse Obliteration Pressure. This summed value is the Peak Inflation Level (this is the level that the cuff should be inflated to for all readings). Record this value on the worksheet. For example: If the Pulse Obliteration Pressure is 80 mmHg, the Peak Inflation Level would be 110 mmHg.

NOTE: All readings on the sphygmomanometer are made to the nearest even digit. Any reading that appears to fall exactly between markings on the column should be read as the marking immediately above, i.e., 2, 4, 6, 8, or 0.

18.C.3. Pulse Measurement

Determine the pulse of the participant:

- Palpate the radial artery on the participant's wrist. Use the participant's right arm for both pulse and blood pressure measurements. If this is not possible, use the left arm. Document which arm is used to measure pulse and blood pressure on the worksheet.



- Ask the participant to sit quietly with their feet flat on the floor, in an erect but comfortable posture for at least five minutes prior to pulse measurement.
- Place the participant's elbow and forearm comfortably on a table with the palm of their hand turned upward.
- Using a stopwatch, count the participant's pulse for exactly 30 seconds and record.
- Multiply this number by 2 and record the product as the heart rate (beats per minute) on the worksheet.

NOTE: Make note of any irregularities observed and notify the Principal Investigator.

18.C.4. Blood Pressure Measurement Procedures (Seated)

The seated blood pressure should be measured three times at each clinic visit. Record all three readings on the Worksheet (the average of the second and third measurements will be recorded on the CRF). Blood pressure equipment should be checked prior to seeing the participant. Once a participant has sat quietly for at least five minutes and Peak Inflation Level and pulse have been determined, blood pressure measurements may be taken. Please follow the instructions below for blood pressure measurement procedures:

- All blood pressure readings taken for the NEPTUNE Study should be performed by trained clinical or research staff at NEPTUNE Study Clinics or satellite offices.
- All blood pressure measurements should be taken using the aneroid Sphygmomanometer.
- Connect the cuff to the aneroid sphygmomanometer device.
- Place the ear pieces of the stethoscope into the ears.

- Apply the bell of the stethoscope over the brachial artery, just below, but not touching the cuff.
- Using the previously determined peak inflation level, rapidly inflate to this level.
- Deflate the cuff, by adjusting the valve, at a constant rate of 2 mmHg per second, listening carefully.
- Note the pressure where the first of two consecutive beats are heard. This is the systolic reading. Record this pressure on the worksheet.
- Note the pressure where the last of two consecutive beats are heard. This is the diastolic reading. Record this pressure on the worksheet.
- Continue deflating until 10 mmHg past the diastolic reading.
- Open the valve to completely deflate and disconnect the tubing.
- Remove the stethoscope earpieces from the ears.
- Repeat the previous steps two more times, taking a total of three blood pressure measurements. Make sure to wait at least 30 seconds after deflation of the cuff prior to re-inflation. Record all three BP measurements on the Worksheet (Baseline Clinical Information [4C] or Follow-Up Clinical Information [13C]. Average the second and third readings and record the Average on the Case Report Form.
- Finally, raise the participant's arm overhead for 15 seconds without the participant's assistance. The arm should be fully supported at the elbow and wrist by the RC and the participant should not help to support the raised arm.

Notes on Determining Systolic and Diastolic Blood Pressure:

Control of the deflation rate is essential for accurate readings. The aneroid dial (pressure dial) should drop at 2 mmHg per second from the maximum pressure until it has dropped 10 mmHg below the last regular sounds can be heard. The systolic value can be identified as the pressure level where the first of two or more consecutive beats are heard in an appropriate rhythm. The diastolic value can be identified as the pressure level where the last two or more consecutive beats are heard.

18.C.5. Standing Pulse and Blood Pressure Measurement

- After completing 3 seated blood pressure readings, raise the bedside table at the participant's immediate right so that unnecessary movement or walking will not occur when the participant is asked to stand.
- Ask the participant to stand quietly with their arms relaxed at their sides for 2 minutes.
- After 2 minutes, raise the participants arm (fully supported overhead) for 15 seconds.
- Place the participant's arm on the bedside table with the palm of the hand turned upward. The bedside table needs to be elevated so the arm can rest at heart level for the standing pulse and blood pressure. If the table can not be elevated to that height, pillows can be added to the table top to bring the arm to heart level.
- Immediately following, palpate the radial artery and count the participant's pulse for thirty seconds. Multiply by two and record the participant's hear rate, in beats per minute, on the case report form.
- Take one standing blood pressure measurement, as described above, and record the values on the worksheet and later on the case report form.

Forgotten Blood Pressure Readings

If for any reason the observer is unable, or has forgotten, to complete any portion of the exam, and the participant is gone, leave the items blank on the worksheet.

If a blood pressure value is missed or forgotten, during the blood pressure reading procedure, completely deflate the cuff and start over with a replacement reading after 30 seconds.

Do not re-inflate the blood pressure cuff during a reading and do not repeat a reading just because the measurement looks unusual to you.

Reporting the Blood Pressure Results to the Participant

If the participant would like to know his or her blood pressure results, average the second and third systolic readings, and second and third diastolic readings, and give these results to the participant.

18.C.6. Edema Assessment

General Information

At baseline and follow up visits RCs will be asked to assess the presence of edema for the participant. In this section, step-by-step procedures for assessment of edema will be presented. Please be sure to follow the procedures outlined below.

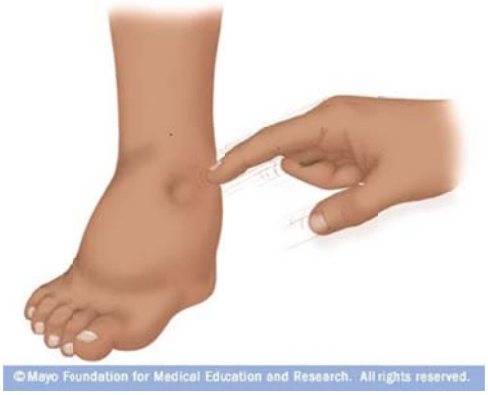
Edema Overview

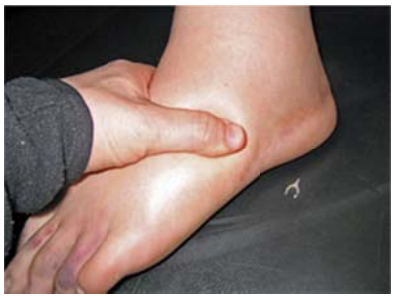
Edema is the swelling of tissues as a result of excess water accumulation. This swelling can occur in a single area on the body, including around the eyes (periorbital edema), in the lower back (sacral edema), in the legs (lower extremity or peripheral edema), or all over the body (anasarca). The NEPTUNE Study is interested in capturing both isolated and generalized edema.

Edema Assessment Step by Step

Please follow the step-by-step instructions below for participant edema assessment.

Lower Extremity Edema Assessment





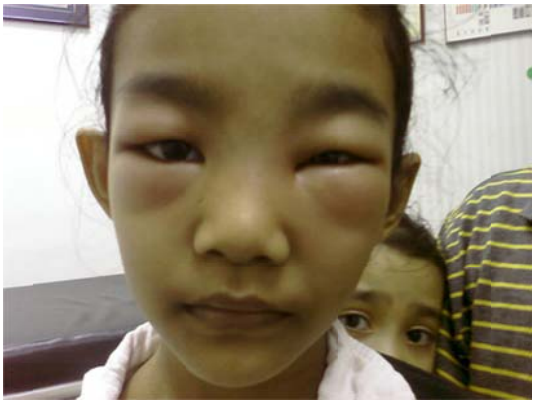
(Reference http://www.ehow.com/how_5607991_test-ascites.html

- Ask the participant, or their legal representative, if they have noticed significant swelling in their legs and whether or not this swelling is currently present.
- Looking at the participant, do both legs appear uniform?
- Looking at the participant, does the skin on the legs appear red, tight, glossy, or swollen?
- Perform a pit test.

Pit Test

- Press firmly on lower extremity below the knee (foot, ankle, or pre-tibial area) with your finger for 10-20 seconds.
- After 10-20 seconds stop pressing, remove your finger, and check for a persistent depression in the skin.
- If a depression, or "pit", is present, the participant is positive for lower extremity edema.
- Please check "Yes" on the worksheet and Case Report Form.
- If the RC is unsure, please consult a physician.

Periorbital Edema Assessment



- Ask the participant, or their legal representative, if they have noticed significant swelling around their eyes and whether or not this swelling is currently present.
- Does the participant's eye or eyes appear significantly swollen? If so, the participant is positive for periorbital edema.
- If the participant is positive for periorbital edema, please check "Yes" on the worksheet and Case Report Form.
- If the RC is unsure, please consult a physician.

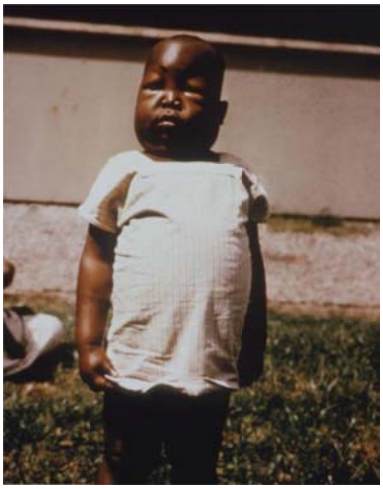
Sacral Edema Assessment

- Ask the participant, or their legal representative, if they have noticed pronounced swelling around their lower back and whether or not this swelling is currently present.
- Looking at the participant, does the skin on the lower back appear red, tight, glossy, or swollen?
- Perform a pit test.

Pit Test

- Press firmly on the sacral area with your finger for 10-20 seconds.
- After 10-20 seconds stop pressing, remove your finger, and check for a persistent depression in the skin.
- If a depression, or "pit", is present, the participant is positive for lower extremity edema.
- Please check "Yes" on the worksheet and Case Report Form.
- If the RC is unsure, please consult a physician.

Anasarca Assessment



http://commons.wikimedia.org/wiki/File:Plasmodium_falciparum_nephrosis_edema_PHIL_3894_lores.jpg

- Does the participant have swelling in many areas (the face, hands, extremities, torso)? This is considered to be generalized edema or anasarca.
- Perform a pit test on upper extremities, thighs, abdomen.
- If the participant has edema in the upper and lower extremities, torso, and/or face, this participant has anasarca. Please check "Yes" for anasarca on the Worksheet and Case Report Form in addition to the individual areas of edema.
- If the RC is unsure, please consult a physician.

19. Appendix F. Quality of Life Questionnaires

19.A. PedsQL™ Administration Guidelines℠

The following guidelines are intended for use by individuals trained in the administration of standardized questionnaires. The PedsQL™ administrator is crucial in developing rapport with the respondents, emphasizing the importance of the questionnaire, addressing concerns, and ensuring that the PedsQL™ is completed accurately and confidentially.

19.A.1. General Protocol

1. The parent/child should first complete the PedsQL™ Generic Core Scales and then complete any additional PedsQL™ Module.
2. Parents, Children (8-12) and Teens (13-18) may self-administer the PedsQL™ after introductory instructions from the administrator. If the administrator determines that the child or teen is unable to self-administer the PedsQL™ (e.g., due to illness, fatigue, reading difficulties), the PedsQL™ should be read aloud to the child or teen. For the Young Child (5-7), the PedsQL™ should be administered by reading the instructions and each item to the young child word for word. At the beginning of each subscale repeat the recall interval instructions (one month or 7 days) to remind the young child to respond only for that specific recall interval. Use the separate page with the three faces response choices to help the young child understand how to answer. When reading items aloud to a child, intonation should be kept neutral to avoid suggesting an answer.
3. If a child has difficulty understanding the age-appropriate PedsQL™, the preceding age group version may be administered to the child (e.g., administering the Young Child (5-7) Self-Report version with the three faces response choices to an 8 year old). However, if a child presents with severe cognitive impairments (as determined by the administrator), the PedsQL™ may not be appropriate for that child. In such cases, only the Parent-Proxy Report should be administered to the child's parent.
4. The parent and child must complete the questionnaires *independently* of one another. Discourage the parent, child, or other family members from consulting with one another during the completion of the questionnaire. Let them know that they can feel free to discuss their answers following completion of the questionnaires, but that it is important to get both the parent's and the child's *individual* perspectives. If you are administering the questionnaire to the child, the child should be facing away from the parent.
5. If the child or parent has a question about what an item means or how they should answer it, do not interpret the question for them. Repeat the item to them verbatim. Ask them to answer the item according to what *they think the question means*. If they have trouble deciding on an answer, ask them to choose the response that comes closest to how they feel. The child and/or the parent has the option of not answering a question if they truly do not understand the question.
6. If a parent/child asks you to interpret the responses, tell her/him that you are not trained to interpret or provide a score for the answers given. If the PedsQL™ is being used for a clinical study, let the parent/child know that their answers will be combined with other participants' answers and analyzed as a group rather than as individual respondents.
7. Document all reasons for refusals and non-completions of the PedsQL™.

19.A.2. Administering the PedsQL™

1. The following scripts have been developed as a guide to introduce the PedsQL™ to the child and his/her parent(s). Modify the language to a style that is most appropriate for you and the respondent.

For the child:

The PedsQL™ asks you questions about how you feel and what you think about your health. It is not a test, and there are no right or wrong answers. It takes about 5 minutes to complete. If you have any questions, please let me know.

For the parent:

The PedsQL™ is a questionnaire that assesses health-related quality of life in children and adolescents. It contains questions about your child's physical, emotional, social, and school functioning ***in the past one month (****or for the Acute version****, in the past 7 days)****.*

The PedsQL™ is brief and typically takes less than 5 minutes to complete. It is not a test, and there are no right or wrong answers. Please be sure to read the instructions carefully and choose the response that is the closest to how you truly feel. Please do not compare your answers with your child's responses. We are interested in your and your child's ***individual*** *perspectives. However, feel free to discuss the questionnaire with your child* ***after*** *you have both completed it and returned it to me. If you have any questions, please let me know.*

2. Provide the respondent with a pen or pencil and a solid writing surface. If a table is not available, the participant should be provided with an item such as a clipboard. Remain nearby should questions or concerns arise.
3. When the parent/child returns the PedsQL™, look it over and check to see that all answers have been completed. Verify that no item has more than one response. If any responses are incomplete, illegible, or there are multiple responses for an item, please ask the parent or child to indicate their response.
4. Ask the participants if they had any difficulties completing the questionnaire or if they have any other comments regarding the questionnaire. Document any important feedback.
5. Thank the parent and child for taking the time to complete the questionnaire. If the study design involves following up with these respondents, let them know that they may be asked to complete the PedsQL™ again at another time. Indicate when they can expect to be contacted again if known.

19.A.3. Study Documentation

The completion or non-completion of the PedsQL should be documented in Form 6A-E and the corresponding CRF. Please review 16.C.11 for documentation instructions.

19.B. SF-36™

19.B.1. Adult Population

This Quality of Life assessment is administered to adult participants only (ages 18+).

19.B.2. General Protocol

The instructions can be read to a participant, or the participant can read the instructions at the top of the survey. Please provide your participant a pencil to complete the survey.

The SF-36 questionnaire will serve as the study source documentation and should remain in the on-site study chart, dated and signed at the completion of the visit.

Instructions for completing the questionnaire: Please answer every question. Some questions may look like others, but each one is different. Please take the time to read and answer each question carefully by filling in the bubble that best represents your response.

19.B.3. Study Documentation

The completion or non-completion of the SF-36 should be documented in Form 6A-E and the corresponding CRF. Please review 16.C.11 for documentation instructions.

19.C. PROMIS – Patient Reported Outcomes Measure Information System

The following instructions are to aid the site investigators and the research coordinators in administration of the Patient Reported Outcome Measurement Information System (PROMIS) in NEPTUNE cohort participants. All NEPTUNE participants, age 8 and above, who are English-speakers, are to be administered the PROMIS survey.

19.C.1. PROMIS Registration

NEPTUNE Research Coordinators must complete registration before entering NEPTUNE participants into the PROMIS Assessment Center. Please contact the NEPTUNE Data Manager, Ellen Woodard at erhw@umich.edu if you did not complete the PROMIS registration during the training period.

Access must be granted by the Data Manager prior to completing the registration.

19.C.1.a. *PROMIS Assessment Center Registration (Research Coordinator)*

Please see pages 4-5.

19.C.1.b. *PROMIS Assessment Center Registration (Participant)*

Please see pages 6-9.

19.C.2. Survey Execution and Data Collection

In order for survey administration to be successful, there must be consistent, uninterrupted access to the internet.

Access the study website using any web browser:

https://www.assessmentcenter.net/ac1/Assessments/NEPTUNE

This website will be used each time the survey is completed so it is recommended that shortcuts be placed on each computer desktop to facilitate navigation.

Participants may complete the PROMIS survey outside the study visit, by being provided the study web link and their Participant User ID and Password (recorded and stored for subsequent study visits on Form 7 by the site research coordinator).

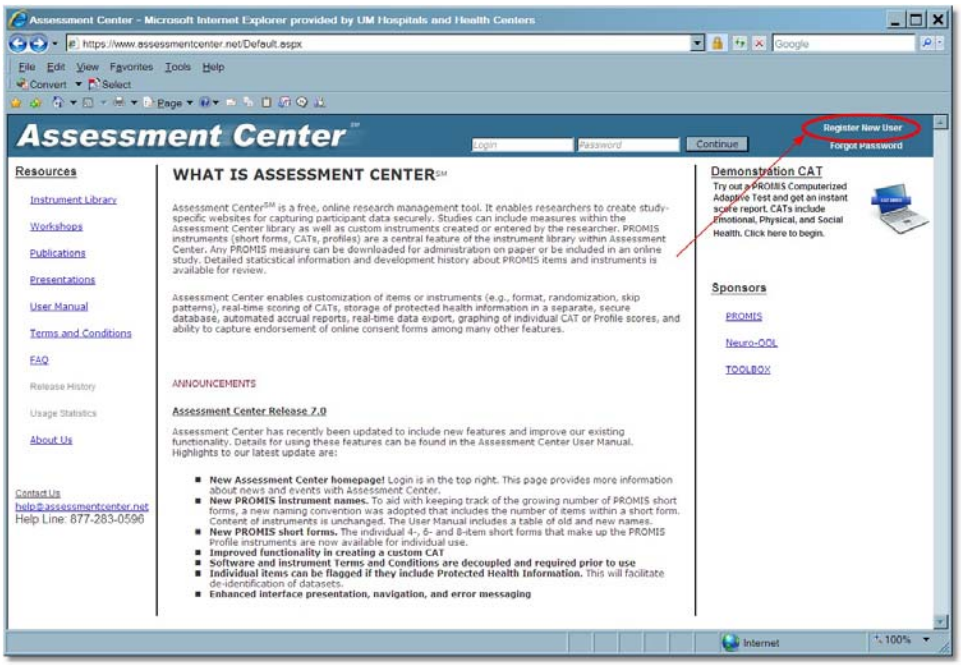
19.C.3. Study Documentation

The completion or non-completion of the PROMIS should be documented in Form 7 and the corresponding CRF. Please review 16.C.11 for documentation instructions.

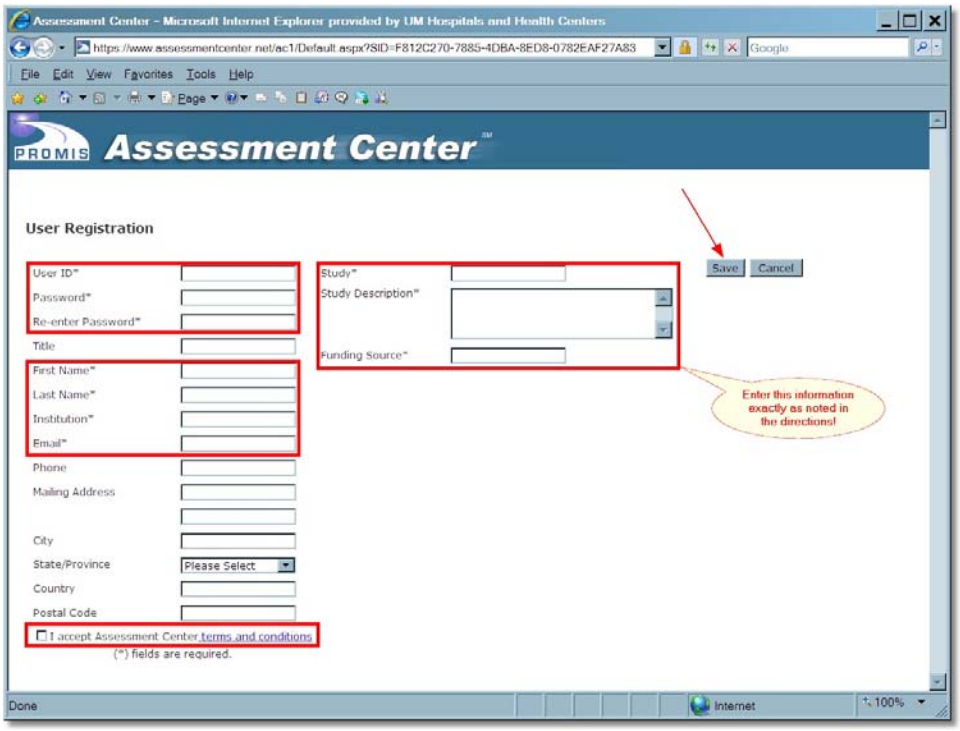
PROMIS Assessment Center

Study Coordinator Registration

1. Go to the Assessment Center web page – www.assessmentcenter.net/ac1 - and click on the "Register New User" link.



2. Complete all fields marked with an asterisk (*).



Make special note of the following:

a. Your password must be at least six (6) characters and can be any combination of letters, numbers or special characters.

b. If another user has already registered with the same combination of last name, first name and institution, you must change at least one of these registration elements.

c. You **MUST** click the box to accept the Assessment Center terms and conditions.

d. Enter EXACTLY the following in the "Study," "Study Description" and "Funding Source" fields:

- Study – NEPTUNE
- Study Description – The Nephrotic Syndrome Study Network is investigating Focal Segmental Glomerulosclerosis, Minimal Change Disease, and Membranous Nephropathy
- Funding Source – NIDDK, ORDR, NephCure Foundation, Univ of MI

3. Click the "Save" button.

To finalize your registration, go to https://www.assessmentcenter.net/ac1/Default.aspx and log in using the User ID and password you specified during the registration process. You can then log right back out again. (**Do NOT skip this step or your registration will be incomplete!**)

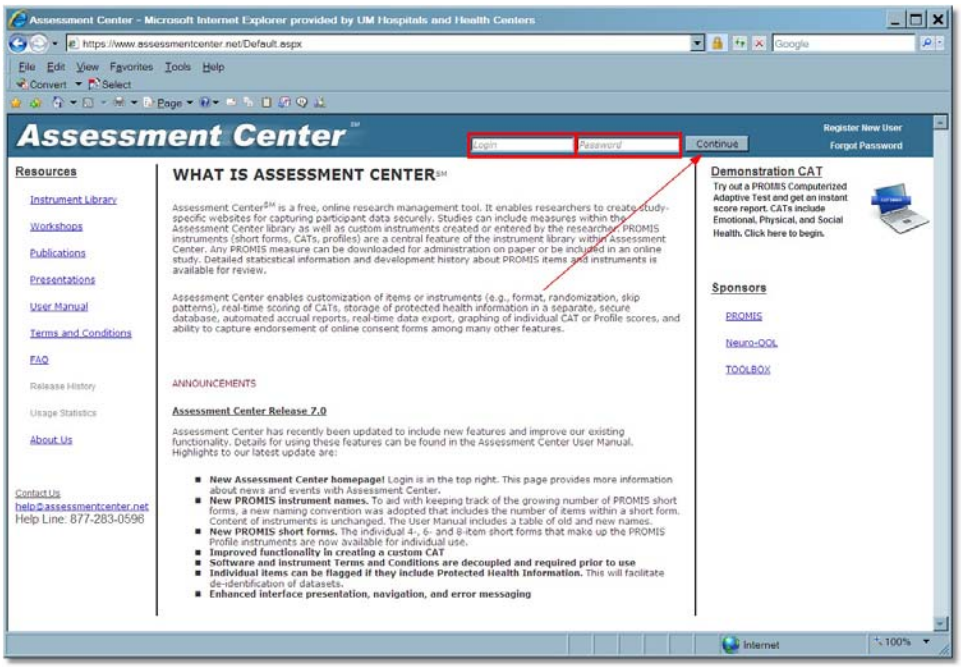
Your registration is now complete. Email Ellen Woodard (erhw@umich.edu) to let her know you've completed your registration so she can add you to the NEPTUNE study team. Once you are added to the NEPTUNE study team you will be able to register study participants (refer to PROMIS_PRegistration.pdf) so they can access and complete the PROMIS survey.

PROMIS Assessment Center

Participant Registration

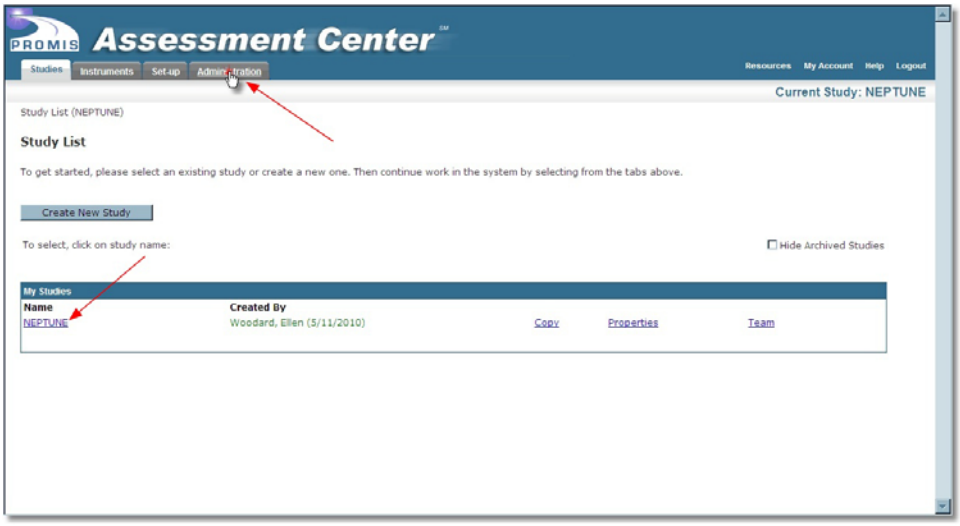
NOTE: You must complete the ***Study Coordinator Registration*** (refer to PROMIS_SCRegistration.pdf) and have notified Ellen Woodard (erhw@umich.edu) that you completed that registration before you can register participants.

1. Go to the Assessment Center web page – www.assessmentcenter.net/ac1 - and enter the User ID and password you supplied upon ***Study Coordinator Registration***, then click the "Continue" button.

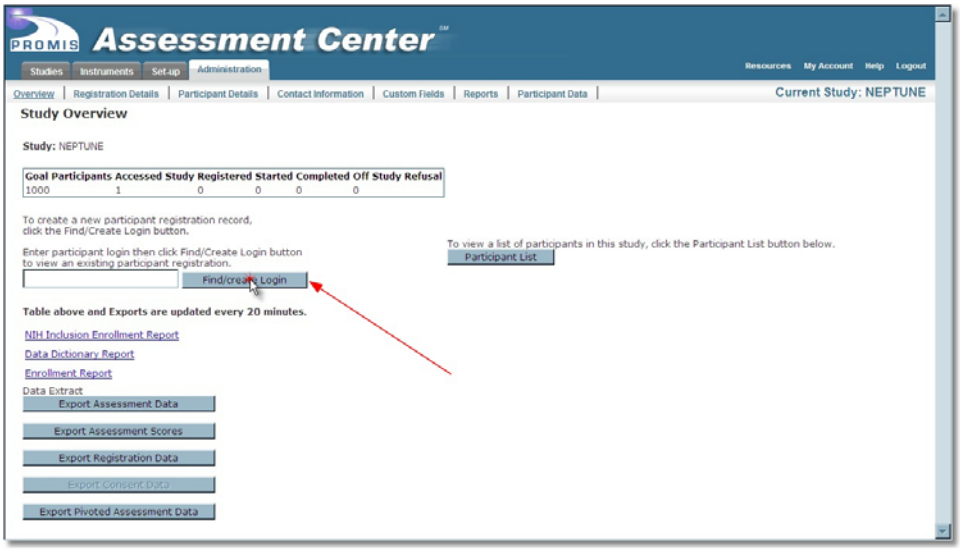


   If you forget your password, click on the "Forgot Password" link and the Assessment Center will automatically email your password to the email address you supplied upon registration.

2. Click on the "Administration" tab. NOTE: You may first need to "select" the study by clicking on "NEPTUNE" in the "Name" column of the "My Studies" list.



3. Click the "Find/Create Login" button.



4. Complete all of the following fields (any field not mentioned is to be left as is):

   a. <u>Registration Details tab</u>

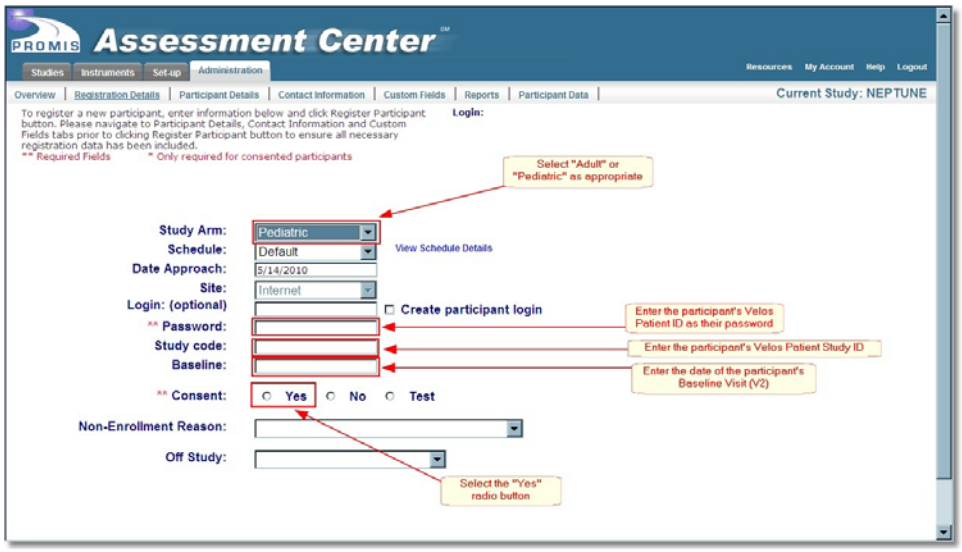
      (1) Study Arm – select "Adult" or "Pediatric" as appropriate

      (2) Password – enter the participant's ***Velos Patient ID*** as their password

      (3) Study code – enter the participant's ***Velos Patient Study ID***

      (4) Baseline – enter the date of the participant's Baseline Visit (V2)

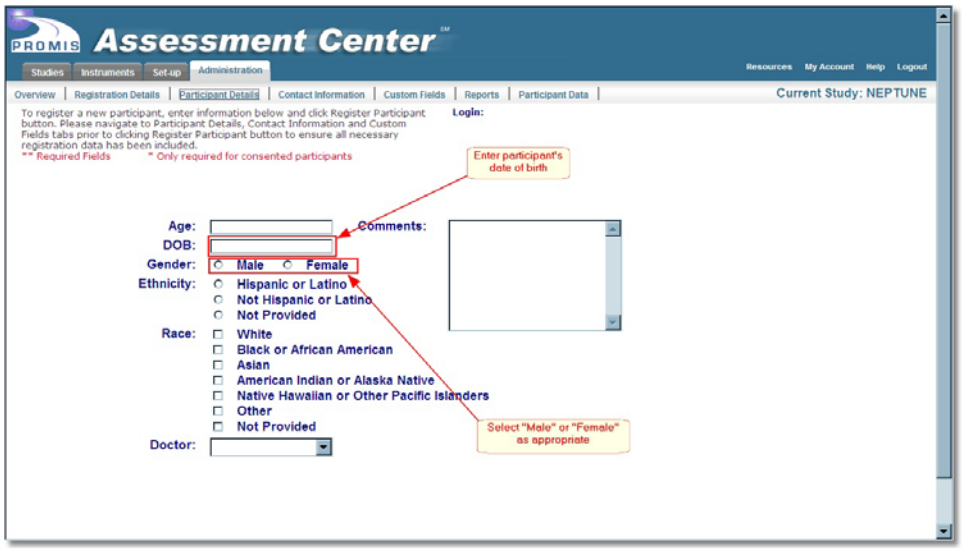
      (5) Consent – select the "Yes" radio button



b. <u>Participant Details tab</u>

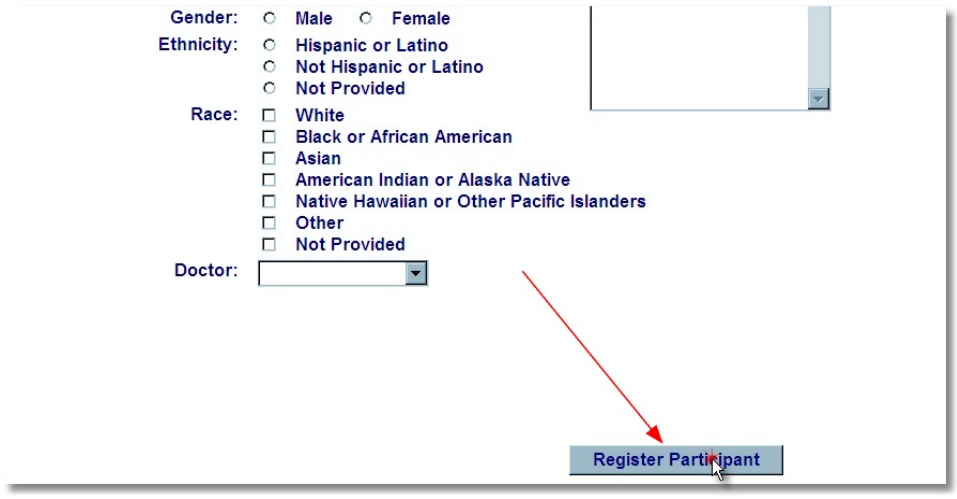
(1) DOB – enter the participant's date of birth

(2) Gender – select "Male" or "Female" as appropriate

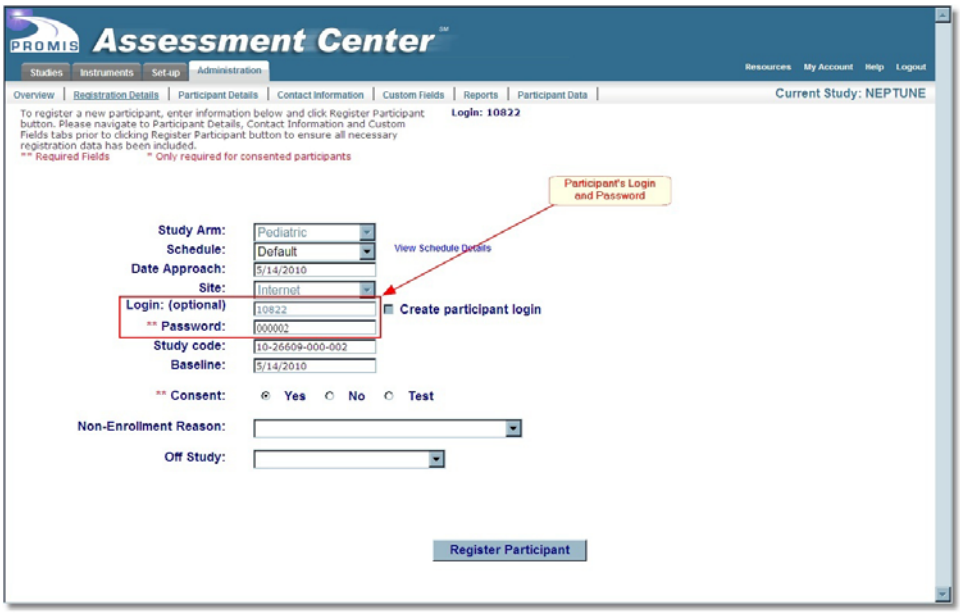


Again, the above-mentioned fields are the only ones that need to be completed. Any field not mentioned is to be left as is.

5. Click the "Register Participant" button.



6. Make note of the participant's assigned Login and password.



7. Click "Logout" (upper right-hand corner of the page) to exit the Assessment Center

You can now provide your participant with their assigned Login and password and they can log in to take the survey at www.assessmentcenter.net/ac1/assessments/NEPTUNE

23. Appendix J. Blood Collection Protocol

23.A. Blood Specimens

Fasting blood draws (Adult baseline max: 100 cc, pediatrics by weight according to tables 1A and 1B below) will be obtained for the NEPTUNE Biorepository and central biochemical laboratory samples. Blood volumes will be reduced in pediatric participants and in all participants, adult and pediatric, with a hematocrit below 28%.

Table 1A. Adults (age 18+):

Visit	Blood Draw Volume
Biopsy [V_{BX}]	40 mL
Baseline [V1]	100 mL
Follow-up	65 mL
Relapse Visit [V_R]	40 mL

Table 1B. Children as follows by weight:

Visit	Blood Draw Volume by Weight (in pounds)		
	< 20 pounds	21-51 pounds	> 52 pounds
Biopsy [V_{BX}]	10 cc	20 cc	40 cc
Baseline [V1]	20 cc	50 cc	100 cc
Follow-up	20 cc	50 cc	65 cc
Relapse Visit [V_R]	10 cc	20 cc	40 cc

Adult participants with a clinically reported hematocrit < 28%; the blood draw should be reduced to the >52 pound children draw at baseline; and 21-51 pound draw at follow-up.

Pediatric participants should be reduced to the next lower weight group; children < 20 pounds will not undergo a blood draw at a visit in which a clinically reported hematocrit < 28% within 30 days of study visit.

Local site phlebotomy practice should be used. Research samples will not be submitted for any clinical measures, so please coordinate with treating physicians if they are in need of standard of care blood draws.

23.B. Biopsy Visit Biospecimen Collection

The data and specimens obtained at the Biopsy Visit has been added to the study procedures effective Protocol V3.0 at each site. This data will serve as the limited baseline study data for the purpose of data analysis and biochemical investigations for participants who do not return to study sites for follow-up. The Baseline Visit should still be pursued for all consented participants with a local pathology diagnosis of FSGS/MCD or MN.

23.B.1. Blood Specimens

Fasting blood draw will be obtained (volumes according to Table 2 below) for the NEPTUNE Biorepository and central biochemical laboratory samples.

Local site phlebotomy practice should be used. Research samples will not be submitted for any clinical measures, so please coordinate with treating physicians if they are in need of standard of care blood draws.

There is no order of prioritization for biopsy/relapse blood draws. Please obtain all tubes indicated here:

Table 2. Biopsy and Relapse Visit Tubes and Volumes

Tube #	Full Draw Priority	Tube Type	**Adult**	**Peds < 21#**	**Peds 21 – 51#**	**Peds >52#**	**Reduced Draw Priority**
16	1	3 mL Serum wrapped in Foil	**3**	**3**	**3**	**3**	**6**
3	2	10 mL SST	**10**			**10**	**3**
18	3	2 mL Sodium Heparin in Foil	**2**		**2**	**2**	**5**
6	4	10 mL EDTA	**10**		**10**	**10**	**1**
14	5	4 ml EDTA	**4**	**4**		**4**	**7**
15	6	6 mL EDTA	**6**			**6**	**8**
12	7	2.5 mL RNA	**2.5**	**2.5**	**2.5**	**2.5**	**2**
20	8	2.5 mL DNA	**2.5**		**2.5**	**2.5**	**4**
Blood Draw Totals:			**40**	**9.5**	**20**	**40**	

23.C. Baseline and Follow-up Biospecimen Collection

The data and specimens obtained at the Baseline Visit will serve as the baseline study data and follow-up biospecimens collected according to the visit schedule serving as longitudinal markers for the purpose of data analysis and biochemical investigations. The study visits will include the following biospecimens procurement according to Tables 3A and 3B.

23.C.1. Blood Specimens Order of Precedence

The blood draw order should follow the order of each tube in the Full Draw Priority column (in the case of pediatric draws based on weight). In the case of a *reduced* blood draw, please use the priority column on the far right of each table.

Local phlebotomy practice should be followed for blood draw attempts and institutional policy should be observed for number of attempts to obtain the research blood draw.

If a participant blood draw is suboptimal (a “difficult stick” or perfusion is weak) please immediately prioritize the tube order to the reduced draw priority list.

The following table is meant as a guide for order of tube draw when a full blood sample is optimal. Please observe the corresponding weight for pediatric participants.

Table 3A. Adult blood procurement

Tube #		Tube Type	**Baseline Visit**	**Full Draw Priority**	**Reduced Draw Priority**	**Follow-up Visits**	**Full Draw Priority**	**Reduced Draw Priority**
1	1	4.5 mL Sodium Citrate	**4.5**	**1**	**4**	**4.5**	**1**	**4**
2	2	10 mL Serum wrapped in foil	**10**	**2**	**6**			
16	3	3 mL Serum wrapped in Foil				**3**	**2**	**6**
3	4	10 mL SST	**10**	**3**	**3**	**10**	**3**	**3**
4	5	10 mL SST wrapped in foil	**10**	**4**	**7**			
17	6	3.5 mL SST wrapped in foil				**3.5**	**4**	**8**
19	7	3.5 mL SST						
5	8	4 mL Sodium Heparin in Foil	**4**	**5**	**5**			**5**
18	9	2 mL Sodium Heparin in Foil				**2**	**5**	
6	10	10 mL EDTA	**10**	**6**	**1**	**10**	**6**	**1**
7	11	10 mL EDTA	**10**	**7**	**9**	**10**	**7**	**9**
8	12	10 mL EDTA	**10**	**8**	**10**	**10**	**8**	**10**
9	13	10 mL EDTA	**10**	**9**	**11**			
10	14	10 mL EDTA	**10**	**10**	**12**			
14	15	4 ml EDTA						
15	16	6 mL EDTA				**6**	**9**	**11**
12	17	2.5 mL RNA	**2.5**	**11**	**2**	**2.5**	**10**	**2**
13	18	8.5 mL DNA	**8.5**	**12**	**8**			
20		2.5 mL DNA				**2.5**	**11**	**7**
Blood Draw Totals:			**99.5**			**64**		

Table 3B. Pediatric participant visit draw order by weight:

Tube #	Tube Type	< 21 pounds	Prioritization
		Baseline & Follow-up	**Full & Reduced Draw Priority**
-	ACD Tube	**4**	**3**
16	3 mL Serum wrapped in Foil	**3**	**5**
17	3.5 mL SST wrapped in foil	**3.5**	**6**
14	4 ml EDTA	**4**	**1**
12	2.5 mL RNA	**2.5**	**2**
20	2.5 mL DNA	**2.5**	**4**
Blood Draw Total:		**19.5**	

*The ACD tubes should only be obtained one time over the course of the study. Once the this draw is complete, for subsequent visits please supplement the 4 mL ACD draw with corresponding follow-up volumes.

Tube #	Tube Type	21-51 pounds	Prioritization	
		Baseline & Follow-up	**Full Draw Priority**	**Reduced Draw Priority**
-	ACD Tube	**4**	**1**	**1**
1	4.5 mL Sodium Citrate	**4.5**	**2**	**5**
2	10 mL Serum wrapped in foil	**10**	**3**	**4**
16	3 mL Serum wrapped in Foil	**3**	**4**	**9**
17	3.5 mL SST wrapped in foil	**7**	**5**	**7**
5	4 mL Sodium Heparin in Foil	**4**	**6**	**8**
14	4 ml EDTA	**4**	**7**	**10**
15	6 mL EDTA	**12**	**8**	**2**
12	2.5 mL RNA	**2.5**	**9**	**3**
20	2.5 mL DNA	**2.5**	**10**	**6**
Blood Draw Total:		**50**		

Tube #	Tube Type	≥ 52 pounds	Prioritization		≥ 52 pounds	Prioritization	
		Baseline	**Full Draw Priority**	**Reduced Draw Priority**	**Follow-up**	**Full Draw Priority**	**Reduced Draw Priority**
-	ACD Tube	**4**	**1**	**4**			
1	4.5 mL Sodium Citrate	**4.5**	**2**	**6**	**4.5**	**1**	**5**
2	10 mL Serum wrapped in foil	**10**	**3**	**14**	**10**	**2**	**9**
16	3 mL Serum wrapped in Foil						
3	10 mL SST	**10**	**4**	**3**	**10**	**3**	**3**
4	10 mL SST wrapped in foil				**10**	**4**	
17	3.5 mL SST wrapped in foil	**7**	**5**	**7**			
5	4 mL Sodium Heparin in Foil	**4**	**6**	**5**	**4**	**5**	**4**
6	10 mL EDTA	**10**	**7**	**1**	**10**	**6**	**1**
7	10 mL EDTA	**10**	**8**	**9**			
8	10 mL EDTA	**10**	**9**	**10**			
9	10 mL EDTA	**10**	**10**	**11**			
14	4 ml EDTA	**4**	**11**	**12**	**4**	**7**	**7**
15	6 mL EDTA	**12**	**12**	**13**	**6**	**8**	**8**
12	2.5 mL RNA	**2.5**	**13**	**2**	**2.5**	**9**	**2**
20	2.5 mL DNA	**2.5**	**14**	**8**	**2.5**	**10**	**6**
Blood Draw Total:		**100.5**			**63.5**		

24. Appendix K. Urine Collection Protocol

In an effort to best accommodate study participants 24-hour collections will be coordinated as necessary with local clinical needs. Determine if the treating physician is requesting a 24-hour urine collection.

If yes:

- Follow your local site's protocol 24-hour urine collection
- Request the study participant to bring their sample to the research visit first.
- Pour off the requested aliquot (see 24-hour MOP); write legibly on the specimen container the volume removed.
- Provide a weight if necessary

If no clinical 24-hour urine collection is necessary, provide the participant a printed copy of the instructions at the end of this appendix, along with the urine collection materials.

Per Protocol V2.5, effective 4/2010 study participants are to be compensated $50.00 for each successful 24-hour urine collection to offset the inconvenience of this necessary study procedure.

24.A. Baseline Urine Collections

The baseline urine collections are necessary components for the primary outcomes of the study. As the current "gold standard" for protein and creatinine measurements, it is essential that the collections are done properly. If for research purposes only, please review the urine collection materials at the end of this appendix with your study participant, providing specimen collection materials including large, unmarked gallon jug and cap, and either a urinal (males) or a urine hat (females and young pediatric participants). These items can be provided by the NEPTUNE Biorepository via the Kit Request Form (See 16. Appendix C).

24.A.1. 24-hour Urine Collection

The 24-hour urine collection is critical for the baseline visit. As the current "gold standard" for protein and creatinine measurements, it is essential that the collection is done properly. If for research purposes only, please review the urine collection materials at the end of this appendix with your study participant, providing specimen collection materials including large, unmarked gallon jug and cap, and either a urinal (males) or a urine hat (females and young pediatric participants). These items can be provided by the NEPTUNE Biorepository via the Kit Request Form (See 16. Appendix C).

24.A.2. Timed Urine Collection

In an effort to best accommodate study participants, timed urine collections and capture will be coordinated as necessary with local clinical needs for children below five years of age and other incontinent participants. Determine if the treating physician is requesting a timed urine collection for the study participant.

Please follow your local site's standard protocol for timed urine collection and urine capture.

The instructions on the last page can be modified to record the necessary details for both a timed urine collection or urine capture.

24.A.3. Baseline Random/Spot Clean Catch Urine Collection

A "clean catch" research urine sample will be collected from all participants. Approximately 60 cc of the "clean catch" urine will be processed and transferred to the NEPTUNE Biorepository.

When the study participant is able, during the course of the study visit, provide a specimen cup.

Using the local site's clean catch protocol, obtain a specimen for processing immediately following the study visit.

A "clean catch" sample may also be known locally as a "spot collection" or "fresh urine". Please be sure to instruct your participants on the necessary procedure for "clean catch" samples.

24.B. Biopsy Random/Spot Clean Catch Urine Collection

A "clean catch" research urine sample will be collected from all participants prior to biopsy. This sample may be obtained up to 7 days prior to biopsy procedure or 7 days post-biopsy. The preferred timing is day of biopsy, prior to procedure. "clean catch" urine samples cannot be obtained post-biopsy for 3 days.

Approximately 60 cc of the "clean catch" urine will be processed and transferred to the NEPTUNE Biorepository.

Using the local site's clean catch protocol, obtain a specimen for processing immediately following the study visit.

A "clean catch" sample may also be known locally as a "spot collection" or "fresh urine". Please be sure to instruct your participants on the necessary procedure for "clean catch" samples.

24.C. Follow-up Urine Collections

The 24-hour urine collection is critical at annual visits. When possible, please obtain a 24-hour urine at every visit, with a minimum collection annually. Determine if the 24-hour urine is necessary for clinical purposes or just research purposes and proceed as described for the baseline 24-hour urine collection. Timed collections are also acceptable as indicated above.

A "clean catch" research urine sample will be collected from all participants at all follow-up visits. Please review the above procedures for each urine type collection.

24.D. 24-Hour Urine Collection Instructions

Dear _____:

Instructions for your 24-hour urine collection

1. **You will be given a 24-hour urine collection bottle. It will need to be kept in the refrigerator or on ice.**

2. **Always start your 24-hour urine collection in the morning once you begin your collection of urine.**

3. **Do not save the first urine of the day, but mark this as your START TIME at the bottom of this sheet.**

4. **Save all urine for the next 24 hours in the collection bottle. In order to get correct results, it is important to save every urine sample. If you forget to save any urine, please report to your NEPTUNE Coordinator:**

   _____
   (please print your name)

   You will need to drink plenty of fluids during this time, 6-8 (8 oz.) glasses if possible.

5. **The last urine sample should be as close to the starting time as possible and must be saved.**

6. **Please remember to keep urine in collection bottle in refrigerator or on ice.**

Bring bottle(s) to: _____
(Study Visit Location)

Please complete the following information:

Patient Name:_____

Collection Start Date:_____ **Start Time:**_____**am/pm**

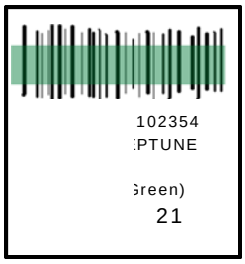
Collection Stop Date:_____ **Stop Time:**_____**am/pm**

Procedure Class:	SPECIMENS
Procedure:	**BIOPSY VISIT [V3]** BIOPSY TISSUE

Procedure Overview
This procedure describes the process for storing renal biopsy tissue for the NEPTUNE Biobank at -80° C. Shipping details are provided in 27. Appendix N Shipping.

Required Supplies:
Included in kit:

- 1 pre-labeled cryovial[1] containing 1.6 mL RNA-Later[2]



Provided by site:

- Renal tissue sample from study participant
- Gloves, goggles, and lab coat

Required Equipment:

- No equipment is necessary for this specimen

Responsible Individuals
The NEPTUNE Research Coordinator is responsible for retrieving samples from consented participants, returning sample to lab, and processing for storage. In the absence of the Research Coordinator, Site PI or designated individual would serve as alternates.

Only a nephrologist or other trained healthcare professional is able to do the renal biopsy.

Procedure
The green-labeled, green-capped cryovial containing RNA-Later MUST be present during the biopsy procedure. Specimen must be placed directly into RNA-later and not be immersed in other fixative solutions prior to storage.

1. Attending physician will procure the renal tissue core during procedure
2. In sterile fashion, the attending physician will procure one full core from specimens obtained
3. In sterile fashion, place the specimen in the green-labeled, green capped cryovial and place lid on, rotating cap to the right to close completely.
4. The specimen in RNA-Later solution is stable at room temperature until return to lab.

Shipping:
Biopsy specimens should be stored with the respective NEPTUNE Participant samples. If Baseline [V2] has not yet occurred, please take care in storing the single cryovial in a -80 freezer. Samples should be shipped with the respective kit.

All specimens should be shipped together **on dry ice** using the NEPTUNE shipping vendor and container provided by the NEPTUNE DACC/Biobank.

Documentation
All corresponding samples for each participant number should be stored together. Please note, effective 6/2012 the number of passes to obtain the research core must be documented in Kidney Specimens Worksheet 11A and documented in the corresponding CRF.

References
1. Cryovials: DOT Scientific Inc. No. T334-6SPR
2. See attached MSDS

Relevant Definitions
None

Applied Biosystems

Ambion®
THE RNA COMPANY®
An Applied Biosystems Business

MSDS PART NUMBER: 4381859 US
OHS PART NUMBER: 00232284
Revision number : A

MATERIAL SAFETY DATA SHEET

1. CHEMICAL PRODUCT AND COMPANY IDENTIFICATION

AMBION, INC
2130 WOODWARD ST.
AUSTIN, TX 78744
(512) 651-0200 (USA)
EMERGENCY CONTACT:
1-800-424-9300 (NORTH AMERICA)
WEB ADDRESS: www.ambion.com
MAIL: techserv@ambion.com

AMBION, INC
LTD HUNTINGDON
CAMBIDGESHIRE, UK PE29 6XY
+44 1480 373 020 (UK)
EMERGENCY CONTACT:
+1-703-527-3887, REVERSE CHARGES (INTERNATIONAL)
MAIL: eurotech@ambion.com

SUBSTANCE: RNAlater®

TRADE NAMES/SYNONYMS:
US MSDS P/N 4381859; P/N AM7020; P/N AM7021; P/N AM7022; P/N AM7023; P/N AM7024; P/N SAM7023; P/N 7020; P/N 7021; P/N 7022; P/N 7023; P/N 7024; P/N 7020G; P/N 7022G1; 00232284

PRODUCT USE: For Research Use Only. Not for use in diagnostic procedures.

CREATION DATE: Nov 13 2006
REVISION DATE: Jan 08 2007

2. COMPOSITION, INFORMATION ON INGREDIENTS

COMPONENT: TRADE SECRET 00232384
CAS NUMBER: Not assigned.
PERCENTAGE: 30-60

COMPONENT: TRADE SECRET 00232322
CAS NUMBER: Not assigned.
PERCENTAGE: <10

COMPONENT: SULFURIC ACID
CAS NUMBER: 7664-93-9

PERCENTAGE: <0.1

3. HAZARDS IDENTIFICATION

NFPA RATINGS (SCALE 0-4): HEALTH=2 FIRE=1 REACTIVITY=0

EMERGENCY OVERVIEW:
PHYSICAL FORM: solution
MAJOR HEALTH HAZARDS: respiratory tract irritation, skin irritation, eye irritation

POTENTIAL HEALTH EFFECTS:
INHALATION:
SHORT TERM EXPOSURE: irritation, cough, sore throat, difficulty breathing, lung congestion
LONG TERM EXPOSURE: irritation
SKIN CONTACT:
SHORT TERM EXPOSURE: irritation
LONG TERM EXPOSURE: irritation
EYE CONTACT:
SHORT TERM EXPOSURE: irritation
LONG TERM EXPOSURE: irritation
INGESTION:
SHORT TERM EXPOSURE: irritation, sore throat, nausea, vomiting, diarrhea, stomach pain, convulsions
LONG TERM EXPOSURE: no information on significant adverse effects

4. FIRST AID MEASURES

INHALATION: If adverse effects occur, remove to uncontaminated area. Give artificial respiration if not breathing. Get immediate medical attention.

SKIN CONTACT: Wash skin with soap and water for at least 15 minutes while removing contaminated clothing and shoes. Get medical attention, if needed. Thoroughly clean and dry contaminated clothing and shoes before reuse.

EYE CONTACT: Flush eyes with plenty of water for at least 15 minutes. Then get immediate medical attention.

INGESTION: If a large amount is swallowed, get medical attention.

5. FIRE FIGHTING MEASURES

FIRE AND EXPLOSION HAZARDS: Slight fire hazard.

EXTINGUISHING MEDIA: carbon dioxide, regular dry chemical, regular foam, water

FIRE FIGHTING: Move container from fire area if it can be done without risk. Avoid inhalation of material or combustion by-products. Stay upwind and keep out of low areas.

FLASH POINT: No data available.

HAZARDOUS COMBUSTION PRODUCTS:
Thermal decomposition products or combustion: oxides of carbon, oxides of nitrogen

6. ACCIDENTAL RELEASE MEASURES

WATER RELEASE:
Subject to California Safe Drinking Water and Toxic Enforcement Act of 1986 (Proposition 65). Keep out of water supplies and sewers.

OCCUPATIONAL RELEASE:
Stop leak if possible without personal risk. Small spills: Absorb with sand or other non-combustible material. Collect spilled material in appropriate container for disposal. Notify Local Emergency Planning Committee and State Emergency Response Commission for release greater than or equal to RQ (U.S. SARA Section 304). If release occurs in the U.S. and is reportable under CERCLA Section 103, notify the National Response Center at (800)424-8802 (USA) or (202)426-2675 (USA).

7. HANDLING AND STORAGE

STORAGE: Store and handle in accordance with all current regulations and standards. See original container for storage recommendations. Keep separated from incompatible substances.

8. EXPOSURE CONTROLS, PERSONAL PROTECTION

EXPOSURE LIMITS:
RNAlater®:
No occupational exposure limits established.

VENTILATION: Provide local exhaust ventilation system. Ensure compliance with applicable exposure limits.

EYE PROTECTION: Wear splash resistant safety goggles with a faceshield. Provide an emergency eye wash fountain and quick drench shower in the immediate work area.

CLOTHING: Wear appropriate chemical resistant clothing.

GLOVES: Wear appropriate chemical resistant gloves.

RESPIRATOR: Under conditions of frequent use or heavy exposure, respiratory protection may be needed. Respiratory protection is ranked in order from minimum to maximum. Consider warning properties before use.
Any chemical cartridge respirator with organic vapor cartridge(s).
Any chemical cartridge respirator with a full facepiece and organic vapor cartridge(s).
Any air-purifying respirator with a full facepiece and an organic vapor canister.
For Unknown Concentrations or Immediately Dangerous to Life or Health -
Any supplied-air respirator with full facepiece and operated in a pressure-demand or other positive-pressure mode in combination with a separate escape supply.
Any self-contained breathing apparatus with a full facepiece.

9. PHYSICAL AND CHEMICAL PROPERTIES

PHYSICAL STATE: liquid
APPEARANCE: clear
PHYSICAL FORM: solution
ODOR: Not available
BOILING POINT: Not available
FREEZING POINT: Not available
VAPOR PRESSURE: Not available
VAPOR DENSITY: Not available
SPECIFIC GRAVITY: Not available
WATER SOLUBILITY: miscible
PH: 4.5
VOLATILITY: Not available
ODOR THRESHOLD: Not available
EVAPORATION RATE: Not available
COEFFICIENT OF WATER/OIL DISTRIBUTION: Not available

10. STABILITY AND REACTIVITY

REACTIVITY: Stable at normal temperatures and pressure.

CONDITIONS TO AVOID: Avoid heat, flames, sparks and other sources of ignition.
Avoid contact with incompatible materials.

INCOMPATIBILITIES: acids, bases, chlorates, chlorine, copper alloys, copper, hypochlorite, nitrates, nitrite salts, oxidizing materials, potassium compounds, zinc

HAZARDOUS DECOMPOSITION:
Thermal decomposition products or combustion: oxides of carbon, oxides of nitrogen

POLYMERIZATION: Will not polymerize.

11. TOXICOLOGICAL INFORMATION

TRADE SECRET 00232384:
IRRITATION DATA: 50 percent/5 day(s)-intermittent skin-mammal
TOXICITY DATA: 2840 mg/kg oral-rat LD50
LOCAL EFFECTS:
Irritant: inhalation, skin, eye
ACUTE TOXICITY LEVEL:
Moderately Toxic: ingestion
MEDICAL CONDITIONS AGGRAVATED BY EXPOSURE: eye disorders, immune system disorders or allergies, respiratory disorders, skin disorders and allergies

TRADE SECRET 00232322:
MEDICAL CONDITIONS AGGRAVATED BY EXPOSURE: high blood pressure, kidney disorders

Additional toxicological data is available on the component(s) of this product. Please call 650-638-5635 or contact MSDS_Inquiry_CCRM@appliedbiosystems.com for more information.

12. ECOLOGICAL INFORMATION

Not available

13. DISPOSAL CONSIDERATIONS

Dispose in accordance with all applicable regulations.

14. TRANSPORT INFORMATION

U.S. DEPARTMENT OF TRANSPORTATION: No classification assigned.

CANADIAN TRANSPORTATION OF DANGEROUS GOODS: No classification assigned.

LAND TRANSPORT ADR: No classification assigned.

LAND TRANSPORT RID: No classification assigned.

AIR TRANSPORT IATA: No classification assigned.

AIR TRANSPORT ICAO: No classification assigned.

MARITIME TRANSPORT IMDG: No classification assigned.

15. REGULATORY INFORMATION

U.S. REGULATIONS:
SARA TITLE III SECTION 302 EXTREMELY HAZARDOUS SUBSTANCES (40 CFR 355.30):
Not regulated.

SARA TITLE III SECTION 304 EXTREMELY HAZARDOUS SUBSTANCES (40 CFR 355.40):
Not regulated.

SARA TITLE III SARA SECTIONS 311/312 HAZARDOUS CATEGORIES (40 CFR 370.21):
ACUTE: Yes
CHRONIC: No
FIRE: No
REACTIVE: No
SUDDEN RELEASE: No

SARA TITLE III SECTION 313 (40 CFR 372.65): Not regulated.

OSHA PROCESS SAFETY (29CFR1910.119): Not regulated.

STATE REGULATIONS:
California Proposition 65:
Known to the state of California to cause the following:
STRONG INORGANIC ACID MISTS CONTAINING SULFURIC ACID
Cancer (Mar 14, 2003)

CANADIAN REGULATIONS:

WHMIS CLASSIFICATION: This product has been classified in accordance with the hazard criteria of the CPR and the MSDS contains all of the information required by the CPR.

NATIONAL INVENTORY STATUS:

U.S. INVENTORY (TSCA): All the components of this substance are listed on or are exempt from the inventory. For purposes of 40 CFR 720.36, this product is for Research and Development (R&D) Use Only.

TSCA 12(b) EXPORT NOTIFICATION: Not listed.

16. OTHER INFORMATION

23.B. BIOPSY VISIT [V3] BLOOD PROCUREMENT AND PROCESSING PROCEDURES Pediatric and Adult Participants

Procedure Overview
This procedure describes the process for collecting and preparing blood samples obtained at the **[V3] Biopsy Visit** for NEPTUNE storage at -80° C. Shipping details are provided in Appendix N of the NEPTUNE Manual of Procedures.

Requires Supplies:
Included in kit:

- 1 x 3.0 mL SST vacutainer[1] (Tube 19)
- 1 x 4.0 mL EDTA vacutainer[2] (Tube 14)
- 1 x RNA PAXgene vacutainer[3] (Tube 12)
- 3 pre-labeled cryovials[4]:
  - 2 red-capped cryovials to store serum from Tube 14
    Pre-labeled **B-E Blood EDTA (Red 1 and 2)**
  - 1 grey-capped cryovial to store serum from Tube 19
    Pre-labeled **B-S Blood SST (Gray**

Provided by site:

- Blood sample from study participant
- Aerosol spray covers for centrifuge buckets
- Pipettors and tips or disposable pipettes (1 ml or 200 µl sizes) or plastic transfer pipettes
- Gloves, goggles, and lab coat

Required Equipment:

- Centrifuge capable of achieving 2000 x g (NOTE: G is not the same as RPM, please consult your centrifuge conversion table if necessary)
- University of Michigan OSEH[5] guidelines designate all work done with biological specimens that could produce spray from pipetting is done in a hood.

Responsible Individuals
The NEPTUNE Research Coordinator is responsible for retrieving blood samples from consented participants, returning sample to lab, and processing for storage. In the absence of the Research Coordinator, the Site PI or designated alternate would provide this service.

Procedure
When possible, blood should be drawn from the participant up to 7 days prior to the biopsy procedure. V3 Biopsy Visit blood may be obtained same day prior or same day post-procedure.

Note the date and start time of the blood draw on V3 Biopsy Specimens Worksheet.

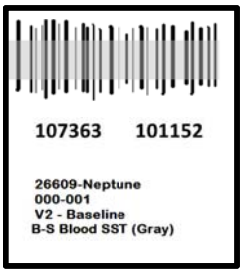
In accordance with the site's protocol for venipuncture, *draw blood tubes in order as follows*: 1 (one) 3.0 mL SST, 1 (one) 4.0 mL EDTA, and 1 (one) RNA PAXgene blood vacutainer from all participants, adult and pediatric.
Following the blood draw the Research Coordinator will return to the lab with the samples.

BLOOD PROCESSING FOR SST (RED/BLACK TIGER TOP) TUBE 19

Procedure Overview
This procedure describes the process for preparing blood samples obtained at the **[V3] Biopsy Visit** in SST blood tubes for NEPTUNE storage at -80° C. Shipping details are provided in Appendix N of the NEPTUNE Manual of Procedures.

Required Supplies:
Included in kit:

- 1 x 3.0 mL SST blood vacutainer[1] (tube 19)
- 1 labeled cryovial[4] (**GRAY CAPS/GRAY LABELS**)
  (Pre-labeled **B-S Blood SST (Gray 3**)



Procedure
When possible, blood should be drawn from the participant up to 7 days prior to the biopsy procedure. V3 Biopsy Visit blood may be obtained same day prior or same day post-procedure.

Note the date and start time of the blood draw on V3 Biopsy Specimens Worksheet.

Sample processing:

1. Following the 30-minute incubation period, balance SST blood tubes per your site's centrifuge protocol and handling policy.
2. Note the time on the V3 Biospecimens worksheet (this should be the time the samples start to spin in the centrifuge).
3. Centrifuge SST tubes at 2000 x g for 12 minutes.

Tube 19

4. After spinning, remove tube from centrifuge keeping tubes upright so as not to disturb the serum*** component.
5. Pipette the serum layer from the SST tube (tube 19) into one approximate 1.6 mL aliquot. This amount can be determined by referencing Figure 1 from 26.A Appendix M Adult Blood Baseline V2. Use the cryovial labeled:

B-S Blood SST (Gray 3

6. Freeze aliquots at -80° C prior to shipping.
7. Discard remaining RBC's per site's OSEH guidelines. X

*** Serum is the top layer of the blood specimen after the centrifugation step. It should be a straw-colored yellow, semi-clear substance. Serum is not the same as plasma.

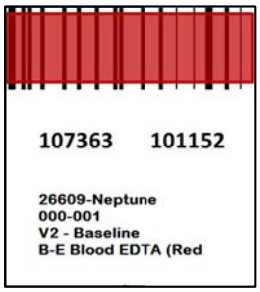
Procedure Class:	SPECIMENS
Procedure:	**BIOPSY VISIT [V3]**BIO-Blood

BLOOD PROCESSING FOR EDTA (PURPLE TOP) TUBE 14

Procedure Overview
This procedure describes the process for preparing blood samples obtained at the **[V3] Biopsy Visit** in EDTA blood tubes for NEPTUNE storage at -80° C. Shipping details are provided at the end of the blood processing MOP.

Required Supplies:
Included in kit:

- 1 x 4.0 mL EDTA blood vacutainer[2] tube (tubes 14)
- 2 labeled cryovials[4] (**RED CAPS/RED LABELS**)
  (Pre-labeled **B-E Blood EDTA (Red 1** and **B-E Blood EDTA (Red 2**)



Procedure
When possible, blood should be drawn from the participant up to 7 days prior to the biopsy procedure. V3 Biopsy Visit blood may be obtained same day prior or same day post-procedure.

Note the date and start time of the blood draw on V3 Biopsy Specimens Worksheet.

Sample processing:

1. Balance EDTA blood tube per site's centrifuge protocol and handling policy.
2. Note the time on the V3 Biospecimens worksheet (this should be the time the samples start to spin in the centrifuge).
3. Centrifuge EDTA tube at 2000 x g for 12 minutes.
4. Remove tubes from centrifuge keeping tubes upright so as not to disturb the plasma* component.
5. After spinning, pipette the plasma layer from the EDTA tube into two (2) approximate 1.6 mL aliquots. This amount can be determined by referencing Figure 1. Use the two cryovials labeled:

   B-E Blood EDTA (Red 1 and **B-E Blood EDTA (Red 2**
6. Freeze aliquots at -80° C prior to shipping.
7. Discard remaining RBC's per site's OSEH guidelines.

* Plasma is the top layer of the blood specimen after the centrifugation step. It should be a straw-colored yellow, semi-clear substance.

Procedure Class:	SPECIMENS
Procedure:	**BIOPSY VISIT [V3]**BIO-Blood

BLOOD PROCESSING FOR RNA PAXGENE (RED/CLEAR TOP) TUBE 12

Procedure Overview
This procedure describes the process for preparing blood samples obtained at the **[V3] Biopsy Visit** in RNA PAXgene blood tube for NEPTUNE storage at -80° C. Shipping details are provided at the end of the blood processing MOP.

Required Supplies:
Included in kit:

- 1 pre-labeled PAXgene RNA Blood Vacutainer^3 (**WHITE LABEL 'D RNA Paxgene (Whit'**) (Tube 12)

Required Equipment:

- No equipment is necessary for processing this specimen

Procedure
When possible, blood should be drawn from the participant up to 7 days prior to the biopsy procedure. V3 Biopsy Visit blood may be obtained same day prior or same day post-procedure.

Note the date and start time of the blood draw on V3 Biopsy Specimens Worksheet.

1. RNA blood vacutainer is marked with a **WHITE LABEL 'D RNA Paxgene (Whit'**

2. Blood draw:
   Summary:
   a. The PAXgene blood RNA vacutainer should be at room temperature prior to use.
   b. Fill the PAXgene RNA tube *after* all other tubes have been drawn.
   c. Collect blood into the PAXgene tube using your institution's recommended standard procedure for venipuncture.

   Blood Collection:
   a. Hold PAXgene tube vertically during blood draw, below point of venipuncture.
   b. Allow at least 10 seconds for complete blood draw; ensuring blood has stopped flowing into tube before removing from vacutainer holder.
   c. After blood collection gently invert tube 8-10 times.
   d. Store tube upright at room temperature for 2 hours.
   e. Following 2 hours room temperature incubation period, place in -80° C freezer upright.

Documentation
If PAXgene RNA tubes are left on shelf (room temp) for greater than 2 hour incubation period, please indicate additional elapsing time on the worksheet and case report forms. Additionally, if PAXgene RNA tubes stabilize at room temp for less than 2 hours, please indicate total time on worksheet and case report forms.

Complete the appropriate Biospecimen CRF corresponding to the study visit.

References

1. 3.5 mL SST Vacutainer: BD, Fisher Scientific ref No. 367981
2. 4.0 mL EDTA Vacutainer: BD, Fisher Scientific ref No. 367861
3. PreAnalytiX PAXgene Blood RNA Tube; 2.5 mL Vacutainer: ref. No. 762165 http://www.preanalytix.com/product-catalog/blood/rna/products/paxgene-blood-rna-tube/
4. Cryovials: DOT Scientific Inc. No. T334-6SPR
5. Follow local OSEH Guidelines
6. See attached MSDS for relevant safety precautions and hazards.

Centrifugation steps were performed in a SORVALL Legend T with radius 15.9 cm. If alternate centrifuges are used, the following equation may be used to calculate appropriate RPM necessary to generate the G-force indicated in above protocol:

$$RCF = 11.18 * \left(\frac{n}{1000}\right)^2 * r$$

RCF = Relative Centrifugal Force (G); a dimensionless number allowing comparison of separation efficiency

r = radius of centrifugation in cm

n = speed in rpm

Procedure Class:	SPECIMENS
Procedure:	**BIOPSY VISIT [V3]**BIO-Blood

Biopsy [V3] Blood Draw for Pediatric and Adult Participants

Tube #	Tube Type	Step 1	Step 2	Step 3	Step 4	Step 5
19	3.0 mL SST	Allow to clot for 30 minutes.	Centrifuge for 12 minutes at 2000 x G	Transfer 1.6 mL of serum into 1 cryovial.	Store aliquots at -80°C	Ship to NEPTUNE Biobank as indicated by site schedule
14	4 mL EDTA	Process within 30 minutes.	Centrifuge for 12 minutes at 2000 x G	Transfer 1.6 mL of serum into 2 cryovials.	Store aliquots at -80°C	Ship to NEPTUNE Biobank as indicated by site schedule
12	2.5 mL RNA	Must sit at room temp for 2 hours	Freeze at -80		Store pre-labeled vacutainer at -80°C	Ship to NEPTUNE Biobank as indicated by site schedule

(Draw tubes in order as they appear in chart)

Procedure Class:	SPECIMENS
Procedure:	BIO-Spot Urine [V3: Biopsy] (SU, AS, AQ and AP)

Procedure Overview
This procedure describes the process for preparing one whole urine sample type (U), two urine supernatant sample types (S, Q) and 2 urine pellet sample types (AP-E and AP-Q) from a spot (random) urine sample for NEPTUNE storage at -80° C. Shipping details are provided in Appendix N of the NEPTUNE Manual of Procedures.

Spot urine is collected in the same fashion for both pediatric and adult study participants. Spot urine samples are obtained at each study visit, including the baseline [V2] and the Biopsy [V3] visits, and all follow-up visits.

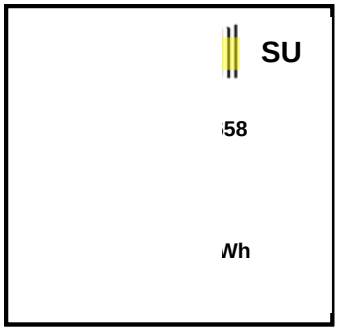
At [V3], the spot urine sample should be obtained PRIOR to the biopsy procedure. Please record this in the [V3] Biospecimen form.

Required Supplies:
Included in kit:

- 2 x 15.0 mL centrifuge tubes[1] empty (marked **"EMPTY"**)
- 2 x 15.0 mL centrifuge tubes containing 15 μL of Protease Inhibitor[2] (marked **"PI"**)
- 16 x 1.8 mL cryovials[3] with yellow caps
  (Pre-labeled as follows)

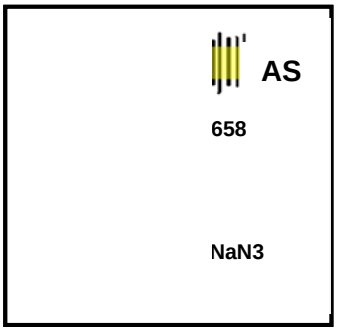
Sample 1:
SU = Whole, Unprocessed Urine

4 pre-labeled, empty cryovials
U-Spot Urine Spot Wh



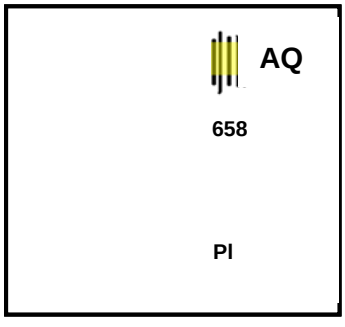
Sample 2:
AS = Processed & NaAzide

4 pre-labeled cryovials containing Sodium Azide[4]
AS Urine Spot SN NaN3



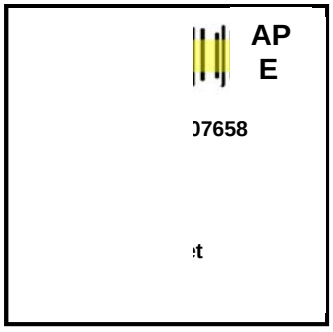
Sample 3:
AQ = Processed & Protease Inhibitor (PI)

4 pre-labeled cryovials for Protease Inhibitor
AQ Urine Spot SN PI



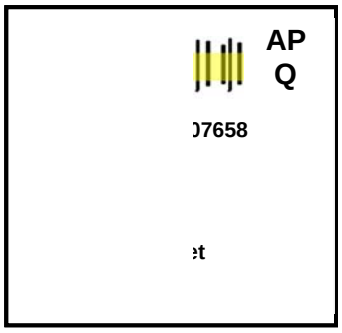
Sample 4:
AP-E = Urine Pellet from 'Empty' centrifuge tubes

2 pre-labeled, empty cryovials
AP-E Urine Pellet



Sample 5:
AP-Q = Urine Pellet from 'PI' containing centrifuge tubes

2 pre-labeled, empty cryovials
AP-Q Urine Pellet



- RNA-Later[5] (expected use per sample: 50-100 μL)

Provided by site:
- Spot Urine sample from study participant
- Pipettes and tips or disposable pipettes (10 ml, 5 ml, 1 ml sizes) or plastic Transfer pipettes
- Gloves, goggles, and lab coat

Required Equipment:
- Centrifuge capable of achieving 2000 x G
- University of Michigan OSEH guidelines designate all work done with biological specimens that could produce spray from pipetting be done in a hood. Please refer to your site specific OSEH policies.

Responsible Individuals
The NEPTUNE Research Coordinator is responsible for retrieving urine sample from consented participants, returning sample to lab, and processing for storage. In the absence of the Research Coordinator, the Site PI or designated, NEPTUNE trained alternate would provide this service.

Procedure:
1. Midstream urine is recovered in sterile specimen container and stored on ice. A minimum of 32 mL of urine is necessary.

2. Note time, fasting state and 1st, 2nd, 3rd, etc urine of the day on study visit worksheet (if patient is able to provide this detail, otherwise, indicate "No recall").

If processing is not able to be completed immediately, please store on ice or refrigerate for a maximum of 4 hours.

Sample processing:

Sample 1

*Requires total spot urine sample of 60 mL minimum; if sample is 50 mL or less please note in worksheet and CRF and do NOT store spot urine for the U-Spot Samples: * NO WHOLE URINE SAMPLE**

3. Using a pipettor, transfer ***whole***, unprocessed urine into the cryovials labeled:

'SU' to the right of the barcode

4. Replace the cap, firmly twisting in a clockwise direction.

Samples 2 & 3

5. Pour the remaining urine into the 4 x 15.0 mL centrifuge tubes in equal 12 milliliter portions (two tubes of each marked "EMPTY" and "Protease Inhibitor (PI)")

   E.g.:

   30 mL of spot urine → 2 – 12.0 mL centrifuge tubes for spinning (Use 1 'Empty' and 1 'PI' tube)

   50 mL of urine → 4 – 12.0 mL centrifuge tube; discard remaining urine (Use 2 of each, 'Empty' and 'PI')

NOTE: Use at least ONE EACH: "EMPTY" and "PI" centrifuge tubes

6. In the tubes labeled "PI", gently invert 8-10 times to dissolve the PI completely. Remove the cap to allow any air bubbles to escape, replace cap tightly.
7. Spin all 4 tubes at 1000 X G for 12 minutes in centrifuge

Sample 2 (AS Urine Spot): From the tubes labeled "EMPTY", transfer urine using a transfer pipettor into the 4 cryovials containing a pre-measured amount of 100 mM Sodium Azide (a biocide) labeled:

'AS' to the right of the barcode

8. When opening each cryovial, take care to place the cap directly in front of the respective tube to retain pre-measured volumes for consistent concentrations in each aliquot.
9. Pipette 1.6 mLs of urine or enough urine to fill the cryovial up to the first ridge on the cryovial (see Figure 1).
10. Replace the cap, firmly twisting in a clockwise direction. After all caps are replaced, invert tubes *at least 2 times* to distribute the sodium azide prior to freezing.

Sample 3 (AQ Urine Spot): From the tubes labeled "PI", transfer urine using a transfer pipettor into the cryovials labeled:

'AQ' to the right of the barcode

11. Pipette 1.6 mLs of urine or enough urine to fill the cryovial up to the first ridge on the cryovial (see Figure 1).

Samples 4 and 5

12. Pour off remaining supernatant from all of the 15.0 mL centrifuge tubes
13. For maximal drainage, gently invert centrifuge tubes on clean paper towels for 10 seconds, take care to not disturb pellet in the centrifuge tube tip.
14. With a clean pipette tip, transfer 25.0-50.0* μL RNA-Later into each centrifuge tube, take care to not touch the inside walls of the centrifuge tube.
15. Gently stir the mixture with the pipette tip, changing tips when moving between the 'Empty' tubes and the 'PI' tubes. **Do not pipette up and down to mix as this could break any cells present in the pellet.**

    Sample 4 (AP-E Urine Pellet): From the centrifuge tubes labeled "EMPTY", pipette one urine pellet from each centrifuge tube into the two cryovials labeled:

    'AP-E' to the right of the barcode

    Sample 5 (AP-Q Urine Pellet): From the centrifuge tubes labeled "PI", pipette one urine pellet from each centrifuge tube into the two cryovials labeled:

    'AP-Q' to the right of the barcode

16. Freeze all samples at -80 C.
17. Residual urine may be disposed per OSEH guidelines enforced at participating institution.

* Amount of RNA-Later will vary depending on size of pellet. RNA-Later should be added to completely submerse the pellet.

Shipping
Samples should be shipped according to the site-specific scheduled interval for shipments.

All specimens should be shipped together **on dry ice** using the shipping instructions found in Appendix N and in the study specific container provided by NEPTUNE.

Documentation
All corresponding samples for each participant ID should be stored together. If specimen does not adequately fill pre-determined number of aliquots, please document to minimize concerns regarding lost aliquots.

Complete the appropriate Biospecimen CRF corresponding to the study visit.

Procedure Class:	SPECIMENS
Procedure:	BIO-Spot Urine [V3: Biopsy] (SU, AS, AQ and AP)

References

1. 15.0 mL orange-capped Corning centrifuge tubes: Fisher Scientific No. 05-538-53D, Corning No. 430052
2. Protease Inhibitor Cocktail: Sigma-Aldrich Catalog: P1860 – 1 mL
3. Cryovials: DOT Scientific Inc. No.: T334-6SPR
4. Sodium Azide (see attached MSDS)
5. Ambion RNA-Later Solution; P/N: Am7021
6. University of Michigan Occupations Safety and Environmental Health (OSEH) guidelines indicate washing urine and its debris down laboratory sink with adequate water.

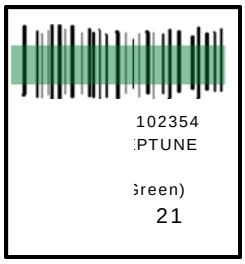
Relevant Definitions: NaN_3: Sodium Azide

Procedure Class:	SPECIMENS
Procedure:	**BIOPSY VISIT [V3]** BIOPSY TISSUE

Procedure Overview
This procedure describes the process for storing renal biopsy tissue for the NEPTUNE Biobank at -80° C. Shipping details are provided in 27. Appendix N Shipping.

Required Supplies:
Included in kit:

- 1 pre-labeled cryovial[1] containing 1.6 mL RNA-Later[2]



Provided by site:

- Renal tissue sample from study participant
- Gloves, goggles, and lab coat

Required Equipment:

- No equipment is necessary for this specimen

Responsible Individuals
The NEPTUNE Research Coordinator is responsible for retrieving samples from consented participants, returning sample to lab, and processing for storage. In the absence of the Research Coordinator, Site PI or designated individual would serve as alternates.

Only a nephrologist or other trained healthcare professional is able to do the renal biopsy.

Procedure
The green-labeled, green-capped cryovial containing RNA-Later MUST be present during the biopsy procedure. Specimen must be placed directly into RNA-later and not be immersed in other fixative solutions prior to storage.

1. Attending physician will procure the renal tissue core during procedure
2. In sterile fashion, the attending physician will procure one full core from specimens obtained
3. In sterile fashion, place the specimen in the green-labeled, green capped cryovial and place lid on, rotating cap to the right to close completely.
4. The specimen in RNA-Later solution is stable at room temperature until return to lab.

Shipping:
Biopsy specimens should be stored with the respective NEPTUNE Participant samples. If Baseline [V2] has not yet occurred, please take care in storing the single cryovial in a -80 freezer. Samples should be shipped with the respective kit.

All specimens should be shipped together **on dry ice** using the NEPTUNE shipping vendor and container provided by the NEPTUNE DACC/Biobank.

Documentation
All corresponding samples for each participant number should be stored together. Please note, effective 6/2012 the number of passes to obtain the research core must be documented in Kidney Specimens Worksheet 11A and documented in the corresponding CRF.

References
1. Cryovials: DOT Scientific Inc. No. T334-6SPR
2. See attached MSDS

Relevant Definitions
None

BIOPSY VISIT [V3] BLOOD PROCUREMENT AND PROCESSING PROCEDURES
Pediatric and Adult Participants

Procedure Overview
This procedure describes the process for collecting and preparing blood samples obtained at the **[V3] Biopsy Visit** for NEPTUNE storage at -80° C. Shipping details are provided in Appendix N of the NEPTUNE Manual of Procedures.

Requires Supplies:
Included in kit:

- 1 x 3.0 mL SST vacutainer[1] (Tube 19)
- 1 x 4.0 mL EDTA vacutainer[2] (Tube 14)
- 1 x RNA PAXgene vacutainer[3] (Tube 12)
- 3 pre-labeled cryovials[4]:
  - 2 red-capped cryovials to store serum from Tube 14
    Pre-labeled **B-E Blood EDTA (Red 1 and 2)**
  - 1 grey-capped cryovial to store serum from Tube 19
    Pre-labeled **B-S Blood SST (Gray**

Provided by site:

- Blood sample from study participant
- Aerosol spray covers for centrifuge buckets
- Pipettors and tips or disposable pipettes (1 ml or 200 µl sizes) or plastic transfer pipettes
- Gloves, goggles, and lab coat

Required Equipment:

- Centrifuge capable of achieving 2000 x g (NOTE: G is not the same as RPM, please consult your centrifuge conversion table if necessary)
- University of Michigan OSEH[5] guidelines designate all work done with biological specimens that could produce spray from pipetting is done in a hood.

Responsible Individuals
The NEPTUNE Research Coordinator is responsible for retrieving blood samples from consented participants, returning sample to lab, and processing for storage. In the absence of the Research Coordinator, the Site PI or designated alternate would provide this service.

Procedure
When possible, blood should be drawn from the participant up to 7 days prior to the biopsy procedure. V3 Biopsy Visit blood may be obtained same day prior or same day post-procedure.

Note the date and start time of the blood draw on V3 Biopsy Specimens Worksheet.

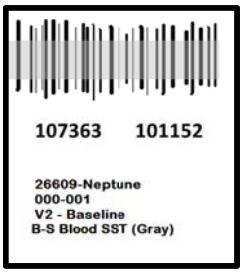
In accordance with the site's protocol for venipuncture, *draw blood tubes in order as follows*: 1 (one) 3.0 mL SST, 1 (one) 4.0 mL EDTA, and 1 (one) RNA PAXgene blood vacutainer from all participants, adult and pediatric.
Following the blood draw the Research Coordinator will return to the lab with the samples.

BLOOD PROCESSING FOR SST (RED/BLACK TIGER TOP) TUBE 19

Procedure Overview
This procedure describes the process for preparing blood samples obtained at the **[V3] Biopsy Visit** in SST blood tubes for NEPTUNE storage at -80° C. Shipping details are provided in Appendix N of the NEPTUNE Manual of Procedures.

Required Supplies:
Included in kit:

- 1 x 3.0 mL SST blood vacutainer[1] (tube 19)
- 1 labeled cryovial[4] (**GRAY CAPS/GRAY LABELS**)
  (Pre-labeled **B-S Blood SST (Gray 3**)



Procedure
When possible, blood should be drawn from the participant up to 7 days prior to the biopsy procedure. V3 Biopsy Visit blood may be obtained same day prior or same day post-procedure.

Note the date and start time of the blood draw on V3 Biopsy Specimens Worksheet.

Sample processing:

1. Following the 30-minute incubation period, balance SST blood tubes per your site's centrifuge protocol and handling policy.
2. Note the time on the V3 Biospecimens worksheet (this should be the time the samples start to spin in the centrifuge).
3. Centrifuge SST tubes at 2000 x g for 12 minutes.

Tube 19

4. After spinning, remove tube from centrifuge keeping tubes upright so as not to disturb the serum*** component.
5. Pipette the serum layer from the SST tube (tube 19) into one approximate 1.6 mL aliquot. This amount can be determined by referencing Figure 1 from 26.A Appendix M Adult Blood Baseline V2. Use the cryovial labeled:

B-S Blood SST (Gray 3

6. Freeze aliquots at -80° C prior to shipping.
7. Discard remaining RBC's per site's OSEH guidelines. X

*** Serum is the top layer of the blood specimen after the centrifugation step. It should be a straw-colored yellow, semi-clear substance. Serum is not the same as plasma.

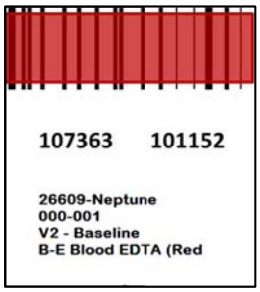
Procedure Class:	SPECIMENS
Procedure:	**BIOPSY VISIT [V3]**BIO-Blood

BLOOD PROCESSING FOR EDTA (PURPLE TOP) TUBE 14

Procedure Overview
This procedure describes the process for preparing blood samples obtained at the **[V3] Biopsy Visit** in EDTA blood tubes for NEPTUNE storage at -80° C. Shipping details are provided at the end of the blood processing MOP.

Required Supplies:
Included in kit:

- 1 x 4.0 mL EDTA blood vacutainer[2] tube (tubes 14)
- 2 labeled cryovials[4] (**RED CAPS/RED LABELS**)
  (Pre-labeled **B-E Blood EDTA (Red 1** and **B-E Blood EDTA (Red 2**)



Procedure
When possible, blood should be drawn from the participant up to 7 days prior to the biopsy procedure. V3 Biopsy Visit blood may be obtained same day prior or same day post-procedure.

Note the date and start time of the blood draw on V3 Biopsy Specimens Worksheet.

Sample processing:

1. Balance EDTA blood tube per site's centrifuge protocol and handling policy.
2. Note the time on the V3 Biospecimens worksheet (this should be the time the samples start to spin in the centrifuge).
3. Centrifuge EDTA tube at 2000 x g for 12 minutes.
4. Remove tubes from centrifuge keeping tubes upright so as not to disturb the plasma* component.
5. After spinning, pipette the plasma layer from the EDTA tube into two (2) approximate 1.6 mL aliquots. This amount can be determined by referencing Figure 1. Use the two cryovials labeled:

   B-E Blood EDTA (Red 1 and **B-E Blood EDTA (Red 2**
6. Freeze aliquots at -80° C prior to shipping.
7. Discard remaining RBC's per site's OSEH guidelines.

* Plasma is the top layer of the blood specimen after the centrifugation step. It should be a straw-colored yellow, semi-clear substance.

Procedure Class:	SPECIMENS
Procedure:	**BIOPSY VISIT [V3]**BIO-Blood

BLOOD PROCESSING FOR RNA PAXGENE (RED/CLEAR TOP) TUBE 12

Procedure Overview
This procedure describes the process for preparing blood samples obtained at the **[V3] Biopsy Visit** in RNA PAXgene blood tube for NEPTUNE storage at -80° C. Shipping details are provided at the end of the blood processing MOP.

Required Supplies:
Included in kit:

- 1 pre-labeled PAXgene RNA Blood Vacutainer[3] (**WHITE LABEL 'D RNA Paxgene (Whit'**) (Tube 12)

Required Equipment:

- No equipment is necessary for processing this specimen

Procedure
When possible, blood should be drawn from the participant up to 7 days prior to the biopsy procedure. V3 Biopsy Visit blood may be obtained same day prior or same day post-procedure.

Note the date and start time of the blood draw on V3 Biopsy Specimens Worksheet.

1. RNA blood vacutainer is marked with a **WHITE LABEL 'D RNA Paxgene (Whit'**

2. Blood draw:
   Summary:
   a. The PAXgene blood RNA vacutainer should be at room temperature prior to use.
   b. Fill the PAXgene RNA tube *after* all other tubes have been drawn.
   c. Collect blood into the PAXgene tube using your institution's recommended standard procedure for venipuncture.

   Blood Collection:
   a. Hold PAXgene tube vertically during blood draw, below point of venipuncture.
   b. Allow at least 10 seconds for complete blood draw; ensuring blood has stopped flowing into tube before removing from vacutainer holder.
   c. After blood collection gently invert tube 8-10 times.
   d. Store tube upright at room temperature for 2 hours.
   e. Following 2 hours room temperature incubation period, place in -80° C freezer upright.

Documentation
If PAXgene RNA tubes are left on shelf (room temp) for greater than 2 hour incubation period, please indicate additional elapsing time on the worksheet and case report forms. Additionally, if PAXgene RNA tubes stabilize at room temp for less than 2 hours, please indicate total time on worksheet and case report forms.

Complete the appropriate Biospecimen CRF corresponding to the study visit.

Procedure Class:	SPECIMENS
Procedure:	**BIOPSY VISIT [V3]**BIO-Blood

References

1. 3.5 mL SST Vacutainer: BD, Fisher Scientific ref No. 367981
2. 4.0 mL EDTA Vacutainer: BD, Fisher Scientific ref No. 367861
3. PreAnalytiX PAXgene Blood RNA Tube; 2.5 mL Vacutainer: ref. No. 762165 http://www.preanalytix.com/product-catalog/blood/rna/products/paxgene-blood-rna-tube/
4. Cryovials: DOT Scientific Inc. No. T334-6SPR
5. Follow local OSEH Guidelines
6. See attached MSDS for relevant safety precautions and hazards.

Centrifugation steps were performed in a SORVALL Legend T with radius 15.9 cm. If alternate centrifuges are used, the following equation may be used to calculate appropriate RPM necessary to generate the G-force indicated in above protocol:

$$RCF = 11.18 * \left(\frac{n}{1000}\right)^2 * r$$

RCF = Relative Centrifugal Force (G); a dimensionless number allowing comparison of separation efficiency

r = radius of centrifugation in cm

n = speed in rpm

Procedure Class:	SPECIMENS
Procedure:	**BIOPSY VISIT [V3]**BIO-Blood

Biopsy [V3] Blood Draw for Pediatric and Adult Participants

Tube #	Tube Type	Step 1	Step 2	Step 3	Step 4	Step 5
19	3.0 mL SST	Allow to clot for 30 minutes.	Centrifuge for 12 minutes at 2000 x G	Transfer 1.6 mL of serum into 1 cryovial.	Store aliquots at -80°C	Ship to NEPTUNE Biobank as indicated by site schedule
14	4 mL EDTA	Process within 30 minutes.	Centrifuge for 12 minutes at 2000 x G	Transfer 1.6 mL of serum into 2 cryovials.	Store aliquots at -80°C	Ship to NEPTUNE Biobank as indicated by site schedule
12	2.5 mL RNA	Must sit at room temp for 2 hours	Freeze at -80		Store pre-labeled vacutainer at -80°C	Ship to NEPTUNE Biobank as indicated by site schedule

(Draw tubes in order as they appear in chart)

Procedure Class:	SPECIMENS
Procedure:	BIO-Spot Urine [V3: Biopsy] (SU, AS, AQ and AP)

Procedure Overview
This procedure describes the process for preparing one whole urine sample type (U), two urine supernatant sample types (S, Q) and 2 urine pellet sample types (AP-E and AP-Q) from a spot (random) urine sample for NEPTUNE storage at -80° C. Shipping details are provided in Appendix N of the NEPTUNE Manual of Procedures.

Spot urine is collected in the same fashion for both pediatric and adult study participants. Spot urine samples are obtained at each study visit, including the baseline [V2] and the Biopsy [V3] visits, and all follow-up visits.

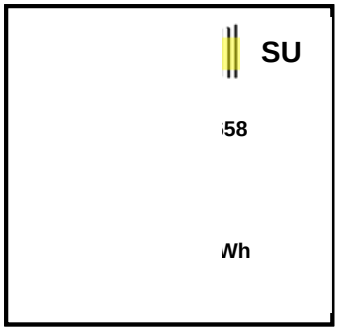
At [V3], the spot urine sample should be obtained PRIOR to the biopsy procedure. Please record this in the [V3] Biospecimen form.

Required Supplies:
Included in kit:

- 2 x 15.0 mL centrifuge tubes[1] empty (marked **"EMPTY"**)
- 2 x 15.0 mL centrifuge tubes containing 15 μL of Protease Inhibitor[2] (marked **"PI"**)
- 16 x 1.8 mL cryovials[3] with yellow caps
  (Pre-labeled as follows)

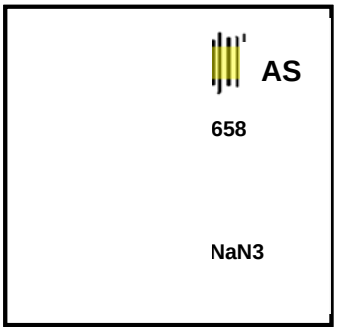
Sample 1:
SU = Whole, Unprocessed Urine

4 pre-labeled, empty cryovials
U-Spot Urine Spot Wh



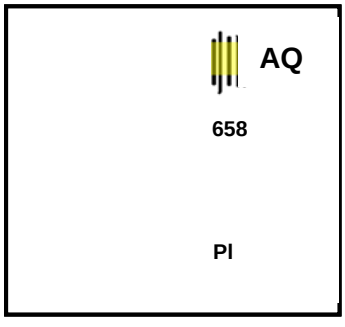
Sample 2:
AS = Processed & NaAzide

4 pre-labeled cryovials containing Sodium Azide[4]
AS Urine Spot SN NaN3



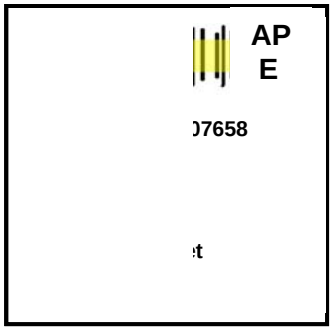
Sample 3:
AQ = Processed & Protease Inhibitor (PI)

4 pre-labeled cryovials for Protease Inhibitor
AQ Urine Spot SN PI



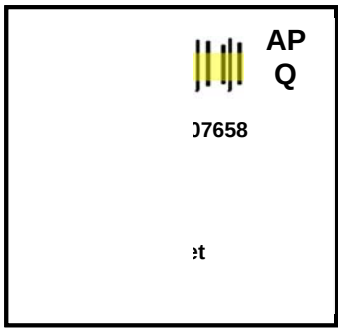
Sample 4:
AP-E = Urine Pellet from 'Empty' centrifuge tubes

2 pre-labeled, empty cryovials
AP-E Urine Pellet



Sample 5:
AP-Q = Urine Pellet from 'PI' containing centrifuge tubes

2 pre-labeled, empty cryovials
AP-Q Urine Pellet



- RNA-Later[5] (expected use per sample: 50-100 μL)

Provided by site:
- Spot Urine sample from study participant
- Pipettes and tips or disposable pipettes (10 ml, 5 ml, 1 ml sizes) or plastic Transfer pipettes
- Gloves, goggles, and lab coat

Required Equipment:
- Centrifuge capable of achieving 2000 x G
- University of Michigan OSEH guidelines designate all work done with biological specimens that could produce spray from pipetting be done in a hood. Please refer to your site specific OSEH policies.

Responsible Individuals
The NEPTUNE Research Coordinator is responsible for retrieving urine sample from consented participants, returning sample to lab, and processing for storage. In the absence of the Research Coordinator, the Site PI or designated, NEPTUNE trained alternate would provide this service.

Procedure:
1. Midstream urine is recovered in sterile specimen container and stored on ice. A minimum of 32 mL of urine is necessary.

2. Note time, fasting state and 1st, 2nd, 3rd, etc urine of the day on study visit worksheet (if patient is able to provide this detail, otherwise, indicate "No recall").

If processing is not able to be completed immediately, please store on ice or refrigerate for a maximum of 4 hours.

Sample processing:

Sample 1

*Requires total spot urine sample of 60 mL minimum; if sample is 50 mL or less please note in worksheet and CRF and do NOT store spot urine for the U-Spot Samples: * NO WHOLE URINE SAMPLE**

3. Using a pipettor, transfer ***whole***, unprocessed urine into the cryovials labeled:

'SU' to the right of the barcode

4. Replace the cap, firmly twisting in a clockwise direction.

Samples 2 & 3

5. Pour the remaining urine into the 4 x 15.0 mL centrifuge tubes in equal 12 milliliter portions (two tubes of each marked "EMPTY" and "Protease Inhibitor (PI)")

   E.g.:

   30 mL of spot urine → 2 – 12.0 mL centrifuge tubes for spinning (Use 1 'Empty' and 1 'PI' tube)

   50 mL of urine → 4 – 12.0 mL centrifuge tube; discard remaining urine (Use 2 of each, 'Empty' and 'PI')

NOTE: Use at least ONE EACH: "EMPTY" and "PI" centrifuge tubes

6. In the tubes labeled "PI", gently invert 8-10 times to dissolve the PI completely. Remove the cap to allow any air bubbles to escape, replace cap tightly.
7. Spin all 4 tubes at 1000 X G for 12 minutes in centrifuge

Sample 2 (AS Urine Spot): From the tubes labeled "EMPTY", transfer urine using a transfer pipettor into the 4 cryovials containing a pre-measured amount of 100 mM Sodium Azide (a biocide) labeled:

'AS' to the right of the barcode

8. When opening each cryovial, take care to place the cap directly in front of the respective tube to retain pre-measured volumes for consistent concentrations in each aliquot.
9. Pipette 1.6 mLs of urine or enough urine to fill the cryovial up to the first ridge on the cryovial (see Figure 1).
10. Replace the cap, firmly twisting in a clockwise direction. After all caps are replaced, invert tubes *at least 2 times* to distribute the sodium azide prior to freezing.

Sample 3 (AQ Urine Spot): From the tubes labeled "PI", transfer urine using a transfer pipettor into the cryovials labeled:

'AQ' to the right of the barcode

11. Pipette 1.6 mLs of urine or enough urine to fill the cryovial up to the first ridge on the cryovial (see Figure 1).

Samples 4 and 5

12. Pour off remaining supernatant from all of the 15.0 mL centrifuge tubes
13. For maximal drainage, gently invert centrifuge tubes on clean paper towels for 10 seconds, take care to not disturb pellet in the centrifuge tube tip.
14. With a clean pipette tip, transfer 25.0-50.0* μL RNA-Later into each centrifuge tube, take care to not touch the inside walls of the centrifuge tube.
15. Gently stir the mixture with the pipette tip, changing tips when moving between the 'Empty' tubes and the 'PI' tubes. **Do not pipette up and down to mix as this could break any cells present in the pellet.**

    Sample 4 (AP-E Urine Pellet): From the centrifuge tubes labeled "EMPTY", pipette one urine pellet from each centrifuge tube into the two cryovials labeled:

    'AP-E' to the right of the barcode

    Sample 5 (AP-Q Urine Pellet): From the centrifuge tubes labeled "PI", pipette one urine pellet from each centrifuge tube into the two cryovials labeled:

    'AP-Q' to the right of the barcode

16. Freeze all samples at -80 C.
17. Residual urine may be disposed per OSEH guidelines enforced at participating institution.

* Amount of RNA-Later will vary depending on size of pellet. RNA-Later should be added to completely submerse the pellet.

Shipping
Samples should be shipped according to the site-specific scheduled interval for shipments.

All specimens should be shipped together **on dry ice** using the shipping instructions found in Appendix N and in the study specific container provided by NEPTUNE.

Documentation
All corresponding samples for each participant ID should be stored together. If specimen does not adequately fill pre-determined number of aliquots, please document to minimize concerns regarding lost aliquots.

Complete the appropriate Biospecimen CRF corresponding to the study visit.

Procedure Class:	SPECIMENS
Procedure:	BIO-Spot Urine [V3: Biopsy] (SU, AS, AQ and AP)

References

1. 15.0 mL orange-capped Corning centrifuge tubes: Fisher Scientific No. 05-538-53D, Corning No. 430052
2. Protease Inhibitor Cocktail: Sigma-Aldrich Catalog: P1860 – 1 mL
3. Cryovials: DOT Scientific Inc. No.: T334-6SPR
4. Sodium Azide (see attached MSDS)
5. Ambion RNA-Later Solution; P/N: Am7021
6. University of Michigan Occupations Safety and Environmental Health (OSEH) guidelines indicate washing urine and its debris down laboratory sink with adequate water.

Relevant Definitions: NaN_3: Sodium Azide

Procedure Class:	SPECIMENS
Procedure:	**BASELINE [V2] ADULT** BIO-Blood (BB, BRF, BS, BSF, BG, BE)

26.A.1. UPDATES TO BLOOD BIOSPECIMEN PROCESSING PROCEDURES:

1. Effective 11/2012 NEPTUNE will use light sensitive dark cryovials in place of the previously used light sensitive eppendorf tubes (conical bottom). Throughout the MOP, these new cryovials will be referred to as the 'dark cryovials'.

   The volume requested for filling dark cryovials is the same as the clear cryovials: 1.6 mLs.

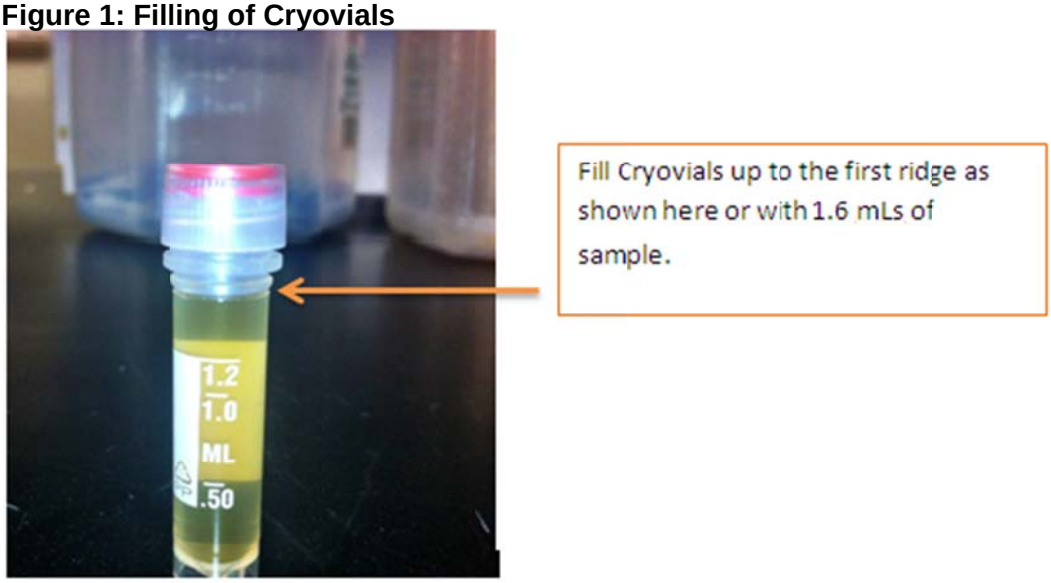
2. Research Coordinators (RCs) will no longer fill sets of cryovials with equal volumes of sample. Effective 11/2012 please fill each cryovial with the maximum volume (1.6 mL) in sequential order (based on the aliquot number indicated on barcoded label) until all corresponding sample in the blood draw tube(s) has been aliquotted. Discard remaining empty cryovials on-site.

3. When transferring blood plasma/serum samples from the blood draw tubes into cryovials for freezer storage, it is imperative that these containers are not overfilled. The following procedures take into account liquid expansion when samples freeze.

   a) <u>Filling cryovials with biospecimens:</u>

   - Digital pipettors: Transfer a total of 1.6 mls of sample into the cryovials.
   - Transfer pipettes (disposable, plastic): Note the two ridges below the cap of the cryovial. Use the lower ridge as a reference point for filling cryovials. Please see Figure 1 below.

Figure 1: Filling of Cryovials



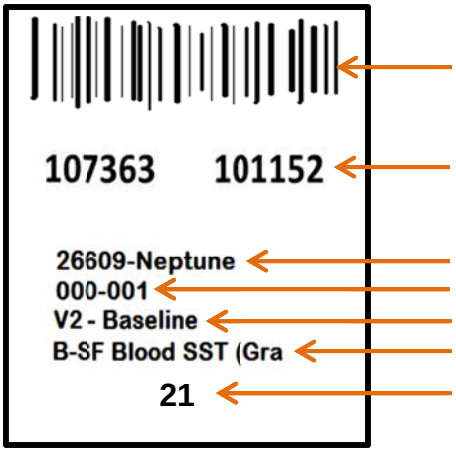
   b) <u>Filling the dark cryovials:</u>

   - Digital pipettors: Transfer a total of 1.60 mLs of sample into each dark cryovial
   - Transfer pipettes (disposable, plastic): one reference available to ensure filling the tubes with the proper volume:
   - Note the ridged pattern around the top part of the black cryovial. 1.6 mLs of liquid reaches the top of that pattern. See Figure 2 below.

Figure 2: Filling of Light Sensitive Dark Cryovials

e ridged
n the dark
1.6 mLs
the top of
l pattern.

New Barcode Label Identifiers



ntifier

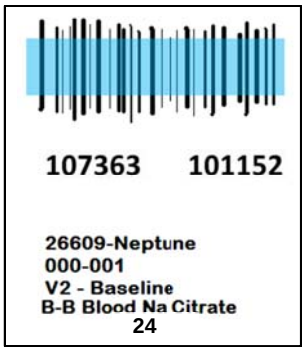
Participant Visit #

Sample Type

Aliquot Number
(this number is subject to change based on visit [Baseline/Follow-up] and participant weight)

26.A.2. BLOOD PROCESSING FOR SODIUM CITRATE (LIGHT BLUE TOP) TUBE 1

Procedure Overview
This procedure describes the process for preparing blood samples obtained at the **Baseline Visit [V2]** in the Sodium Citrate blood tube for NEPTUNE storage at -80° C. Shipping details are provided in Appendix N of the NEPTUNE Manual of Procedures.



Required Supplies:
Included in kit:

- 1 x 4.5 mL Sodium Citrate blood vacutainer tubes[1] (Tube 1)
- 2 labeled cryovials[2] (**BLUE CAPS/BLUE LABELS)**
  (Both Pre-labeled with **B-B Blood Na Citra ##**

Provided by site:

- Blood sample from study participant
- Aerosol spray covers for centrifuge buckets
- Pipettors and tips or disposable pipettes (1 ml or 200 µl sizes) or plastic transfer pipettes
- Gloves, goggles, and lab coat

Required Equipment:

- Centrifuge capable of achieving 2000 x g (NOTE: G is not the same as RPM, please consult your centrifuge conversion table if necessary)
- University of Michigan OSEH[3] guidelines designate all work done with biological specimens that could produce spray from pipetting is done in a hood.

Responsible Individuals
The NEPTUNE Research Coordinator is responsible for retrieving blood samples from consented participants, returning sample to lab, and processing for storage. In the absence of the Research Coordinator, the Site PI or designated alternate would provide this service.

Procedure
All baseline blood samples should be drawn prior to or within thirty days of the renal biopsy.

Note the date and start time of the blood draw (Tube 1) on Biospecimen Worksheet.
In accordance with the site's protocol for venipuncture, draw 1 (one) 4.5 mL Sodium Citrate blood vacutainers from adult participants. Following the blood draw the Research Coordinator will return to the lab with the samples.

1. Balance Sodium Citrate blood tube per your site's centrifuge protocol and handling policy.
2. Note the time on the Biospecimen worksheet (this should be the time the samples start to spin in the centrifuge).
3. Centrifuge the tube at 2000 x g for 12 minutes.

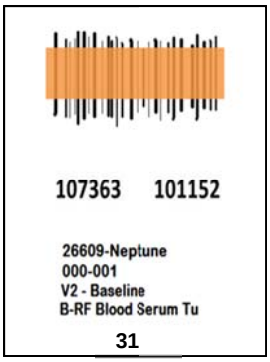
Sample processing:

4. After spinning, pipette plasma* from the Sodium Citrate tube into two equal aliquots of 1.6 mLs each (reference Figure 1 to determine proper volume). Use the cryovials labeled:
   B-B Blood Na Citrat ## and **B-B Blood Na Citrat ##**
5. Freeze aliquots at -80° C prior to shipping.
6. Discard remaining RBC's per OSEH guidelines

*Plasma is the top layer of the blood specimen after the centrifugation step. It should be a straw-colored yellow, semi-clear substance.

26.A.3. BLOOD PROCESSING FOR SERUM (BURNT ORANGE) TUBE 2

Procedure Overview
This procedure describes the process for preparing blood samples obtained at the **Baseline Visit [V2]** in the Serum blood tube for NEPTUNE storage at -80° C. Shipping details are provided in Appendix N of the NEPTUNE Manual of Procedures.



Required Supplies:
Included in kit:

- 1 x 10.0 mL Serum blood vacutainer tube[4] wrapped in foil** (Tube 2)
- 3 labeled light sensitive Dark Cryovials[5] (**ORANGE LABELS**)
  (3 Pre-labeled **B-RF Blood Serum Tu ##**)

  ** *Sample must be protected from light (do **not** remove foil*)

Provided by site:

- Blood sample from study participant
- Aerosol spray covers for centrifuge buckets
- Pipettors and tips or disposable pipettes (1 ml or 200 µl sizes) or plastic transfer pipettes
- Gloves, goggles, and lab coat

Required Equipment:

- Centrifuge capable of achieving 2000 x g (NOTE: G is not the same as RPM, please consult your centrifuge conversion table if necessary)
- University of Michigan OSEH[3] guidelines designate all work done with biological specimens that could produce spray from pipetting is done in a hood.

Responsible Individuals
The NEPTUNE Research Coordinator is responsible for retrieving blood samples from consented participants, returning sample to lab, and processing for storage. In the absence of the Research Coordinator, the Site PI or designated alternate would provide this service.

Procedure
All blood samples should be drawn prior to or within thirty days of the renal biopsy.

Note the date and start time of the blood draw (Tube 2) on Biospecimen Worksheet.
In accordance with the site's protocol for venipuncture, draw 1 (one) 10.0 mL Serum blood vacutainers from adult participants. Following the blood draw the Research Coordinator will return to the lab with the samples.

1. Balance Serum blood tube (tube 2) per your site's centrifuge protocol and handling policy.
2. Note the time on the Biospecimen worksheet (this should be the time the samples start to spin in the centrifuge).
3. Centrifuge the tube at 2000 x g for 12 minutes.

Sample processing:

4. After spinning, pipette serum*** from the tube into three aliquots of 1.60 mLs each (see Figure 2 to determine proper volume). Use the light-sensitive Dark cryovials labeled:

   B-RF Blood Serum Tu ## **B-RF Blood Serum Tu ##**
   B-RF Blood Serum Tu ##

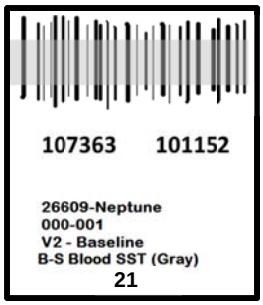
5. Freeze aliquots at -80° C prior to shipping.
6. Discard remaining RBC's per OSEH guidelines

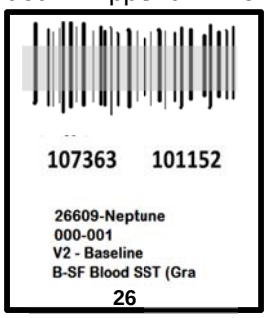
Procedure Class:	SPECIMENS
Procedure:	**BASELINE [V2] ADULT** BIO-Blood (BB, BRF, BS, BSF, BG, BE)

*** Serum is the top layer of the blood specimen after the centrifugation step. It should be a straw-colored yellow, semi-clear substance. Serum is not the same as plasma.

26.A.4. BLOOD PROCESSING FOR SST (RED/BLACK TIGER TOP) TUBES 3 and 4

Procedure Overview
This procedure describes the process for preparing blood samples obtained at the **Baseline Visit [V2]** in SST blood tubes for NEPTUNE storage at -80° C. Shipping details are provided in Appendix N of the NEPTUNE Manual of Procedures.





Required Supplies:
Included in kit:

- 1 x 10.0 mL SST blood vacutainer tubes[6] (Tube 3)
- 3 labeled cryovials[2] (**GRAY CAPS/GRAY LABELS**)
  (Pre-labeled **B-S Blood SST (Gray ##**)

- 1 x 10.0 mL SST blood vacutainer tube[6] wrapped in foil** (Tube 4)
- 3 labeled light sensitive dark cryovials[5] (**Black, light prohibitive/ GREY LABELS**)
  (Pre-labeled **B-SF Blood SST (Gra ##)**

  ** *Sample must be protected from light (do **not** remove foil*)

Provided by site:

- Blood sample from study participant
- Aerosol spray covers for centrifuge buckets
- Pipettors and tips or disposable pipettes (1 ml or 200 µl sizes) or plastic transfer pipettes
- Gloves, goggles, and lab coat

Required Equipment:

- Centrifuge capable of achieving 2000 x g (NOTE: G is not the same as RPM, please consult your centrifuge conversion table if necessary)
- University of Michigan OSEH[3] guidelines designate all work done with biological specimens that could produce spray from pipetting is done in a hood.

Responsible Individuals
The NEPTUNE Research Coordinator is responsible for retrieving blood samples from consented participants, returning sample to lab, and processing for storage. In the absence of the Research Coordinator, the Site PI or designated alternate would provide this service.

Procedure
All baseline blood samples should be drawn prior to or within thirty days of the renal biopsy.

At least one SST tube must be wrapped in aluminum foil *prior* to and following the blood draw, as well as while the sample is processed.

Note the date and start time of the blood draw (tubes 3-4) on Biospecimen Worksheet.

In accordance with the site's protocol for venipuncture, draw 2 (two) 10.0 mL SST blood vacutainers from adult participants. Following the blood draw the Research Coordinator will return to the lab with the samples.
The SST tubes must be processed following a 30-minute incubation period, placed in cryovials, and placed in the -80°C freezer.

Procedure Class:	SPECIMENS
Procedure:	**BASELINE [V2] ADULT** BIO-Blood (BB, BRF, BS, BSF, BG, BE)

Sample processing:

1. Following the 30-minute incubation period, balance SST blood tubes per your site's centrifuge protocol and handling policy.
2. Note the time on the Biospecimen worksheet (this should be the time the samples start to spin in the centrifuge).
3. Centrifuge SST tubes at 2000 x g for 12 minutes.

Tube 3

4. After spinning, remove tube from centrifuge keeping tubes upright so as not to disturb the serum*** component.
5. Pipette the serum layer from the un-foiled SST tubes (tube 3) into approximate 1.6 mL aliquots. This amount can be determined by referencing Figure 1. Use the cryovials labeled:

B-S Blood SST (Gray ## through **B-S Blood SST (Gray ##**

Tube 4

6. After spinning, pipette serum*** from the foiled SST tube (tube 4) into approximate 1.60 mL aliquots. This amount can be determined by referencing Figure 2. Use the light sensitive Dark cryovials labeled:

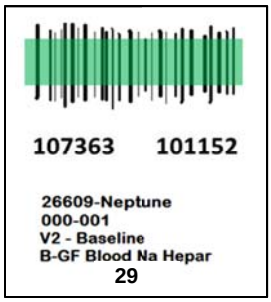
B-SF Blood SST (Gra ## through **B-SF Blood SST (Gra ##**

7. Freeze all aliquots at -80° C prior to shipping.
8. Discard remaining RBC's per OSEH guidelines

*** Serum is the top layer of the blood specimen after the centrifugation step. It should be a straw-colored yellow, semi-clear substance. Serum is not the same as plasma.

26.A.5. BLOOD PROCESSING FOR SODIUM HEPARIN (GREEN TOP) TUBE 5

Procedure Overview
This procedure describes the process for preparing blood samples obtained at the **Baseline Visit [V2]** in the Sodium Heparin blood tubes for NEPTUNE storage at -80° C. Shipping details are provided in Appendix N of the NEPTUNE Manual of Procedures.



Required Supplies:
Included in kit:

- 1 x 4.0 mL Sodium Heparin blood vacutainer tube[7] wrapped in foil** (Tube 5)
- 2 labeled light sensitive dark cryovials[5] (**Black, light prohibitive/GREEN LABELS**) (Pre-labeled **B-GF Blood Na Hepar ##** and **B-GF Blood Na Hepar ##**)

*** Sample must be protected from light (do **not** remove foil*)

Provided by site:

- Blood sample from study participant
- Aerosol spray covers for centrifuge buckets
- Pipettes and tips or Disposable pipettes (1 ml or 200 µl sizes) or plastic Transfer pipettes
- Gloves, goggles, and lab coat

Required Equipment:

- Centrifuge capable of achieving 2000 x g (NOTE: G is not the same as RPM, please consult your centrifuge conversion table if necessary)
- University of Michigan OSEH[3] guidelines designate all work done with biological specimens that could produce spray from pipetting is done in a hood.

Responsible Individuals
The NEPTUNE Research Coordinator is responsible for retrieving blood samples from consented participants, returning sample to lab, and processing for storage. In the absence of the Research Coordinator, the Site PI or designated alternate would provide this service.

Procedure
All baseline blood samples should be drawn prior to or within thirty days of the renal biopsy.

Note the date and start time of the blood draw (tube 5) on Biospecimen Worksheet.

In accordance with the site's protocol for venipuncture, draw 1 (one) 4.0 mL Sodium Citrate blood vacutainer (tube 5) from adult participants. Following the blood draw the Research Coordinator will return to the lab with the samples.

7. Balance Sodium Heparin blood tube (tube 6) per your site's centrifuge protocol and handling policy.
8. Note the time on the Biospecimen worksheet (this should be the time the samples start to spin in the centrifuge).
9. Centrifuge the tube at 2000 x g for 12 minutes.

Sample processing:

10. After spinning, pipette plasma* from the Sodium Heparin tube into two equal 1.60 mL aliquots, which can be determined using Figure 2. Use the two light sensitive Dark cryovials labeled:

B-GF Blood Na Hepar ## and **B-GF Blood Na Hepar ##**

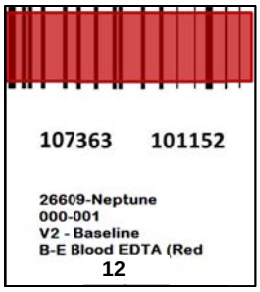
11. Freeze aliquots at -80° C prior to shipping.
12. Discard remaining RBC's per OSEH guidelines

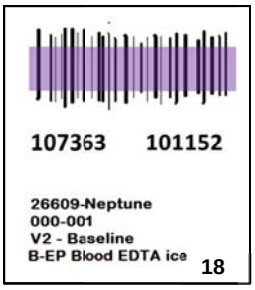
* Plasma is the top layer of the blood specimen after the centrifugation step. It should be a straw-colored yellow, semi-clear substance.

Procedure Class:	SPECIMENS
Procedure:	**BASELINE [V2] ADULT** BIO-Blood (BE, BS, BSF, BR, BB, BGF)

26.A.6. BLOOD PROCESSING FOR EDTA (PURPLE TOP) TUBES 6-11

Procedure Overview
This procedure describes the process for preparing blood samples obtained at the **Baseline Visit [V2]** in EDTA blood tubes for NEPTUNE storage at -80° C. Shipping details are provided in Appendix N of the NEPTUNE Manual of Procedures.





Required Supplies:
Included in kit:

- 6 x 10.0 mL EDTA blood vacutainer tubes[8] (tubes 6-11)
- 17 labeled cryovials[2] (**RED CAPS/RED LABELS**)
  (Pre-labeled **B-E Blood EDTA (Red 1** through **B-E Blood EDTA (Red 17**)
- 3 labeled cryovials[2] (**PURPLE CAPS/PURPLE LABELS**)
  (Pre-labeled **B-EP Blood EDTA Ic 18** through **B-EP Blood EDTA Ic 20**)

Provided by site:

- Blood sample from study participant
- Aerosol spray covers for centrifuge buckets
- Pipettes and tips or Disposable pipettes (1 ml or 200 µl sizes) or plastic Transfer pipettes
- Gloves, goggles, and lab coat
- Ice and ice bucket

Required Equipment:

- Centrifuge capable of achieving 2000 x g (NOTE: G is not the same as RPM, please consult your centrifuge conversion table if necessary)
- University of Michigan OSEH[3] guidelines designate all work done with biological specimens that could produce spray from pipetting is done in a hood.

Responsible Individuals
The NEPTUNE Research Coordinator is responsible for retrieving blood samples from consented participants, returning sample to lab, and processing for storage. In the absence of the Research Coordinator the Site PI or designated alternate would provide this service.

Procedure
All blood samples should be drawn prior to or within thirty days of the renal biopsy visit [V3].

At least one EDTA tube must be on ice *prior* to the blood draw.

Note the date and start time of the blood draw (tubes 6-11) and fasting state on Biospecimen Worksheet.

In accordance with the site's protocol for venipuncture, draw 6 (six) 10.0 mL EDTA blood vacutainers from adult participants. The EDTA tubes must be PLACED ON ICE, processed, placed in cryovials, and placed in the -80°C freezer within 30 minutes. Following the blood draw the Research Coordinator will return to the lab with the samples.

Sample processing:

1. Balance EDTA blood tubes per site's centrifuge protocol and handling policy.
2. Note the time on the Biospecimen worksheet (this should be the time the samples start to spin in the centrifuge).
3. Centrifuge EDTA tubes at 2000 x g for 12 minutes.
4. Remove tubes from centrifuge keeping tubes upright so as not to disturb the plasma* component.

5. After spinning, pipette the plasma layer from all EDTA tubes into approximate 1.6 mL aliquots. This amount can be determined by referencing Figure 1. Use the cryovials labeled:

   B-E Blood EDTA (Red 1 through **B-E Blood EDTA (Red 17**

6. The EDTA tube which was "iced" prior to the blood draw and following should be spun as above.
7. After spinning, iced plasma should be put 1.6 mL aliquots into 3 cryovials labeled:
   B-EP Blood EDTA Ic 18 through **B-EP Blood EDTA Ic 20**
8. Freeze aliquots at -80° C prior to shipping.
9. Discard remaining RBC's per site's OSEH guidelines.

* Plasma is the top layer of the blood specimen after the centrifugation step. It should be a straw-colored yellow, semi-clear substance.

Procedure Class:	SPECIMENS
Procedure:	**BASELINE [V2] ADULT** BIO-Blood (BE, BS, BSF, BR, BB, BGF)

26.A.7. BLOOD PROCESSING FOR RNA PAXGENE (RED/CLEAR TOP) TUBE 12

Procedure Overview
This procedure describes the process for preparing blood samples obtained at the **Baseline Visit [V2]** in RNA PAXgene blood tubes for NEPTUNE storage at -80° C. Shipping details are provided in Appendix N of the NEPTUNE Manual of Procedures.

Required Supplies:
Included in kit:

- 1 pre-labeled PAXgene RNA Blood Vacutainer[9] (**WHITE LABEL 'D RNA Paxgene (Whit'**) (Tube 12)

Provided by site:

- Blood sample from study participant
- Gloves, goggles, and lab coat

Required Equipment:

- No equipment is necessary for processing this specimen

Responsible Individuals
The Biobank Study Coordinator is responsible for retrieving blood samples from consented participants, returning sample to lab, and processing for storage. In the absence of the Study Coordinator, the Biobank Lab Manager, followed by the Biobank Program Director/Site PI would serve as alternates.

Procedure
All blood samples should be drawn prior to renal biopsy, if biopsy is indicated. Following the biopsy, the Biobank Study Coordinator will return to the lab with the samples.

1. RNA blood vacutainer is marked with a **WHITE LABEL 'D RNA Paxgene (Whit'**
2. Blood draw:
   Summary:
   a. The PAXgene blood RNA vacutainer should be at room temperature prior to use.
   b. Fill the PAXgene RNA tube *after* all other tubes have been drawn.
   c. Collect blood into the PAXgene tube using your institution's recommended standard procedure for venipuncture.

   Blood Collection:
   a. Hold PAXgene tube vertically during blood draw, below point of venipuncture.
   b. Allow at least 10 seconds for complete blood draw; ensuring blood has stopped flowing into tube before removing from vacutainer holder.
   c. After blood collection gently invert tube 8-10 times.
   d. Store tube upright at room temperature for 2 hours.
   e. Following 2 hours room temperature incubation period, place in -80° C freezer upright.

Documentation
If PAXGene RNA tubes are left on shelf (room temp) for greater than 2 hour incubation period, please indicate additional elapsing time on the worksheet and case report forms. Additionally, if PAXgene RNA tubes stabilize at room temp for less than 2 hours, please indicate total time on worksheet and case report forms.

Procedure Class:	SPECIMENS
Procedure:	**BASELINE [V2] ADULT** BIO-Blood (BE, BS, BSF, BR, BB, BGF)

27.A.8. BLOOD PROCESSING FOR DNA PAXGENE (BLUE/CLEAR TOP) TUBE 13

Procedure Overview
This procedure describes the process for preparing blood samples obtained at the **Baseline Visit [V2]** in DNA PAXgene blood tubes for NEPTUNE storage at -80° C. Shipping details are provided in Appendix N of the NEPTUNE Manual of Procedures.

Required Supplies:
Included in kit:

- 1 pre-labeled PAXgene DNA Blood Vacutainer[10] (BLUE LABEL 'C DNA Paxgene (Blue') (Tube 13)

Provided by site:
Blood sample from study participant

- Gloves, goggles, and lab coat

Required Equipment:

- No equipment is necessary for this specimen

Responsible Individuals
The Biobank Study Coordinator is responsible for retrieving blood samples from consented participants, returning sample to lab, and processing for storage. In the absence of the Study Coordinator, the Biobank Lab Manager, followed by the Biobank Program Director/Site PI would serve as alternates.

Procedure
All blood samples should be drawn prior to renal biopsy, if biopsy is indicated. Following the biopsy, the Biobank Study Coordinator will return to the lab with the samples.

3. DNA blood vacutainer is marked with a **BLUE LABEL 'C DNA Paxgene (Blue'**
4. Blood draw:
   Summary:
   a. The PAXgene blood DNA vacutainer should be at room temperature prior to use.
   b. Fill the PAXgene DNA tube *after* all other tubes have been drawn.
   c. Collect blood into the PAXgene tube using your institution's recommended standard procedure for venipuncture.

   Blood Collection:
   a. Hold PAXgene tube vertically during blood draw, below point of venipuncture.
   b. Allow at least 10 seconds for complete blood draw; ensuring blood has stopped flowing into tube before removing from vacutainer holder.
   c. After blood collection gently invert tube 8-10 times.
   d. Store tube upright at room temperature for 2 hours.
   e. Following 2 hours room temperature incubation period, please in -80° C freezer upright.

Documentation
If PAXgene DNA tubes are left on shelf (room temp) for greater than 2 hour incubation period, please indicate additional elapsing time on the worksheet and case report forms. Additionally, if PAXgene DNA tubes stabilize at room temp for less than 2 hours, please indicate total time on worksheet and case report forms.

All corresponding samples for each sample number should be stored together. If specimen does not adequately fill pre-determined number of aliquots, please document to minimize concerns regarding lost aliquots.

Complete the appropriate Biospecimen CRF corresponding to the study visit.

27.A.9. References

1. 4.5 mL Sodium Citrate Vacutainer: BD, Fisher Scientific ref No. 366415
2. Cryovials: DOT Scientific Inc. No. T334-6SPR
3. Check your local OSEH Guidelines
4. 10 mL Serum Vacutainer: BD, Fisher Scientific ref No. 366441
5. Light Sensitive Dark Cryovials: USA Scientific No. 1420-9709
6. 10 mL SST Vacutainer: BD, Fisher Scientific ref No. 367985
7. 4.0 mL Sodium Heparin Vacutainer: BD, Fisher Scientific ref No. 367871
8. 10 mL EDTA Vacutainer: BD, Fisher Scientific ref No. 366643
9. PreAnalytiX PAXgene Blood RNA Tube; 2.5 mL Vacutainer: ref. No. 762165
   http://www.preanalytix.com/product-catalog/blood/rna/products/paxgene-blood-rna-tube/
10. PreAnalytiX PAXgene DNA Tube; 8.5 mL Vacutainer: Cat. No. 761115
    http://www.preanalytix.com/DNA.asp
11. See attached MSDS for relevant safety precautions and hazards.

Centrifugation steps were performed in a SORVALL Legend T with radius 15.9 cm. If alternate centrifuges are used, the following equation may be used to calculate appropriate RPM necessary to generate the G-force indicated in above protocol:

$$RCF = 11.18 * \left(\frac{n}{1000}\right)^2 * r$$

RCF = Relative Centrifugal Force (G); a dimensionless number allowing comparison of separation efficiency

r = radius of centrifugation in cm

n = speed in rpm

Procedure Class:	SPECIMENS
Procedure:	**BASELINE [V2] ADULT** BIO-Blood (BE, BS, BSF, BR, BB, BGF)

110 cc Adult Baseline Blood Draw

Tube #	Tube Type	Step 1	Step 2	Step 3	Step 4	Step 5
1	4.5 mL Sodium Citrate	Process within one hour.	Centrifuge for 12 minutes at 2000 x G	Transfer 1.6 mL aliquots of serum into 2 cryovials	Store aliquots at -80°C	Ship to NEPTUNE Biobank as indicated by site schedule
2	10 mL Serum Wrapped in Foil	Avoid exposure to light. Allow to clot for 30 minutes.	Centrifuge for 12 minutes at 2000 x G	Transfer 1.60 mL aliquots of serum into 3 light sensitive Dark cryovials	Store aliquots at -80°C	Ship to NEPTUNE Biobank as indicated by site schedule
3	10 mL SST	Allow to clot for 30 minutes.	Centrifuge for 12 minutes at 2000 x G	Transfer 1.6 mL of serum into 3 cryovials.	Store aliquots at -80°C	Ship to NEPTUNE Biobank as indicated by site schedule
4	10 mL SST Wrapped in Foil	Avoid exposure to light. Allow to clot for 30 minutes.	Centrifuge for 12 minutes at 2000 x G	Transfer 1.60 mL aliquots of serum into 3 light sensitive Dark cryovials.	Store aliquots at -80°C	Ship to NEPTUNE Biobank as indicated by site schedule
5	4 mL Sodium Heparin Wrapped in Foil	Avoid exposure to light.	Centrifuge for 12 minutes at 2000 x G	Transfer 1.60 ml aliquots of serum to 2 light sensitive Dark cryovials.	Store aliquots at -80°C	Ship to NEPTUNE Biobank as indicated by site schedule
6	10 mL EDTA	Keep on ice. Process within 30 minutes.	Centrifuge for 12 minutes at 2000 x G	Transfer 1.6 mL of serum into 3 cryovials.	Store aliquots at -80°C	Ship to NEPTUNE Biobank as indicated by site schedule
7	10 mL EDTA	Keep on ice. Process within 30 minutes.	Centrifuge for 12 minutes at 2000 x G	Transfer 1.6 mL of serum into 3 cryovials.	Store aliquots at -80°C	Ship to NEPTUNE Biobank as indicated by site schedule
8	10 mL EDTA	Keep on ice. Process within 30 minutes.	Centrifuge for 12 minutes at 2000 x G	Transfer 1.6 mL of serum into 3 cryovials.	Store aliquots at -80°C	Ship to NEPTUNE Biobank as indicated by site schedule
9	10 mL EDTA	Keep on ice. Process within 30 minutes.	Centrifuge for 12 minutes at 2000 x G	Transfer 1.6 mL of serum into 3 cryovials.	Store aliquots at -80°C	Ship to NEPTUNE Biobank as indicated by site schedule
10	10 mL EDTA	Keep on ice. Process within 30 minutes.	Centrifuge for 12 minutes at 2000 x G	Transfer 1.6 mL of serum into 3 cryovials..	Store aliquots at -80°C	Ship to NEPTUNE Biobank as indicated by site schedule
11	10 mL EDTA	Keep on ice. Process within 30 minutes.	Centrifuge for 12 minutes at 2000 x G	Transfer 1.6 mL of serum into 3 cryovials.	Store aliquots at -80°C	Ship to NEPTUNE Biobank as indicated by site schedule
12	2.5 mL RNA	Must sit at room temp for 2 hours	Freeze at -80		Store pre-labeled vacutainer at -80°C	Ship to NEPTUNE Biobank as indicated by site schedule
13	8.5 mL DNA	Must sit at room temp for 2 hours	Freeze at -80		Store pre-labeled vacutainer at -80°C	Ship to NEPTUNE Biobank as indicated by site schedule

* approximate

Adult Blood Draw Guide

Tube #	Tube Type	Adults	
		Baseline [V2]	Follow-Up [V4-V13]
1	4.5 mL Na Citrate	4.5	4.5
	Cryovials:	*2: **blue** caps*	
2	10 mL Serum - Foil	10	
16	3 mL Serum - Foil		3
	Light Sensitive Cryovials:	*3 – **Orange** Cap*	*1 – **Orange** Cap*
3	10 mL SST	10	10
	Cryovials:	*3- Grey Caps*	
4	10 mL SST - Foil	10	
17	3.5 mL SST - Foil		3.5
	Light Sensitive Cryovials:	*3 – Grey Caps*	*1 – Grey Cap*
5	4 mL Na Heparin- Foil	4	
18	2 mL Na Heparin- Foil		2
	Light Sensitive Cryovials:	*2 – **Green** Caps*	*1 – **Green** Cap*
6	10 mL EDTA	10	10
7	10 mL EDTA	10	10
8	10 mL EDTA	10	10
9	10 mL EDTA	10	*10 (iced)
10	10 mL EDTA	10	
11	10 mL EDTA	*10 (iced)	
14	4 mL EDTA (*Iced for peds >21 lbs)		
15	6 mL EDTA		
	Cryovials:	*17- **Red** Caps* **3 – **Purple***	*11- **Red** Caps* **3 – **Purple***
12	2.5 mL RNA PAXGene	2.5	2.5
13	8.5 mL DNA PAXGene	8.5	
Blood Draw Totals (mLs)		**110**	**65.5**

26.A.11. Pediatric Blood Draw Guide

Tube #	Tube Type	< 21 pounds	21-51 pounds	≥ 52 pounds	
		Baseline[V2] & Follow-Up [V4-V13}	Baseline[V2] & Follow-Up [V4-V13]	Baseline [V2]	Follow-Up [V4-V13]
1	4.5 mL Na Citrate		4.5	4.5	4.5
	Cryovials:		*2: **blue** caps*	*2: **blue** caps*	
2	10 mL Serum - Foil		10	10	10
16	3 mL Serum - Foil	3			
	Light Sensitive Cryovials:	*1 – **Orange** Cap*	*3 – **Orange** Caps*	*3 – **Orange** Caps*	
3	10 mL SST			10	10
	Cryovials:			*3- Grey Caps*	
4	10 mL SST - Foil		10	10	10
17	3.5 mL SST - Foil	3.5			
	Light Sensitive Cryovials:	*1 – Grey Cap*	*3 – Grey Caps*	*3 – Grey Caps*	
5	4 mL Na Heparin- Foil		4	4	4
18	2 mL Na Heparin- Foil	2			
	Light Sensitive Cryovials:	*1 – **Green** Cap*	*2 – **Green** Caps*	*2 – **Green** Caps*	
6	10 mL EDTA		10	10	10
7	10 mL EDTA			10	10
8	10 mL EDTA			10	
9	10 mL EDTA			10	
14	4 mL EDTA (*Iced for peds >21 lbs)	4	*4	*4	*4
15	6 mL EDTA	5	5	6	
	Cryovials:	*3 – **Red** Caps*	*5 – **Red** Caps,*	*14 – **Red** Caps* **2 – **Purple***	*6- **Red** Caps* **2 – **Purple***
12	2.5 mL RNA PAXGene	2.5	2.5	2.5	2.5
13	8.5 mL DNA PAXGene			8.5	
Blood Draw Totals (mLs)		**20**	**50**	**99.5**	**65**

The DNA tube should only be obtained one time over the course of the study. Once the DNA draw is complete, the subsequent visits please supplement the 8.5 mL DNA draw with a complete 10 mL EDTA tube draw. (This applies for the 21-51 pound participant follow-up visits)

Procedure Class:	SPECIMENS
Procedure:	BIO-Spot Urine (SU, AS, AQ and AP)

26.B.1. UPDATES TO SPOT URINE PROCESSING PROCEDURES:

Effective 11/2012

1. Research Coordinators (RCs) will no longer fill sets of cryovials with equal volumes of sample. Effective 11/2012 please fill each cryovial with the maximum volume (1.6 mL) in sequential order (based on the aliquot number indicated on barcoded label) until all corresponding urine sample has been aliquotted. Discard remaining empty cryovials on-site.

2. When transferring urine from the original collection container or the processing tube for freezer storage, it is imperative that these containers are not overfilled. The following procedures take into account liquid expansion when samples freeze.

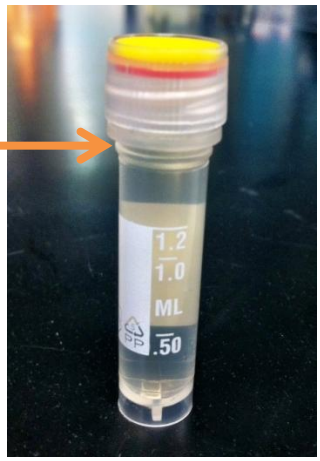
   a) Filling cryovials with biospecimens:

   - Digital pipettors: Transfer a total of 1.6 mls of sample into the cryovials.
   - Transfer pipettes (disposable, plastic): Note the two ridges below the cap of the cryovial. Use the lower ridge as a reference point for filling cryovials. Please see Figure 1 below.

When transferring urine samples from the centrifuge tubes into cryovials for freezer storage, it is imperative that these containers are not overfilled. The following directions take into account urine volume expansion when frozen.

Figure 1: Filling of Cryovials

The small, lowest ridge on the cryovial should be used for reference. Fill cryovial to this point, or with 1.6 mLs of sample.



Procedure Class:	SPECIMENS
Procedure:	BIO-Spot Urine (SU, AS, AQ and AP)

26.B.2. Spot/Random Clean Catch Urine Specimen Processing Procedure

Procedure Overview

This procedure describes the process for preparing one whole urine sample type (U), two urine supernatant sample types (S, Q) and 2 urine pellet sample types(AP-E and AP-Q) from a spot (random) urine sample for NEPTUNE storage at -80° C. Shipping details are provided in Appendix N of the NEPTUNE Manual of Procedures.

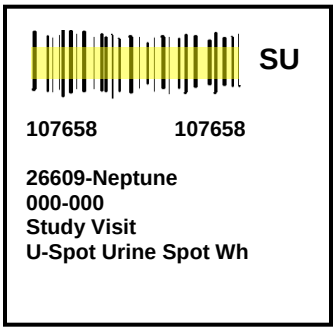
Spot urine is collected in the same fashion for both pediatric and adult study participants. Spot urine samples are obtained at each study visit, including the baseline [V2] and the Biopsy [V3] visits, and all follow-up visits.

Required Supplies:
Included in kit:

- 2 x 15.0 mL centrifuge tubes[1] empty (marked **"EMPTY"**)
- 2 x 15.0 mL centrifuge tubes containing 15 μL of Protease Inhibitor[2] (marked **"PI"**)
- 16 x 1.8 mL cryovials[3] with yellow caps
  (Pre-labeled as follows)

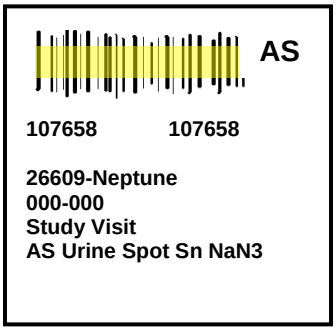
Sample 1:
SU = Whole, Unprocessed Urine

4 pre-labeled, empty cryovials
U-Spot Urine Spot Wh



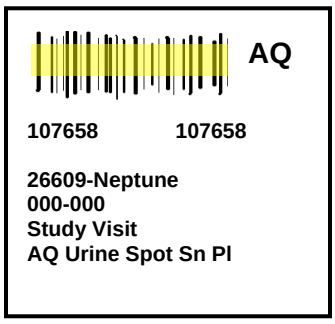
Sample 2:
AS = Processed & NaAzide

4 pre-labeled cryovials containing Sodium Azide[4]
AS Urine Spot SN NaN3



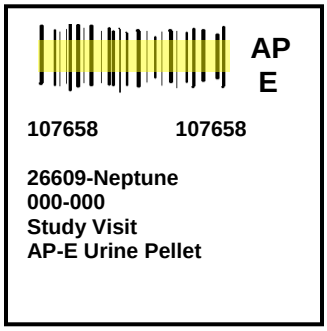
Sample 3:
AQ = Processed & Protease Inhibitor (PI)

4 pre-labeled cryovials for Protease Inhibitor
AQ Urine Spot SN PI



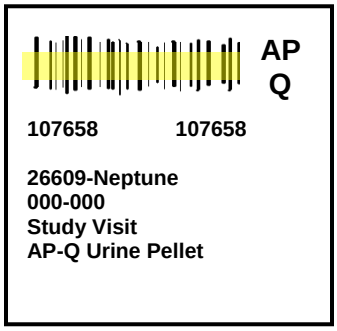
Sample 4:
AP-E = Urine Pellet from 'Empty' centrifuge tubes

2 pre-labeled, empty cryovials
AP-E Urine Pellet



Sample 5:
AP-Q = Urine Pellet from 'PI' containing centrifuge tubes

2 pre-labeled, empty cryovials
AP-Q Urine Pellet



- RNA-Later[5] (expected use per sample: 50-100 μL)

Provided by site:
- Spot Urine sample from study participant
- Pipettes and tips or disposable pipettes (10 ml, 5 ml, 1 ml sizes) or plastic Transfer pipettes
- Gloves, goggles, and lab coat

Required Equipment:
- Centrifuge capable of achieving 2000 x G
- University of Michigan OSEH guidelines designate all work done with biological specimens that could produce spray from pipetting be done in a hood. Please refer to your site specific OSEH policies.

Responsible Individuals
The NEPTUNE Research Coordinator is responsible for retrieving urine sample from consented participants, returning sample to lab, and processing for storage. In the absence of the Research Coordinator, the Site PI or designated, NEPTUNE trained alternate would provide this service.

Procedure:
1. Midstream urine is recovered in sterile specimen container and stored on ice. A minimum of 32 mL of urine is necessary.
2. Note time, fasting state and 1st, 2nd, 3rd, etc urine of the day on study visit worksheet (if patient is able to provide this detail, otherwise, indicate "No recall").

Procedure Class:	SPECIMENS
Procedure:	BIO-Spot Urine (SU, AS, AQ and AP)

If processing is not able to be completed immediately, please store on ice or refrigerate for a maximum of 4 hours.

Sample processing:

Sample 1

*Requires total spot urine sample of 60 mL minimum; if sample is 50 mL or less please note in worksheet and CRF and do NOT store spot urine for the U-Spot Samples: * NO WHOLE URINE SAMPLE**

3. Using a pipettor, transfer ***whole***, unprocessed urine into the cryovials labeled:

'SU' to the right of the barcode

4. Replace the cap, firmly twisting in a clockwise direction.

Samples 2 & 3

5. Pour the remaining urine into the 4 x 15.0 mL centrifuge tubes in equal 12 milliliter portions (two tubes of each marked "EMPTY" and "Protease Inhibitor (PI)")

   E.g.:

   30 mL of spot urine → 2 – 12.0 mL centrifuge tubes for spinning (Use 1 'Empty' and 1 'PI' tube)

   50 mL of urine → 4 – 12.0 mL centrifuge tube; discard remaining urine (Use 2 of each, 'Empty' and 'PI')

NOTE: Use at least ONE EACH: "EMPTY" and "PI" centrifuge tubes

6. In the tubes labeled "PI", gently invert 8-10 times to dissolve the PI completely. Remove the cap to allow any air bubbles to escape, replace cap tightly.
7. Spin all 4 tubes at 1000 X G for 12 minutes in centrifuge

Sample 2 (AS Urine Spot): From the tubes labeled "EMPTY", transfer urine using a transfer pipettor into the 4 cryovials containing a pre-measured amount of 100 mM Sodium Azide (a biocide) labeled:

'AS' to the right of the barcode

8. When opening each cryovial, take care to place the cap directly in front of the respective tube to retain pre-measured volumes for consistent concentrations in each aliquot.
9. Pipette 1.6 mLs of urine or enough urine to fill the cryovial up to the first ridge on the cryovial (see Figure 1).
10. Replace the cap, firmly twisting in a clockwise direction. After all caps are replaced, invert tubes *at least 2 times* to distribute the sodium azide prior to freezing.

Sample 3 (AQ Urine Spot): From the tubes labeled "PI", transfer urine using a transfer pipettor into the cryovials labeled:

'AQ' to the right of the barcode

11. Pipette 1.6 mLs of urine or enough urine to fill the cryovial up to the first ridge on the cryovial (see Figure 1).

Samples 4 and 5

12. Pour off remaining supernatant from all of the 15.0 mL centrifuge tubes

13. For maximal drainage, gently invert centrifuge tubes on clean paper towels for 10 seconds, take care to not disturb pellet in the centrifuge tube tip.
14. With a clean pipette tip, transfer 25.0-50.0* μL RNA-Later into each centrifuge tube, take care to not touch the inside walls of the centrifuge tube.
15. Gently stir the mixture with the pipette tip, changing tips when moving between the 'Empty' tubes and the 'PI' tubes. **Do not pipette up and down to mix as this could break any cells present in the pellet.**

    Sample 4 (AP-E Urine Pellet): From the centrifuge tubes labeled "EMPTY", pipette one urine pellet from each centrifuge tube into the two cryovials labeled:

    'AP-E' to the right of the barcode

    Sample 5 (AP-Q Urine Pellet): From the centrifuge tubes labeled "PI", pipette one urine pellet from each centrifuge tube into the two cryovials labeled:

    'AP-Q' to the right of the barcode

16. Freeze all samples at -80 C.
17. Residual urine may be disposed per OSEH guidelines enforced at participating institution.

* Amount of RNA-Later will vary depending on size of pellet. RNA-Later should be added to completely submerse the pellet.

Shipping
Samples should be shipped according to the site-specific scheduled interval for shipments.

All specimens should be shipped together **on dry ice** using the shipping instructions found in Appendix N and in the study specific container provided by NEPTUNE.

Documentation
All corresponding samples for each participant ID should be stored together. If specimen does not adequately fill pre-determined number of aliquots, please document to minimize concerns regarding lost aliquots.

Complete the appropriate Biospecimen CRF corresponding to the study visit.

26.B.3. References

1. 15.0 mL orange-capped Corning centrifuge tubes: Fisher Scientific No. 05-538-53D, Corning No. 430052
2. Protease Inhibitor Cocktail: Sigma-Aldrich Catalog: P1860 – 1 mL
3. Cryovials: DOT Scientific Inc. No.: T334-6SPR
4. Sodium Azide (see attached MSDS)
5. Ambion RNA-Later Solution; P/N: Am7021
6. University of Michigan Occupations Safety and Environmental Health (OSEH) guidelines indicate washing urine and its debris down laboratory sink with adequate water.

Relevant Definitions: NaN_3: Sodium Azide

SIGMA-ALDRICH

sigma-aldrich.com

Material Safety Data Sheet

Version 4.1
Revision Date 01/19/2012
Print Date 11/29/2012

1. PRODUCT AND COMPANY IDENTIFICATION

Product name	:	Protease Inhibitor Cocktail
Product Number	:	P1860
Brand	:	Sigma
Supplier	:	Sigma-Aldrich 3050 Spruce Street SAINT LOUIS MO 63103 USA
Telephone	:	+1 800-325-5832
Fax	:	+1 800-325-5052
Emergency Phone # (For both supplier and manufacturer)	:	(314) 776-6555
Preparation Information	:	Sigma-Aldrich Corporation Product Safety - Americas Region 1-800-521-8956

2. HAZARDS IDENTIFICATION

Emergency Overview

OSHA Hazards
Combustible Liquid, Target Organ Effect

Target Organs

Eyes, Skin

GHS Classification
Flammable liquids (Category 4)

GHS Label elements, including precautionary statements

Pictogram	none
Signal word	Warning
Hazard statement(s)	
H227	Combustible liquid
Precautionary statement(s)	none

HMIS Classification

Health hazard:	0
Chronic Health Hazard:	*
Flammability:	2
Physical hazards:	0

NFPA Rating

Health hazard:	0
Fire:	2
Reactivity Hazard:	0

Potential Health Effects

Inhalation	May be harmful if inhaled. Causes respiratory tract irritation.
Skin	May be harmful if absorbed through skin. Causes skin irritation.

Eyes	Causes eye irritation.
Ingestion	May be harmful if swallowed.

3. COMPOSITION/INFORMATION ON INGREDIENTS

Component		Classification	Concentration
Dimethyl sulfoxide			
CAS-No. EC-No.	67-68-5 200-664-3		60 - 100 %

4. FIRST AID MEASURES

General advice
Consult a physician. Show this safety data sheet to the doctor in attendance.Move out of dangerous area.

If inhaled
If breathed in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician.

In case of skin contact
Wash off with soap and plenty of water. Consult a physician.

In case of eye contact
Rinse thoroughly with plenty of water for at least 15 minutes and consult a physician.

If swallowed
Do NOT induce vomiting. Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.

5. FIREFIGHTING MEASURES

Suitable extinguishing media
For small (incipient) fires, use media such as "alcohol" foam, dry chemical, or carbon dioxide. For large fires, apply water from as far as possible. Use very large quantities (flooding) of water applied as a mist or spray; solid streams of water may be ineffective. Cool all affected containers with flooding quantities of water.

Special protective equipment for firefighters
Wear self contained breathing apparatus for fire fighting if necessary.

Hazardous combustion products
Hazardous decomposition products formed under fire conditions. - Carbon oxides, Sulphur oxides

Further information
Use water spray to cool unopened containers.

6. ACCIDENTAL RELEASE MEASURES

Personal precautions
Use personal protective equipment. Avoid breathing vapors, mist or gas. Ensure adequate ventilation. Remove all sources of ignition. Beware of vapours accumulating to form explosive concentrations. Vapours can accumulate in low areas.

Environmental precautions
Prevent further leakage or spillage if safe to do so. Do not let product enter drains.

Methods and materials for containment and cleaning up
Contain spillage, and then collect with an electrically protected vacuum cleaner or by wet-brushing and place in container for disposal according to local regulations (see section 13). Keep in suitable, closed containers for disposal.

7. HANDLING AND STORAGE

Precautions for safe handling
Avoid contact with skin and eyes. Avoid inhalation of vapour or mist.
Keep away from sources of ignition - No smoking. Take measures to prevent the build up of electrostatic charge.

Conditions for safe storage
Keep container tightly closed in a dry and well-ventilated place.

Recommended storage temperature: -20 °C

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

Components with workplace control parameters

Components	CAS-No.	Value	Control parameters	Basis
Dimethyl sulfoxide	67-68-5	TWA	250 ppm	USA. Workplace Environmental Exposure Levels (WEEL)

Personal protective equipment

Respiratory protection
Where risk assessment shows air-purifying respirators are appropriate use a full-face respirator with multi-purpose combination (US) or type ABEK (EN 14387) respirator cartridges as a backup to engineering controls. If the respirator is the sole means of protection, use a full-face supplied air respirator. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

Hand protection
Handle with gloves. Gloves must be inspected prior to use. Use proper glove removal technique (without touching glove's outer surface) to avoid skin contact with this product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices. Wash and dry hands.

Eye protection
Safety glasses with side-shields conforming to EN166 Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU).

Skin and body protection
impervious clothing, The type of protective equipment must be selected according to the concentration and amount of the dangerous substance at the specific workplace.

Hygiene measures
Handle in accordance with good industrial hygiene and safety practice. Wash hands before breaks and at the end of workday.

9. PHYSICAL AND CHEMICAL PROPERTIES

Appearance

Form	liquid
Colour	no data available

Safety data

pH	no data available
Melting point/freezing point	no data available
Boiling point	no data available
Flash point	87 °C (189 °F)
Ignition temperature	no data available
Autoignition temperature	no data available
Lower explosion limit	no data available
Upper explosion limit	no data available
Vapour pressure	no data available
Density	no data available
Water solubility	no data available
Partition coefficient:	no data available

n-octanol/water	
Relative vapour density	no data available
Odour	no data available
Odour Threshold	no data available
Evaporation rate	no data available

10. STABILITY AND REACTIVITY

Chemical stability
Stable under recommended storage conditions.

Possibility of hazardous reactions
no data available

Conditions to avoid
Heat, flames and sparks.

Materials to avoid
no data available

Hazardous decomposition products
Hazardous decomposition products formed under fire conditions. - Carbon oxides, Sulphur oxides
Other decomposition products - no data available

11. TOXICOLOGICAL INFORMATION

Acute toxicity

Oral LD50
no data available

Inhalation LC50
no data available

Dermal LD50
no data available

Other information on acute toxicity
no data available

Skin corrosion/irritation
no data available

Serious eye damage/eye irritation
Eyes: no data available

Respiratory or skin sensitization
no data available

Germ cell mutagenicity

no data available

Carcinogenicity

IARC:	No component of this product present at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by IARC.
ACGIH:	No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by ACGIH.
NTP:	No component of this product present at levels greater than or equal to 0.1% is identified as a known or anticipated carcinogen by NTP.
OSHA:	No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by OSHA.

Reproductive toxicity

no data available

Teratogenicity

no data available

Specific target organ toxicity - single exposure (Globally Harmonized System)
no data available

Specific target organ toxicity - repeated exposure (Globally Harmonized System)
no data available

Aspiration hazard
no data available

Potential health effects

Inhalation	May be harmful if inhaled. Causes respiratory tract irritation.
Ingestion	May be harmful if swallowed.
Skin	May be harmful if absorbed through skin. Causes skin irritation.
Eyes	Causes eye irritation.

Synergistic effects
no data available

Additional Information
RTECS: Not available

12. ECOLOGICAL INFORMATION

Toxicity

no data available

Persistence and degradability
no data available

Bioaccumulative potential
no data available

Mobility in soil
no data available

PBT and vPvB assessment
no data available

Other adverse effects

no data available

13. DISPOSAL CONSIDERATIONS

Product
This combustible material may be burned in a chemical incinerator equipped with an afterburner and scrubber. Offer surplus and non-recyclable solutions to a licensed disposal company. Contact a licensed professional waste disposal service to dispose of this material.

Contaminated packaging
Dispose of as unused product.

14. TRANSPORT INFORMATION

DOT (US)

NA-Number: 1993 Class: CBL Packing group: III
Proper shipping name: Combustible liquid, n.o.s. (Dimethyl sulfoxide)
Marine pollutant: No
Poison Inhalation Hazard: No

IMDG
Not dangerous goods

IATA
Not dangerous goods

15. REGULATORY INFORMATION

OSHA Hazards
Combustible Liquid, Target Organ Effect

SARA 302 Components
SARA 302: No chemicals in this material are subject to the reporting requirements of SARA Title III, Section 302.

SARA 313 Components
SARA 313: This material does not contain any chemical components with known CAS numbers that exceed the threshold (De Minimis) reporting levels established by SARA Title III, Section 313.

SARA 311/312 Hazards
Fire Hazard, Chronic Health Hazard

Massachusetts Right To Know Components
No components are subject to the Massachusetts Right to Know Act.

Pennsylvania Right To Know Components

	CAS-No.	Revision Date
Dimethyl sulfoxide	67-68-5	2007-03-01

New Jersey Right To Know Components

	CAS-No.	Revision Date
Dimethyl sulfoxide	67-68-5	2007-03-01

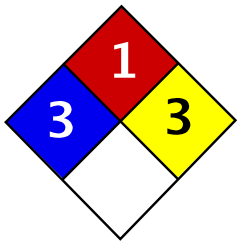
California Prop. 65 Components
This product does not contain any chemicals known to State of California to cause cancer, birth defects, or any other reproductive harm.

16. OTHER INFORMATION

Further information

The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide. The information in this document is based on the present state of our knowledge and is applicable to the product with regard to appropriate safety precautions. It does not represent any guarantee of the properties of the product. Sigma-Aldrich Corporation and its Affiliates shall not be held liable for any damage resulting from handling or from contact with the above product. See www.sigma-aldrich.com and/or the reverse side of invoice or packing slip for additional terms and conditions of sale.

ScienceLab.com
Chemicals & Laboratory Equipment



Health	3
Fire	1
Reactivity	3
Personal Protection	E

Material Safety Data Sheet
Sodium azide MSDS

Section 1: Chemical Product and Company Identification

Product Name: Sodium azide

Catalog Codes: SLS1363

CAS#: 26628-22-8

RTECS: VY8050000

TSCA: TSCA 8(b) inventory: Sodium azide

CI#: Not available.

Synonym:

Chemical Name: Hydrazoic Acid, Sodium Salt

Chemical Formula: NaN3

Contact Information:

Sciencelab.com, Inc.
14025 Smith Rd.
Houston, Texas 77396

US Sales: **1-800-901-7247**
International Sales: **1-281-441-4400**

Order Online: ScienceLab.com

CHEMTREC (24HR Emergency Telephone), call:
1-800-424-9300

International CHEMTREC, call: 1-703-527-3887

For non-emergency assistance, call: 1-281-441-4400

Section 2: Composition and Information on Ingredients

Composition:

Name	CAS #	% by Weight
Sodium azide	26628-22-8	100

Toxicological Data on Ingredients: Sodium azide: ORAL (LD50): Acute: 27 mg/kg [Rat]. 27 mg/kg [Mouse]. DERMAL (LD50): Acute: 20 mg/kg [Rabbit].

Section 3: Hazards Identification

Potential Acute Health Effects:
Very hazardous in case of skin contact (irritant), of eye contact (irritant). Hazardous in case of ingestion, of inhalation. Slightly hazardous in case of skin contact (permeator). Severe over-exposure can result in death. Inflammation of the eye is characterized by redness, watering, and itching. Skin inflammation is characterized by itching, scaling, reddening, or, occasionally, blistering.

Potential Chronic Health Effects:
CARCINOGENIC EFFECTS: Not available.
MUTAGENIC EFFECTS: Not available.
TERATOGENIC EFFECTS: Not available.
DEVELOPMENTAL TOXICITY: Not available.
Repeated exposure to an highly toxic material may produce general deterioration of health by an accumulation in one or many human organs.

Section 4: First Aid Measures

Eye Contact:
Check for and remove any contact lenses. Immediately flush eyes with running water for at least 15 minutes, keeping eyelids open. Cold water may be used. Do not use an eye ointment. Seek medical attention.

Skin Contact:
After contact with skin, wash immediately with plenty of water. Gently and thoroughly wash the contaminated skin with running water and non-abrasive soap. Be particularly careful to clean folds, crevices, creases and groin. Cold water may be used. Cover the irritated skin with an emollient. If irritation persists, seek medical attention. Wash contaminated clothing before reusing.

Serious Skin Contact:
Wash with a disinfectant soap and cover the contaminated skin with an anti-bacterial cream. Seek immediate medical attention.

Inhalation: Allow the victim to rest in a well ventilated area. Seek immediate medical attention.

Serious Inhalation:
Evacuate the victim to a safe area as soon as possible. Loosen tight clothing such as a collar, tie, belt or waistband. If breathing is difficult, administer oxygen. If the victim is not breathing, perform mouth-to-mouth resuscitation. Seek medical attention.

Ingestion:
Do not induce vomiting. Examine the lips and mouth to ascertain whether the tissues are damaged, a possible indication that the toxic material was ingested; the absence of such signs, however, is not conclusive. Loosen tight clothing such as a collar, tie, belt or waistband. If the victim is not breathing, perform mouth-to-mouth resuscitation. Seek immediate medical attention.

Serious Ingestion: Not available.

Section 5: Fire and Explosion Data

Flammability of the Product: May be combustible at high temperature.

Auto-Ignition Temperature: Not available.

Flash Points: Not available.

Flammable Limits: Not available.

Products of Combustion: Some metallic oxides.

Fire Hazards in Presence of Various Substances: Highly flammable in presence of shocks.

Explosion Hazards in Presence of Various Substances:
Risks of explosion of the product in presence of static discharge: Not available.
Highly explosive in presence of shocks, of metals.

Fire Fighting Media and Instructions:
SMALL FIRE: Use DRY chemical powder.
LARGE FIRE: Use water spray, fog or foam. Do not use water jet.

Special Remarks on Fire Hazards: Not available.

Special Remarks on Explosion Hazards: Not available.

Section 6: Accidental Release Measures

Small Spill: Use appropriate tools to put the spilled solid in a convenient waste disposal container.

Large Spill:
Use a shovel to put the material into a convenient waste disposal container. Be careful that the product is not present at a concentration level above TLV. Check TLV on the MSDS and with local authorities.

Section 7: Handling and Storage

Precautions:
Keep locked up Keep away from heat. Keep away from sources of ignition. Empty containers pose a fire risk, evaporate the residue under a fume hood. Ground all equipment containing material. Do not ingest. Do not breathe dust. Take precautionary measures against electrostatic discharges. In case of insufficient ventilation, wear suitable respiratory equipment If ingested, seek medical advice immediately and show the container or the label. Avoid contact with skin and eyes Keep away from incompatibles such as metals.

Storage:
Keep container dry. Keep in a cool place. Ground all equipment containing material. Keep container tightly closed. Keep in a cool, well-ventilated place. Highly toxic or infectious materials should be stored in a separate locked safety storage cabinet or room.

Section 8: Exposure Controls/Personal Protection

Engineering Controls:
Use process enclosures, local exhaust ventilation, or other engineering controls to keep airborne levels below recommended exposure limits. If user operations generate dust, fume or mist, use ventilation to keep exposure to airborne contaminants below the exposure limit.

Personal Protection:
Splash goggles. Lab coat. Dust respirator. Be sure to use an approved/certified respirator or equivalent. Gloves.

Personal Protection in Case of a Large Spill:
Splash goggles. Full suit. Dust respirator. Boots. Gloves. A self contained breathing apparatus should be used to avoid inhalation of the product. Suggested protective clothing might not be sufficient; consult a specialist BEFORE handling this product.

Exposure Limits: TWA: 0.29 (mg/m3) from ACGIHConsult local authorities for acceptable exposure limits.

Section 9: Physical and Chemical Properties

Physical state and appearance: Solid.

Odor: Not available.

Taste: Not available.

Molecular Weight: 65.02 g/mole

Color: Not available.

pH (1% soln/water): Not available.

Boiling Point: Not available.

Melting Point: Decomposes.

Critical Temperature: Not available.

Specific Gravity: 1.846 (Water = 1)

Vapor Pressure: Not applicable.

Vapor Density: Not available.

Volatility: Not available.

Odor Threshold: Not available.

Water/Oil Dist. Coeff.: Not available.

Ionicity (in Water): Not available.

Dispersion Properties: See solubility in water.

Solubility: Soluble in cold water.

Section 10: Stability and Reactivity Data

Stability: Unstable.

Instability Temperature: Not available.

Conditions of Instability: Not available.

Incompatibility with various substances: Extremely reactive or incompatible with metals.

Corrosivity: Non-corrosive in presence of glass.

Special Remarks on Reactivity: Not available.

Special Remarks on Corrosivity: Not available.

Polymerization: No.

Section 11: Toxicological Information

Routes of Entry: Eye contact. Inhalation. Ingestion.

Toxicity to Animals:
Acute oral toxicity (LD50): 27 mg/kg [Mouse].
Acute dermal toxicity (LD50): 20 mg/kg [Rabbit].

Chronic Effects on Humans: Not available.

Other Toxic Effects on Humans:
Very hazardous in case of skin contact (irritant).
Hazardous in case of ingestion, of inhalation.
Slightly hazardous in case of skin contact (permeator).

Special Remarks on Toxicity to Animals: Not available.

Special Remarks on Chronic Effects on Humans: Not available.

Special Remarks on other Toxic Effects on Humans: Not available.

Section 12: Ecological Information

Ecotoxicity: Not available.

BOD5 and COD: Not available.

Products of Biodegradation: Possibly hazardous short/long term degradation products are to be expected.

Toxicity of the Products of Biodegradation: The products of degradation are more toxic.

Special Remarks on the Products of Biodegradation: Not available.

Section 13: Disposal Considerations

Waste Disposal:

Section 14: Transport Information

DOT Classification: CLASS 6.1: Poisonous material.

Identification: : Sodium azide : UN1867 PG: II

Special Provisions for Transport: Not available.

Section 15: Other Regulatory Information

Federal and State Regulations:
Pennsylvania RTK: Sodium azide
Massachusetts RTK: Sodium azide
TSCA 8(b) inventory: Sodium azide
SARA 302/304/311/312 extremely hazardous substances: Sodium azide
SARA 313 toxic chemical notification and release reporting: Sodium azide
CERCLA: Hazardous substances.: Sodium azide

Other Regulations: OSHA: Hazardous by definition of Hazard Communication Standard (29 CFR 1910.1200).

Other Classifications:

WHMIS (Canada): CLASS D-1A: Material causing immediate and serious toxic effects (VERY TOXIC).

DSCL (EEC):
R38- Irritating to skin.
R41- Risk of serious damage to eyes.

HMIS (U.S.A.):

Health Hazard: 3

Fire Hazard: 1

Reactivity: 3

Personal Protection: E

National Fire Protection Association (U.S.A.):

Health: 3

Flammability: 1

Reactivity: 3

Specific hazard:

Protective Equipment:
Gloves.
Lab coat.
Dust respirator. Be sure to use an approved/certified respirator or equivalent. Wear appropriate respirator when ventilation is inadequate.
Splash goggles.

Section 16: Other Information

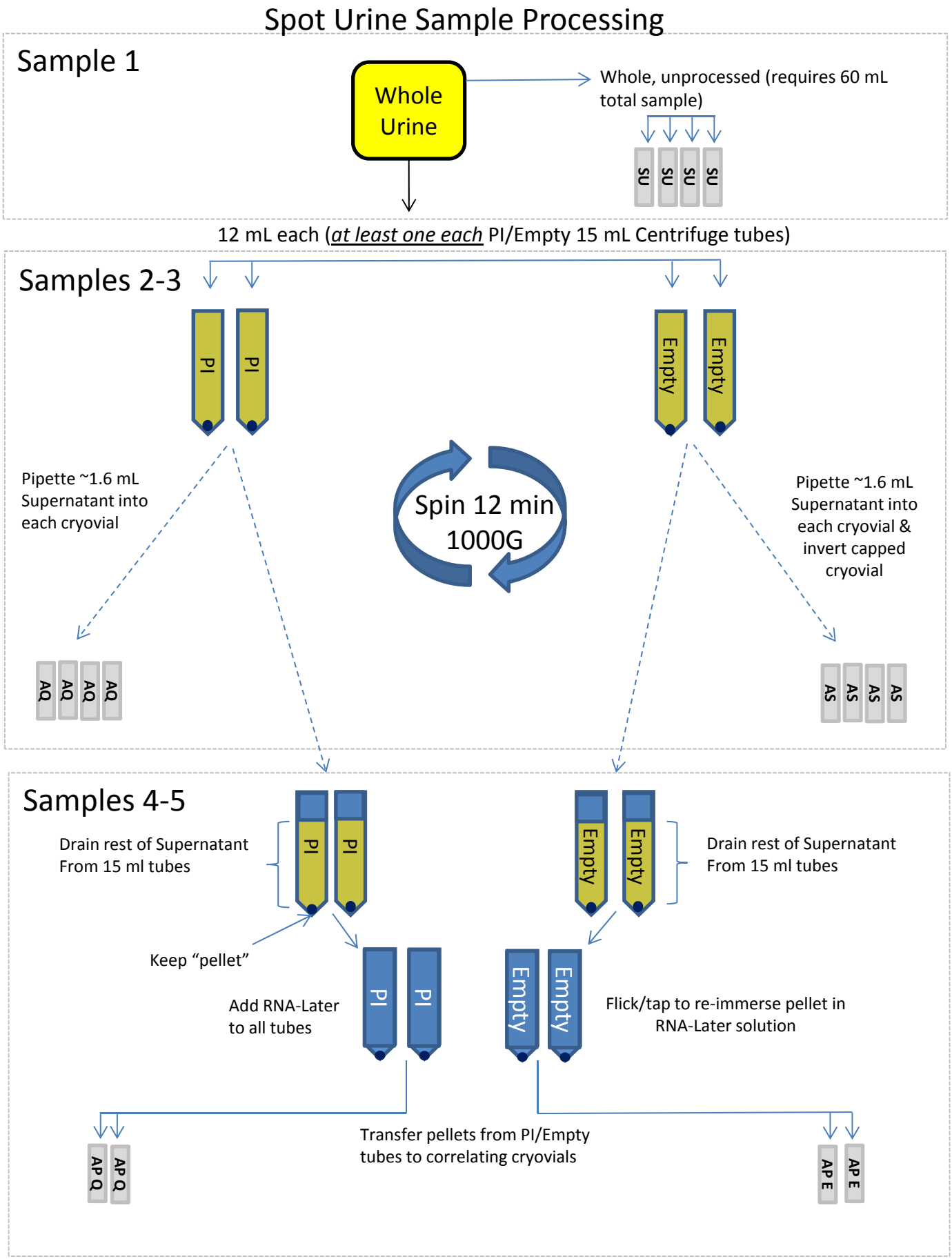
References: Not available.

Other Special Considerations: Not available.

Created: 10/11/2005 12:32 PM

Last Updated: 11/06/2008 12:00 PM

The information above is believed to be accurate and represents the best information currently available to us. However, we make no warranty of merchantability or any other warranty, express or implied, with respect to such information, and we assume no liability resulting from its use. Users should make their own investigations to determine the suitability of the information for their particular purposes. In no event shall ScienceLab.com be liable for any claims, losses, or damages of any third party or for lost profits or any special, indirect, incidental, consequential or exemplary damages, howsoever arising, even if ScienceLab.com has been advised of the possibility of such damages.


Spot Urine Sample Processing
Sample 1
Whole Urine
Whole, unprocessed (requires 60 mL total sample)
SU
SU
SU
SU
12 mL each (at least one each PI/Empty 15 mL Centrifuge tubes)
Samples 2-3
PI
PI
Empty
Empty
Pipette ~1.6 mL Supernatant into each cryovial
Spin 12 min 1000G
Pipette ~1.6 mL Supernatant into each cryovial & invert capped cryovial
AQ
AQ
AQ
AQ
AS
AS
AS
AS
Samples 4-5
Drain rest of Supernatant From 15 ml tubes
PI
PI
Empty
Empty
Drain rest of Supernatant From 15 ml tubes
Keep "pellet"
Add RNA-Later to all tubes
PI
PI
Empty
Empty
Flick/tap to re-immerse pellet in RNA-Later solution
Transfer pellets from PI/Empty tubes to correlating cryovials
AP Q
AP Q
AP E
AP E

Procedure Class:	SPECIMENS
Procedure:	BIO-24-Urine (U, UQ)

26.C.1. UPDATES TO 24-HOUR URINE SAMPLE PROCESSING PROCEDURES

Effective 11/2012

1. Research Coordinators (RCs) will no longer fill sets of centrifuge tubes or cryovials with equal volumes of sample. Effective 11/2012 please fill each tube/cryovial with the maximum volume (40.0 mL and 4.5 mL, respectively) in sequential order (based on the aliquot number indicated on barcoded label) until all corresponding urine sample has been aliquotted. Discard remaining empty cryovials on-site.

2. When transferring urine from the original collection container or the processing tube for freezer storage, it is imperative that these containers are not overfilled. The following procedures take into account liquid expansion when samples freeze.

   When transferring 24-hour samples into the 50 and 5 mL storage tubes, it is imperative that the tubes are not overfilled. The following directions take into account urine volume expansion when frozen.

   a) 50 mL centrifuge tubes: Do not fill beyond 40 mLs of urine (Figure 1)
   b) 5 ml cryovials: Do not fill beyond 4.5 mls of urine (Figure 2)

(Please references the images below)

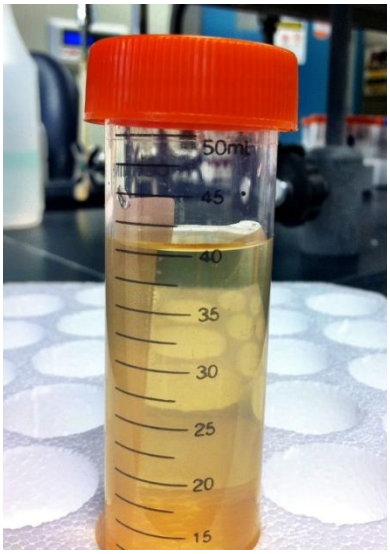


Figure 1: 50 mL centrifuge tube
Filled properly with 40 mLs of urine

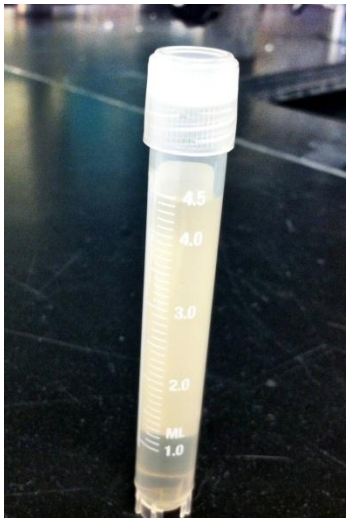


Figure 2: 5.0 mL cryovial
Filled properly with 4.5 mLs of urine

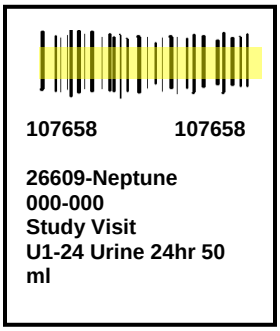
Procedure Class:	SPECIMENS
Procedure:	BIO-24-Urine (U, UQ)

26.C.2. 24-Hour or Timed Urine Processing Procedure

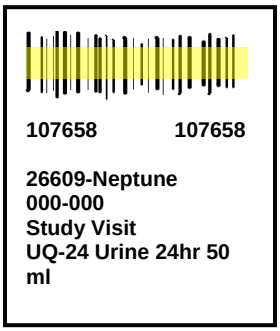
Procedure Overview
This procedure describes the process for preparing aliquots of the 24-hour urine sample for NEPTUNE storage at -80° C. Shipping details are provided in Appendix N of the NEPTUNE Manual of Procedures.

Required Supplies:
Included in kit:

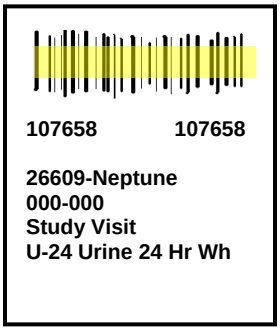
- 2 x 50 mL orange top centrifuge tubes
- 5 x 5.0 mL cryovials
  (Pre-labeled as follows)
- 1 pre-labeled, empty 50 mL centrifuge tube[1] labeled as follows:



- 1 pre-labeled, 50 mL centrifuge tube containing 6 μL of Protease Inhibitor[2]



- 5 pre-labeled 5.0 mL cryovials[3] numbered as follows:



- Empty 24-hour urine container for tare

Provided by site:

- 24-hour urine sample from study participant
- Scale
- Pipettes and tips or disposable pipettes (10 ml, 5 ml, 1 ml sizes) or plastic transfer pipettes
- Gloves, goggles, and lab coat

Required Equipment:

- University of Michigan OSEH[4] guidelines designate all work done with biological specimens that could produce spray from pipetting be done in a hood, please refer to your site specific OSEH policies.

Responsible Individuals
The NEPTUNE Research Coordinator is responsible for retrieving urine sample from consented participants, returning sample to lab, and processing for storage. In the absence of the Research Coordinator, the Site PI or designated, NEPTUNE trained alternate would provide this service. Local Research Coordinators need to be vigilantly aware of the participant's clinical care needs. Determine in advance if the 24-hour urine is also required for clinical care and coordinate the research sample aliquots appropriately.

Procedure

1. The participant should have been instructed on collecting a 24-hour urine sample. See Appendix K for detailed instructions on 24-hour urine collection.
2. Note start and end time for a maximum timed collection, or "first morning urine" or "random urine collection" for participants unable to provide a full 24-hour sample.

If processing is not able to be completed immediately, please store on ice or refrigerate for a maximum of 4 hours.

3. Weigh the full 24-hour urine collection and note the weight on the study visit worksheet.
4. Weigh the empty 24-hour urine collection container provided for *tare* and enter the tare value on the study visit worksheet.
5. If 24-hour urine sample is larger than one container, the samples must be combined into a single, total urine collection prior to aliquotting. (NOTE: If 2 containers are used, the tare weight must be doubled on the worksheet).

No processing should be done to the 24-hour urine sample – this should be whole, unprocessed/unspun urine.

6. Pour off two 40.0 mL aliquots into the pre-labeled centrifuge tubes:
U1-24 Urine 24hr 50 ml and UQ-24 Urine 24hr 50 ml
7. Gently invert tube **UQ1** 8-10 times to allow dissolution of the protease inhibitor. Remove the cap to allow any air bubbles to escape, replace cap tightly. Place samples aside to complete processing the whole urine sample.
8. Pipette 5 aliquots of 4.5 mLs from the 24-hour urine sample, into the cryovials labeled:
U-24 Urine 24hr Wh
9. Replace the caps, firmly twisting in a clockwise direction.
10. If residual urine does not need to be returned to local labs for clinical care, it may be disposed per OSEH guidelines enforced at participating institution.
11. Note time processed and freeze at -80° C.

Shipping:
Samples should be shipped according to the site-specific scheduled interval for shipments.

All corresponding specimens for each participant ID and visit should be shipped together **on dry ice** following directions in Appendix N for shipping and container provided by NEPTUNE.

Documentation
All corresponding samples for each participant ID should be stored together. If specimen does not adequately fill pre-determined number of aliquots, please document to minimize concerns regarding lost aliquots.

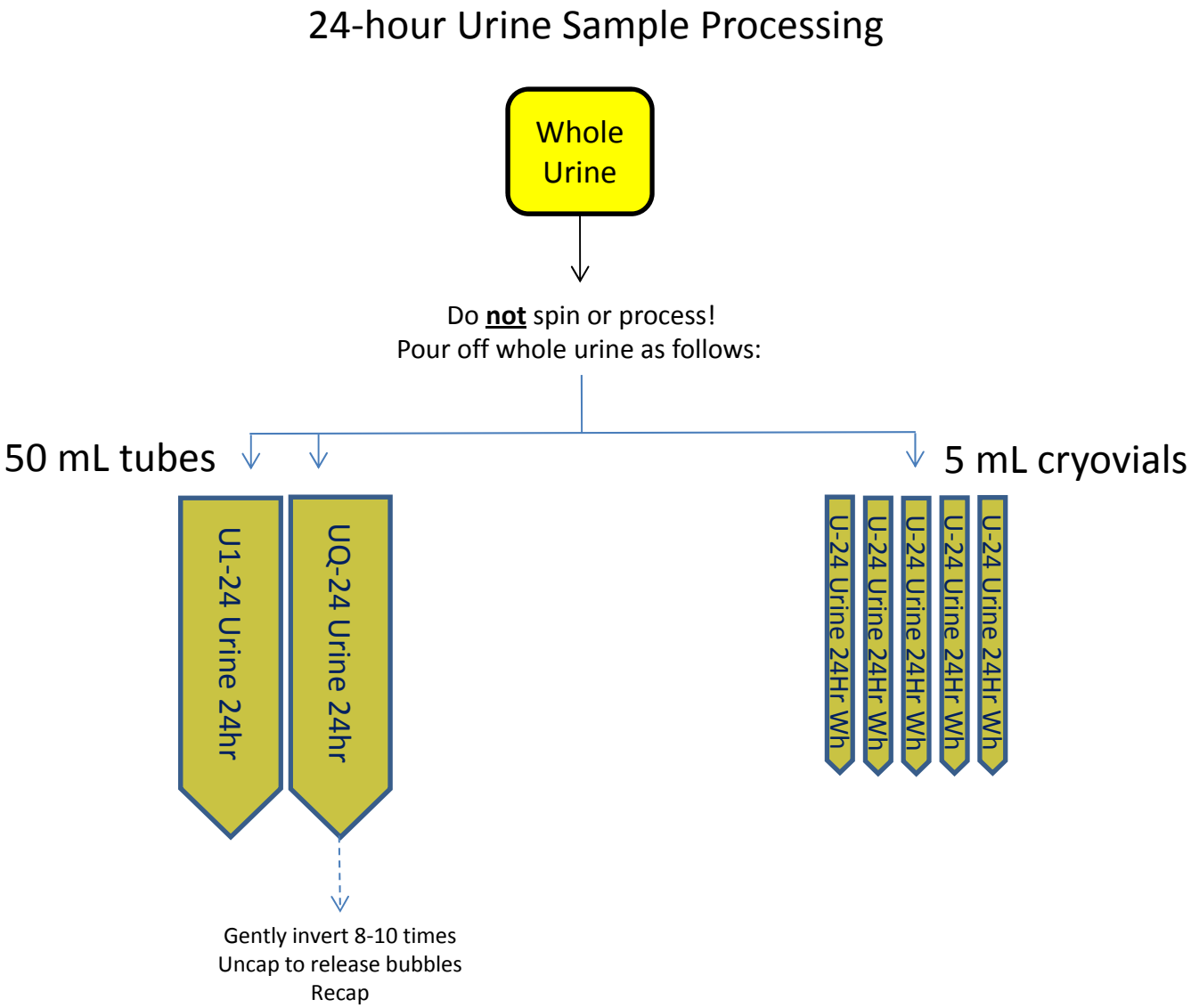
Complete the appropriate Biospecimen CRF corresponding to the study visit, noting any deviations from specimen collection or processing.

26.C.3. References

1. 50.0 mL orange-capped Corning Centrifuge tubes: Corning no. 43290
2. Protease Inhibitor Cocktail: Sigma-Aldrich Catalog: P1860 – 1 mL
3. 5.0 mL Cryovials: Fisher Scientific No. 12-565-167N
4. University of Michigan Occupations Safety and Environmental Health (OSEH) guidelines indicate washing urine and its debris down laboratory sink with adequate water.

Relevant Definitions - None

26.C.4. For Protease Inhibitor Cocktail MSDS See 26.B.4.


24-hour Urine Sample Processing
Whole Urine
Do not spin or process!
Pour off whole urine as follows:
50 mL tubes
5 mL cryovials
U1-24 Urine 24hr
UQ-24 Urine 24hr
U-24 Urine 24Hr Wh
U-24 Urine 24Hr Wh
U-24 Urine 24Hr Wh
U-24 Urine 24Hr Wh
U-24 Urine 24Hr Wh
Gently invert 8-10 times
Uncap to release bubbles
Recap

27. Appendix N. Shipping

27.A. Shipments with Biological Substances Category B ("Full" specimen kits)

For the purposes of NEPTUNE, shipments with "Biological Substances, Category B" will be sent from participating NEPTUNE sites to the University of Michigan.

These shipments contain processed blood, urine and kidney tissue specimens for use by the NEPTUNE study.

27.B. Maximum Kits Per Shipment

Shipments may contain up to 4 baseline kits and/or 6 return visits for a **maximum combined total of up to 6 kits per shipment**. More than one visit from the same participant should not be combined in a single shipment. Shipments containing "full" specimen kits will be packaged in the following manner.

27.C. Items Required for Proper Shipment

27.C.1. Study Provided

- NEPTUNE styrofoam box
- NEPTUNE cardboard box
- 95 kPa pressure tested bags
- Specimen kits sealed in provided biohazard specimen bags with orange study ID inserts
- NEPTUNE Shipping Manifest
- Dry Ice and Biological Substances, Class B Labels

27.C.2. Site Provided

- 4.5 kg of dry ice (about the weight of a gallon of milk)
- Packing tape
- Absorbent material (e.g. paper towel)

27.D. Shipment Preparation Instructions

1. Wrap PAXgene tubes individually in absorbent material (paper towel or absorbent sleeves sent with the NEPTUNE kits).
2. Place wrapped PAXgene tubes and blood cryovials in the **"Blood"** (red barcoded) biohazard specimen bag and all urine cryovials and tubes in the **"Urine"** (yellow barcoded) biohazard specimen bag
3. Place absorbent material (paper towel) in the specimen bags and close by pressing the zipper seal.
4. Place the pre-labeled specimen bags into a 95 kPa pressure tested bag and seal per the manufacturer's instructions on page 4).
5. Place ½ of the total dry ice at the bottom of the Styrofoam box.
6. Place the specimen-filled 95 kPa pressure tested bags on top of the dry ice.
7. Place the remaining ½ of dry ice on top of the 95 kPa pressure tested bags.
8. Place the lid on the Styrofoam box.

Do not tape the Styrofoam box shut!

9. Place the Styrofoam box into the cardboard box.
10. If nail specimens need to be shipped, they are to be placed **outside and on top of the Styrofoam box**, prior to taping the cardboard box closed.
11. Close and tape the cardboard box for shipping while making sure **not** to seal all box edges. This will allow CO2 released from the dry ice to escape the package.
12. Weigh the cardboard box and note the weight.
13. Attach the Biological Specimens Category B and Dry Ice labels to the cardboard box.
14. Record the weight of the dry ice on the Dry Ice label.

27.E. UPS Shipment Instructions – Biological Specimens

The NEPTUNE Data Analysis and Coordinating Center has created an account for shipping NEPTUNE materials via UPS, with access for creating and billing shipments through the internet. If your site is not scheduled for regular UPS pick-ups, please schedule a pick-up as indicated in the on-line shipment guide below.

27.E.1. Creating a UPS Shipment for pick-up and invoice

1. Go to UPS link:
   https://www.campusship.ups.com/login/umstrategiccon
2. Sign in using the following information:
   Username: boridley
   Password: NEPTUNE

   You will be prompted by the "Begin Your Shipment" box.
3. Box #1
   a) "Where is the shipment going?"
   b) Select the shipment's destination "NEPTUNE Biorepository" by using the drop down box located under "Address Book."
4. Box #2
   c) "Where is the shipment coming from?"
   d) Click on the "Edit" link:
   Using the drop down box located under "Address Book", select your location and name. Make sure the address is correct. If not correct, please contact Kyle at spottsk@umich.edu
   e) Scroll down and click on the "Update" button at the bottom right-hand side of the "Address Information" box.

   You should now see the "Begin Your Shipment" box.
5. Box #3
   f) "What are you shipping?"
   g) Select the number of packages from the drop down box.
   h) Under "Packaging Type", select "Other Packaging".
   i) Under "Weight" Enter the weight of your package in pounds (lbs.)
6. Box #4
   j) "How would you like to ship?"
   k) Using the drop down box that says "Select Service," choose Next Day Air. All Category B Biological Substances must be sent via Next Day Air.
   l) Make sure the "Send E-mail Notifications" and "Dry Ice" boxes have check marks.

7. Box #5 “Would you like to add reference numbers to this shipment?”
   Enter 062136 under “Short code Required” if it didn’t automatically populate.
8. Box #6 “How would you like to pay?”
   Select “A07R07 – Internal Medicine/Nephrology” if it didn’t automatically populate
9. Box #7 “Would you like to schedule a pick-up?”
   If your site has a daily UPS shipment pick-up, please use it. Otherwise, you will need to check the “Schedule a Pickup” box or drop off the package at a UPS store.

   Click “Next”
10. A new #1 box “Send E-mail Notifications Using Quantum View Notify” should appear
    Enter your email address, the person you are shipping to, and spottks@umich.edu into the fields if not automatically populated.
    Check the “Ship”, “Exception” and “Delivery” column boxes for all the email addresses.
11. Under “Personal E-mail Message:” enter a list of all kits contained within the shipment.
    For Example: BL: 000-007, 001-008 RV5: 000-003 RV6: 000-001
12. Box #2 Dry Ice
    Enter the amount of dry ice added to the shipping box in lbs or kg. The amount of dry ice required is 4.5kg or 9.9lbs (about the weight of a gallon of milk).

    Click “Next”
13. A screen will prompt you to “Review your shipment details.” Please make sure all of the information on this page is correct then click “Ship Now” at the bottom right corner of the box.
14. Your printer dialogue box should appear (pop-ups must be enabled).
15. Please print the shipping label and the receipt.
16. Cut or fold the shipping label to fit the NEPTUNE box.
17. Tape the shipping label to the top of the NEPTUNE box.
18. Keep the receipt for your records.
19. Your package is ready to ship!

CLOSING INSTRUCTIONS
FOR THESE VONSEAL® SPECIMEN SHIPPING BAGS

The specimen shipping bags in this carton are designed to perform at high internal pressure should an airliner lose it's cabin pressure.

In order for the bag to perform to it's design capabilities, it is imperative that the bag be closed properly after insertion of the specimen.

PLEASE FOLLOW THE PROCEDURES ILLUSTRATED BELOW

FIG. #1

Figure 1: shows the bag laying flat on a counter with the tape side up and the tape end towards the person closing the bag.

FIG. #2

Figure 2: shows the specimen at the bottom of the bag and the area above the specimen being flattened in preparation for the adhesive tape to fold over.

FIG. #3

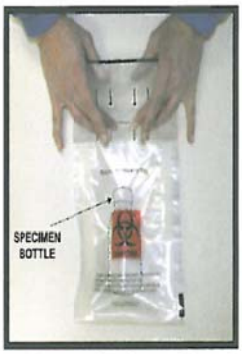


Figure 3: shows the bag after the paper adhesive cover has been removed and with the middle fingers touching the adhesive at the middle of the bag below the slit opening. It also shows the thumbs under the end of the bag ready to fold the top of the bag over.

FIG. #4

Figure 4: shows the bag after the top is folded at the slit opening and the thumbs pressing the folded end of the bag.

FIG #5

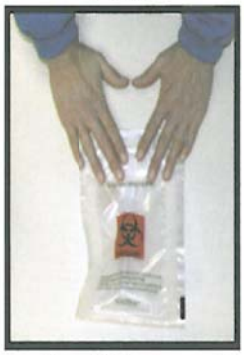


Figure 5: shows the middle finger of both hands being used to press the adhesive together. Start at the center of the fold and work outward. Please be sure to firmly press the entire adhesive area, especially the folded edge and corners.

This is a high performance bag. Thank you for making the effort to close it properly.

27.F. Pathology Slide/Materials Shipping

For the purposes of NEPTUNE, biopsy slide shipments will be sent from participating NEPTUNE sites to the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK).

These shipments are considered non-biological specimens. Dry ice and special shipping precautions are not necessary.

Please be sure pathology materials are properly de-identified, including any letterhead information, physician names, etc. as specified in 15. Appendix B Pathology Manual of Procedures.

27.G. Items Required for Proper Shipment

27.G.1. Study Provided

- Padded, bubble mailer
- Slide holders
- Blank CD

27.G.2. Site Provided

- Study labeled and de-identified NEPTUNE participant slides
- Study labeled CD with de-identified EM and path report data
- Hardcopy de-identified pathology report with Participant Study ID

27.H. Shipment Preparation Instructions

1. Place labeled and de-identified slides in provided slide holders
2. Label each slide holder with the correct participant ID
3. Place all slide holders that will comfortably fit in the provided bubble mailing envelope and seal.

27.I. UPS Shipment Instructions – Non-Biological Specimens

The NEPTUNE Data Analysis and Coordinating Center has created an account for shipping NEPTUNE materials via UPS, with access for creating and billing shipments through the internet. If your site is not scheduled for regular UPS pick-ups, please schedule a pick-up as indicated in the shipment guide below.

27.I.1. Creating a UPS Shipment for pick-up and invoice

1. Go to UPS link:
   https://www.campusship.ups.com/login/umstrategiccon

2. Sign in using the following information:
   Username: boridley
   Password: NEPTUNE

   You will be prompted by the “Begin Your Shipment” box.

3. Box #1
   “Where is the shipment going?”
   m) Select the shipment’s destination “**Lisa Swearinger - NIH”** by using the drop down box located under “**Address Book**.”

4. Box #2
   "Where is the shipment coming from?"
   n) Click on the "Edit" link:

      Using the drop down box located under "Address Book", select your location and name. Make sure the address is correct. If not correct, please contact Kyle at spottsk@umich.edu
   o) Scroll down and click on the "Update" button at the bottom right-hand side of the "Address Information" box.

   You should now see the "Begin Your Shipment" box.

5. Box #3
   "What are you shipping?"
   p) Select the number of packages from the drop down box.
   q) Under "Packaging Type", select "Other Packaging".
   r) Under "Weight" Enter the weight of your package in pounds (lbs.)

6. Box #4
   "How would you like to ship?"
   s) Using the drop down box that says "Select Service," please select "ground".
   t) Make sure the "Send E-mail Notifications" box has a check mark.

7. Box #5
   "Would you like to add reference numbers to this shipment?"
   u) Enter 062136 under "Short code Required" if this information did not auto-populate.

8. Box #6
   "How would you like to pay?"
   v) Select "A07R07 – Internal Medicine/Nephrology" if this information did not auto-populate.

9. Box #7
   "Would you like to schedule a pick-up?"
   w) If your site has a daily UPS shipment pick-up, please use it. Otherwise, you will need to check the "Schedule a Pickup" box or drop off the package at a UPS store.

   Click "Next"

10. A new #1 box "Send E-mail Notifications Using Quantum View Notify" should appear
    If not automatically populated, enter:
    x) Your email address
    y) The person you are shipping to (Lisa Swearinger - NIH), and spottsk@umich.edu into the fields if not automatically populated.
    z) Check the "Ship", "Exception" and "Delivery" column boxes for all the email addresses.

11. Under "Personal E-mail Message:" enter a list of all participant ID slides contained within the shipment.
    For Example: 000-001 through 000-004

Click “Next”

12. A screen will prompt you to “Review your shipment details.” Please make sure all of the information on this page is correct then click “Ship Now” at the bottom right corner of the box.

13. Your printer dialogue box should appear (you may need to enable pop-ups if previously disabled).

14. Please print the shipping label and the receipt.

15. Cut or fold the shipping label to fit the bubble mailer.

16. Tape the shipping label to the top of the bubble mailer.

17. Keep the receipt for your records.

18. Your package is ready to ship!

27.J. International Shipping

Please discuss with the NEPTUNE DACC for international shipping considerations.