Manual of Operations

Anti-CD20 Trial

DRAFT

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TrialNet Anti-CD20 Trial Manual of Operations

Chapter 1. Anti-CD20 Trial

1. Overview

The purpose of the Anti-CD20 Trial is to assess the safety, efficacy, and mode of action of rituximab, anti-CD20 monoclonal antibody, for the treatment of individuals with new onset type 1 diabetes. This trial is implemented by the Type 1 Diabetes TrialNet at participating clinical sites.

The Anti-CD20 Trial is a two-arm, multicenter, randomized, double-masked, placebo-controlled comparison of rituximab versus placebo. The study design involves 2 arms with 44 participants in the active treatment group and 22 participants in the placebo group. The study will include a total of 66 evaluable participants, ages 8-45 with new onset type 1 diabetes. Study participants enrolled will be those who meet the eligibility criteria and provide written informed consent.

Study Outcomes:
The primary outcome of each participant is the area under the stimulated C-peptide curve over the first 2 hours of a 4-hour mixed meal tolerance test (MMTT) conducted at the one-year visit.

The secondary outcome of this research study will involve the development and examination of surrogate markers for immunologic effects of the treatment on disease-specific outcomes and immunological outcomes.

1.1. Mechanistic Studies

Throughout the Anti-CD20 Trial, blood will also be collected for mechanistic studies. These studies will measure, (but are not necessarily limited to) expression of RNA and its protein products (proteomics), T-lymphocyte functional assessment including cytokine expression, DNA for genotyping for

novel diabetes-associated genes, and pancreatic autoantibody isotypes. These mechanistic samples will be maintained at the NIDDK Repository site.

Chapter 2. Project Organization

1. Committees

1.1. Steering Committee

The Steering Committee has overall responsibility for the design, planning, execution, and publication of the research performed by the TrialNet Study Group. The Steering Committee will approve all protocols, changes to protocols, and study procedures.

1.2. Executive Committee

The Executive Committee manages the day-to-day operations of TrialNet, and coordinates the fiscal aspects of the grants funding the TrialNet and advises the Steering Committee of its actions. The Executive Committee, together with NIDDK, is responsible for the addition or the deletion of Clinical Centers.

1.3. TrialNet Coordinating Center (TNCC)

The TrialNet Coordinating Center has both scientific and administrative functions. In providing scientific support, the TNCC provides overall leadership to the TrialNet study group to include protocol and manual preparation and development; manuscript coordination, tracking, submission; developing meeting agendas; and overseeing the performance of quality control audits. Scientific functions also include review of all proposed protocols and development of statistical design for each study; analysis of study results; review of all manuscripts for statistical considerations; development and testing of predictive models for disease progression; and conduct of statistical research to meet the needs of the study. Additional

scientific functions are providing for central registration and random assignment of all participants enrolled in trials; preparation of data management aids; maintaining participant and protocol files; providing statistical reports on progress of trials at all meetings; and serving on all TrialNet administrative committees.

Administrative functions include coordinating interactions among the Clinical Centers, Core Laboratories, Committees, and NIDDK; financial management; interactions with sponsoring agencies; communication with TrialNet membership, including circulation of TrialNet information, newsletters, operations manuals, protocols, manuscripts; maintenance of membership rosters and committee lists.

1.4. Core Laboratories

The TNCC is responsible for the coordination of all laboratories used in the TrialNet studies. These laboratories are responsible for measurement of the critical variables in the study protocols. The director of each laboratory will represent their laboratory at TrialNet meetings.

During the study, each laboratory is responsible for storage of additional material from specimens for future testing or in the event re-testing becomes necessary. Each lab is required to participate in the Split Duplicate Quality Assurance/ Quality Control Program being developed for all laboratories participating in TrialNet studies.

The core laboratories for the Anti-CD20 Trial include:

1.4.1. Core Screening Laboratory (ICA Lab)

The Core Screening Laboratory serves as the central laboratory for measurement of Islet Cell Autoantibodies by Indirect Immunofluorescence. The Core Screening Laboratory for TrialNet is:

University of Florida
Department of Pathology, Immunology & Laboratory Medicine
4800 SW 35th Drive
Gainesville, Florida 32608
Phone: (352) 265-9900
Fax: (352) 265-9901
Director: William Winter, MD

1.4.2. Core HLA/DNA Extraction Laboratory

This laboratory extracts and preserves DNA from all participants staged for eligibility for enrollment in intervention protocols, and determines the presence or absence of certain HLA genotypes as genetic markers that may influence susceptibility or progression of IDDM.

UCHSC at Fitzsimons
Attn: Sunanda Babu PhD
1775 N. Ursula Street, M20-4201 C
Aurora, CO 80045
Phone: (303) 724-6806
Fax: (303) 315-4892
Director: George Eisenbarth, MD, PhD.

1.4.3. Core Autoantibody Laboratory (BAA Lab)

The Core Autoantibody Laboratory serves as the central laboratory for testing for the presence of serum antibody levels for one or more of anti-GAD65, ICA512, anti-insulin (IAA) and/ or islet cell cytoplasmic autoantibodies (ICA).

University of Colorado Health Science Center
Barbara Davis Center for Childhood Diseases
Attn: Liping Yu
1775 N. Ursula Street, M20-4201 E
Aurora, CO 80045
Phone: (303) 724-6809
Director: Liping Yu MD

1.4.4. Core β-Cell Function Laboratory

The Core Beta Cell Function Core Laboratory serves as the central laboratory for assessment of Beta Cell Function by measuring Immunoreactive Insulin and C-peptide, Plasma Glucose, and Glycosylated Hemoglobin (HbA1c).

Specimen Processing, Northwest Lipid Research Laboratories
401 Queen Anne Avenue North
Seattle, Washington 98109
Phone: (206) 685-3327
Director: Jerry Palmer, MD

1.4.5. Core Biochemistry Laboratory

The Core Biochemistry Laboratory serves as the central laboratory for assessment of Comprehensive Blood Chemistry Panels and Immunology Panels. This lab also performs serological testing for Hepatitis B and C, and HIV.

Specimen Processing, Northwest Lipid Research Laboratories
401 Queen Anne Ave., North
Seattle, WA 98109
Phone: (206) 685-3327
Director: Santica Marcovina, PhD, ScD

1.4.6. Core Viral Laboratory

The Core Viral Laboratory serves as the central laboratory for Serological testing, assessment of Tetanus and Hepatitis A antibody, and EBV/CMV Monitoring.

University of Colorado Hospital
Clinical Lab – LOB room 253
12401 E. 17th Ave.
Aurora, CO 80045
Phone: (720) 848-4401
Director: Adriana Weinberg, MD

1.5. Contract Laboratories

The contract laboratories for the Anti-CD20 Trial include:

1.5.1. Covance Laboratory

Covance Laboratory serves as the contract laboratory for measuring the serum levels of antibody to rituximab in specimens collected at the clinical sites.

Covance Laboratories Inc.
3635 Concorde Parkway, Suite 100
Chantilly, VA 20151
Phone: (703) 245- 2200 ext. 5478
Director: Michael Hirsch, MD

1.5.2. The Molecular Diagnostics Laboratory

The Molecular Diagnostics Laboratory serves as the contract laboratory for supplying phiX174 (phage) for the Anti-CD20 Trial and for measuring the serum levels of antibody to phiX174 (phage) in specimens collected at the clinical sites.

The Molecular Diagnostics Laboratory
Attn: Marge Young
307 Westlake Ave. N., Suite 300
Seattle, WA 98109
Phone: (206) 987- 7442
Director: Hans Ochs, MD

1.5.3 The University of Alabama, Division of Clinical Immunology and Rheumatology, HLA Laboratory

The HLA Laboratory serves as the contract laboratory for identifying the Fc Receptor genotypes of study participants. DNA specimens will be shipped, batched and frozen, from the core HLA laboratory in Colorado to the FcR laboratory for typing.

University of Alabama at Birmingham
Division of Clinical Immunology and Rheumatology
Attn: Deborrah McDuffie and Lifeng Zhang
c/o Jeffrey C. Edberg, PhD
SIBR 276
1825 University Boulevard
Birmingham, Alabama 35294
Phone: (205) 996- 4478
Director: Jeff C. Edberg, PhD
Director: Robert P. Kimberly, MD

1.5.4. Roswell Park Cancer Institute Flow Core

The Roswell Park Cancer Institute Flow Core serves as the contract laboratory for identifying antigenic markers on the surface of lymphocytes isolated from specimens collected from clinical sites. This laboratory uses immunophenotyping monoclonal antibody panels for flow cytometric analysis.

Laboratory of Flow Cytometry
Roswell Park Cancer Institute
Elm and Carlton Streets
Buffalo, NY 14263
Phone: (716) 845- 8471
Director: Paul K. Wallace, PhD

1.6. T Cell Laboratories

The T cell laboratories for the Anti-CD20 Trial include:

1.6.1. Cytokine ELISpot Laboratory

This laboratory will serve as the central laboratory for identifying and enumerating cytokine secreting peptide-reactive T cells from specimens collected at clinical sites. Interleukin-10 production will also be monitored by cytokine ELISpot, which may demonstrate the presence of cells with a regulatory phenotype.

UCHSC, BDC
Attn: Rebecca Wagner
1775 N. Ursula Street
Room 4201U
Aurora, CO 80045
Phone: (303) 724- 6804
Director: Peter Gottleib, MD

1.6.2. Cellular Immunoblot Laboratory

This laboratory will serve as the central laboratory for measuring study participant's T cell proliferative responses to a pool of antigens prepared from human islet cells.

VA Medical Center
1660 S. Columbia Way
Building 1, Room 609
Seattle, WA 98108
Phone: (206) 764- 2696 or (206) 764- 2616
Director: Barbara Brooks- Worrell, PhD

1.6.3. Tetramer Laboratory

This laboratory will perform a MHC Class II/Peptide Tetramer study specific for HLA-DR4 and DR3 alleles, and GAD65 and proinsulin peptides. These peptide tetramers will identify the presence of effector CD4+ T cells specific for a given complex of peptide fragments of antigen (GAD65 and proinsulin) bound to self MHC molecules (HLA-DR4 and HLA-DR3).

Clinical Core Lab
Benaroya Research Institute
1201 Ninth Avenue
Seattle, WA 98101
Phone: (206) 341- 1986
Director: Helena Reijonen, PhD

1.6.4. Toronto T Cell Laboratory

This laboratory will serve as the central laboratory to perform the *Toronto Assay* using a pilot of antigens (GAD65, Insulin B, inactive Tetanus Toxin) and a subset of the lab's established and tested autoantigens, as the cell yields allow, to test reactivity of the T cell population.

The Hospital for Sick Children
555 University Ave., Elm Wing, Room 10128
Toronto, ON
Canada M5G 1X8
Phone: (416) 813- 6260
Director: H. Michael Dosch, MD

1.7. Specimen Storage: NIDDK Repository

The NIDDK Repository serves as the storage facility for all TrialNet residual specimens, DNA, RNA, plasma, and PBMC's. These specimens are collected from TrialNet studies and are stored at - 80° C until they are retrieved for further testing or destroyed under the approval and supervision of TNCC.

The Immune Tolerance Network (ITN) will coordinate the logistics (specimen collection, shipping, and storage) for all specimens that will be stored at the NIDDK (RNA, plasma, PBMC, residual DNA) for the Anti-CD20 Trial.

All of the pertinent study information is recorded for each specimen stored at the NIDDK, as well as the precise location of each cryovial, facilitating specimen recovery. Upon request, the specific specimen can be located and readily retrieved for analysis. A copy of the data spreadsheet, listing all of the TrialNet specimens by study, is available from the NIDDK.

NIDDK Repository
Fisher BioServices
20301 Century Blvd.
Building 6, Suite 400
Germantown, MD 20874
Phone: (240) 686- 4702
Director: Heather Higgins

1.8. Clinical Sites and Major Affiliates

Clinical sites and major affiliates will be designated sites with responsibility for the screening of potential participants, enrollment of participants, and conduct of the protocols of the TrialNet. The Type 1 Diabetes Network consists of clinical sites and major affiliates throughout the United States and Canada, as well as international sites. Each clinical site and major affiliate will have a Principal Investigator, a full time Trial Coordinator, other investigators, and ancillary personnel as needed. The Principal Investigator will work with the TrialNet Coordinating Center, Protocol Chairman, and NIH Staff assigned to this project to conduct the study in accordance with the Protocol and Manual of Operations. A list of participating sites is given in Appendix A.

Chapter 3. Recruitment

1. Overview

The Type 1 Diabetes Network consists of clinical sites throughout the United States and Canada, as well as international sites.

Trial Coordinators, Site Coordinators and Recruitment Coordinators should:

- generate general **awareness** about the study
- generate an **interest in participating** for those eligible,
- provide **education** on the goals of TrialNet.

Several tools for recruitment will be developed by TrialNet for use at the local level. They include editable posters and study brochures. Clinical sites should make use of each of the tools developed to recruit new participants.

2. TrialNet Call Center Operation

The mission of the TrialNet Call Center is to provide comprehensive, accurate information about TrialNet to people calling the 1-800 number or accessing the call center over the Internet. The Call Center refers all inquiries to the appropriate TrialNet Clinical Centers. The toll free number to the Call Center is 1-800-425-8361. The Call Center will serve as a bridge between TrialNet sites and the public to accelerate and simplify the recruitment process. The Call Center will collect contact information and prescreen callers/callers' family members on basic inclusion/exclusion criteria (e.g., age; length of diabetes) for TrialNet studies. It will operate in the United States and Canada. The TrialNet Call Center is operated by Matthews Media Group, Inc. (MMG) and located in Rockville, MD.

Information Specialists will answer and return calls using a standardized, IRB-approved script to pre-screen callers for TrialNet studies. Callers who may be eligible for a TrialNet study will be referred to the TrialNet site closest to them.

3. Participant Recruitment

Participants will be recruited through participating TrialNet sites. Selection of participants for enrollment in the study will be done through a screening process that will occur over a two to four week period. The screening process will occur between 3 weeks (21 days) and 3 months (100 days) from the date of diagnosis of type 1 diabetes in order to ensure that the mixed meal test results will not be biased. The screening process may be divided into an initial screening visit and then a mixed meal tolerance test one week later. In cases where participants must travel some distance, steps will be taken to expedite the process so that the initial screening and the mixed meal tolerance test can be conducted within one visit to the study clinic.

Chapter 4. Site Certification and Requirements

Participating clinical sites must fulfill specific requirements prior to the start of any protocol activity. When a site has met all requirements to start the study, a written notice of **"Site Certification"** will be issued by the TrialNet Coordinating Center. Once authorized to start protocol enrollment, the site will have a continuing responsibility to update the TrialNet Coordinating Center of any changes. Reminders for annual renewals of documents will be sent to the Principal Investigators and Study Coordinators two months in advance of renewal dates.

The following items are collected and maintained at the TrialNet Coordinating Center (TNCC):

- IRB approval for study (includes IRB letter of approval for protocol and informed consents; additionally, sites in the United States will need to provide a copy of the IRB approved Research Subject Authorization Form)
- Annual renewal of IRB approvals
- Executed Letter of Agreement (LOA) with The George Washington University (GWU)
- Confidentiality Agreement completed by each Research Staff member
- Duality of Interest Forms (DU1 and DU7) completed by each Research Staff member
- Annual update to DU1 by completed DU2 or DU3
- NIH Education on Human Subjects Protections or equivalent proof of training for each Research Staff member
- Research staff names, mailing addresses, email addresses, office FAX/telephone numbers
- Curriculum vitae (signed and dated) for the Investigator and all Sub-Investigators listed on the 1572.
- Completed and signed Statement of Investigator (Form FDA 1572)
- Completed and signed Financial Disclosure Form if applicable (Form FDA 3455)

- Name and location of laboratory utilized for laboratory assays and other facilities conducting tests, including a copy of the laboratory certificate
- List of normal laboratory values (CBC with Differential)
- Mailing address and contact information for on-site pharmacy where shipments of study medication will be received
- Signed Investigator Brochure acceptance form
- Availability of –70/-80 C degree freezer at on-site pharmacy for storage of PhiX174 immunization

1. IRB Approvals

A copy of IRB approved documents (letter of approval from site IRB, approved informed consent forms, and approved Research Subject Authorization Form) must be received and filed at the TNCC. The IRB documents must reflect the appropriate version number and date of the Protocol. To maintain requirements for continuing participation in TrialNet, the site must provide their annual renewal of IRB approval for the study.

2. Letter of Agreement (LOA)

The Letter of Agreement is a formal document that outlines the responsibilities of all parties for the conduct of the TrialNet studies. The LOA is addressed to the Principal Investigator at the institution and includes appendices of required forms that must be completed by the institution and its research staff that will be involved in TrialNet studies.

The LOA must be executed between The George Washington University (GWU) on behalf of the TNCC and Regional Clinical Centers and Affiliate Sites in order to establish the payment records for reimbursement of study related participant care costs. Only one LOA with GWU is required from the site. The LOA remains in effect throughout the duration of TrialNet.

3. Research Staff Requirements

Each TrialNet Research Staff member at the site is required to sign a TrialNet Confidentiality Agreement, Duality of Interest Form (DU1 and DU7), and complete the NIH Education on Human Subjects Protections or equivalent training. Annually, Research Staff will be requested to provide either a statement of no change (DU3) or conflict (DU2) to their original DU1. The TNCC will send reminders to research staff or Trial Coordinators of impending renewal dates.

The site is expected to contact the TNCC on an ongoing basis of any changes or additions to Research Staff.

4. Clinical Staff Requirements

Certification of staff is required for the Anti-CD20 Trial. At least one person (preferably and typically the Study Coordinator) at the site must be certified in these components to satisfy requirements for Site Certification and initiate protocol enrollment activities.

Certification is an important step in ensuring that study procedures are performed consistently across all TrialNet sites. The Anti-CD20 Trial certification includes the following:

- **Metabolic Tests Quiz** - This quiz applies to all TrialNet studies and only needs to be taken once by all study personnel who will be performing MMTTs on participants.
- **Shipping Procedures Quiz** - This quiz applies to all TrialNet studies and only needs to be taken once by all personnel who will collect specimens and/or prepare samples for shipment to the core laboratories at each site.

- **Protocol Quiz** - This quiz is specific to the Anti-CD20 Trial. For each new TrialNet study that a site participates in, the relevant staff must complete a separate protocol quiz.
- **Web Randomization System** – All study personnel who will be randomizing participants into the study will be required to randomize a "mock" participant by logging into the system and following the procedures correctly. Refer to Chapter 9 for information on completing a mock randomization.

The Trial or Study Coordinator at each clinical site must be certified in all required components. In addition to the Trial/Study Coordinator, there are two general categories of staff that must be certified:

1) **Research Staff** - work directly with the Trial or Study Coordinator and are involved with the completion of case report forms and research participant files, consenting research participants, communicating with the TNCC, conducting participant visits, randomizing participants, and/or collecting and shipping specimens. These staff must complete all of the certification components listed above.

2) **Other Staff** - nurses and other staff at the GCRC or CRC who will collect specimens and/or prepare samples for shipment to the core laboratories. These staff must complete the Metabolic Tests Quiz and the Shipping Procedures Quiz, but they do not have to complete the protocol quiz or the "mock" randomization.

4.1. Components of Staff Certification

Written Quizzes (3)

The Metabolic Tests and Shipping Procedures quizzes cover standard study procedures. These include:

- collection of serum for autoantibody measurement
- collection of blood for HbA1c measurement
- collection of blood for HLA typing
- Mixed Meal Tolerance Test (MMTT)

The Anti-CD20 Protocol Quiz covers key points of the Anti-CD20 Protocol, such as eligibility criteria and visit procedures, as well as informed consent issues.

Before taking the quizzes, the relevant study documents should be reviewed (see Table 1). Although staff may refer to these documents while taking quizzes, candidates should be familiar with the concepts covered in them.

Table 1. Documents for Review Prior to Certification Quizzes

• Protocol • Informed Consent/Assent Forms (Screening and Intervention) • Case Report Forms • Specimen Transmittal Forms • Patient Handbook • Rituximab Investigator Brochure • Volunteer Quiz

The three quizzes may be taken at the same time or separately. Allow at least 30 minutes to 1 hour for each quiz. Use of a calculator may be necessary for the 'Metabolic Tests' quiz.

Completed quizzes are sent to The Coordinating Center for scoring. The Coordinating Center notifies the candidate of any incorrect responses. If there are incorrect items, the Protocol Research Associate/Assistant will discuss and review these items with the individual to ensure that he/she understands the material. In some cases, retesting may be necessary.

5. Notification of Certification

The Coordinating Center tracks all completed certification components at each site. The Coordinating Center will notify individuals of their successful certification and authorize sites to start protocol enrollment. A Site Initiation Activities Checklist is given in Appendix C.

Chapter 5. Informed Consent and Research Subject Authorization Form

1. Overview

At the beginning of the screening visit, participants will be given a written consent form by qualified study personnel (the Trial Coordinator and/or Investigator or other designee). The personnel will understand the research study, and will complete any necessary courses required by their Institutional Review Board prior to implementing the consent process. The consent process will occur in a quiet setting, and the participant will be given time to review the written consent form and ask questions prior to the initiation of study procedures. The consent form for the Anti-CD20 Trial will be reviewed with participants and signed prior to performing any study related assessments. This ensures that the participant understands that participation is voluntary and that they may choose to end participation at any time.

Participants under 18 years of age will be given the opportunity to discuss the study and consent form independently from their parent or guardian, which will allow these participants to ask questions they might not have felt comfortable asking previously. In addition, the parent/guardian of the adolescent participants will be given the opportunity to discuss the study independently from the participant. One or both parents/legal guardians (depending on institutional policies) will be required to sign the Informed Consent Forms. At some sites, the participant will also be required to sign an Assent Form. Care should be taken to explain the study to the participant on a level that is understandable. Specific questions should be addressed to the participant to help ensure that the study is completely understood.

Essential components of the informed consent process include the following:

- participant understanding that participation in the research study is **voluntary**

- a participant may **choose to end** participation at any time
- consent for screening for the Anti-CD20 Trial **does not imply commitment to full study participation**, and that an *intervention informed consent will be required to proceed beyond the screening phase*

Study personnel must provide the participant with:

- an overview of the full study
- the **inclusion** and **exclusion** criteria
- information on the **procedures** involved
- a description of the potential visits
- required **time commitments** for participating in the study

A signature should only be obtained on the Informed Consent Form after a thorough discussion of the study. A copy of the signed Informed Consent, Assent Form and Research Subject Authorization Form (if in the United States) should be provided to the participant. **The original signed documents should remain at the clinical site**. These original and signed documents should **not** be sent to the TrialNet Coordinating center.

Consent for participants 18 years of age or older: The participant needs to **initial the bottom of each page** of the consent and **sign the last page.** The participant's signature, the current date, and the printed name of the participant are all required. Next, the person obtaining the consent (the person who has explained the study to the participant), must sign the form, provide the current date and print his/her name. Please ensure that the printed areas are completed legibly.

Consent for participants less than 18 years of age: The parent of the legal guardian needs to **initial the bottom of each page** of the consent and **sign the last page.** Sufficient evidence must be provided to show that the person

giving consent for the minor does, in fact, have the legal right to serve as the participant's guardian. The parent/guardian must sign and date the form as well as print his/her name legibly. One or two parental signatures will be required as per the requirements of the local institution. A witness must also sign, date and print his/her name.

**Note: The ages specified in the Anti-CD20 Trial Manual of Operations and on the Model Informed Consent Forms may not be the same age limits set by local institutional boards and ethics committees.*

Investigator Statement Section: The Principal Investigator at the clinical site must complete The Investigator Statement section for the Anti-CD20 Trial.

1.1. Assent Form

In the event that the participant is between **8 and 17 years of age**, the participant/child may also be required to sign an Assent Form in addition to the parent/legal guardian signing the Informed Consent Form, depending on institutional policies. In this case, the parent/guardian will grant informed permission (consent) for the minor to participate in the study after being presented with all the information about the study. Parents must demonstrate that they fully understand the study and the commitments required by the child. Assent, or the willingness to participate, will also be required from the child following an age appropriate discussion of the study procedures, risks and benefits. A child 8 to 17 years of age is capable of having a limited understanding of the study and is capable of agreeing to participate. A witness must also sign, date and print his/her name on the assent form.

The original signed Assent Form should remain at the site and a copy should be provided to the participant/parent.

1.2. Research Subject Authorization Form (RSAF)

Research Subject Authorization is one aspect of the Health Insurance Portability and Accountability Act of 1996 (HIPAA) for sites in the United States. HIPAA is a federal law in the United States that, among other things, protects the privacy of protected health information (PHI). Under HIPAA regulations, PHI may not be used for research purposes unless the participant gives written authorization in advance. Although it is not the intent of TrialNet to request such Protected Health Information (PHI) some local IRBs will still require TrialNet Anti-CD20 Trial participants to give written authorization.

Sites will need to follow their institutional requirements for Research Subject Authorization. The RSAF or similar form only needs to be signed at the beginning of a research study, this process does not need to be repeated (unless required by the institution). Regardless of institutional variations on the procedures for obtaining this authorization, a copy of the signed authorization must be provided to the participant. It is acceptable to incorporate the RSAF into the informed consent forms. The original should be kept at the clinical site with the original signed Informed Consent Form for a participant. These forms should not be sent to the TrialNet Coordinating Center.

2. Informed Consent Process

Before initiating screening activities (data collection, specimen collection, or tests), participants will be given the Screening Informed Consent Form, which provides details about the procedures involved with being screened and allows participants to be screened for the study without committing to full study participation. Participants will also be given the Intervention Informed Consent Form prior to screening, which will need to be reviewed and signed before participants proceed beyond the screening phase of the study. All participants must read, and have their site's IRB approved Screening and

Intervention Informed Consent Forms explained to them by qualified study personnel (the Trial Coordinator and/or Investigator or other designee).

As part of the informed consent process, the participant will also be required to complete a short, written Volunteer Understanding Quiz that is designed to ensure that the participant understands the study, as well as what is being asked of him/her. The quiz should be given to the participant following a description of the study and after the Screening Informed Consent Form has been signed, but before the Intervention Informed Consent Form has been signed. If the participant is under the age of 18, the participant's parent/guardian will be required to complete the Volunteer Understanding Quiz independently from the participant. Study personnel will review the completed quiz with the participant (and his/her parent/guardian in the case of an adolescent participant), taking special care to review any questions the participant answered incorrectly and answer any questions about the study. The purpose of the quiz is to enhance informed consent. The key for the Volunteer Understanding Quiz is given in Appendix D.

In addition to the standard procedures and tests associated with the study, participants will be asked for permission to store their remaining blood samples including genetic samples for future studies to learn more about factors associated with risk for the development of type 1 diabetes. The participant must indicate their choice on the Informed Consent Forms and initial in the appropriate area.

Note: Participants are **not** required to provide consent for stored samples in order to participate in the study. Participants also have the right to withdraw their consent to store samples at any time and to have their stored samples destroyed to the extent possible.

Additional consent for testing for reportable conditions such as HIV or Hepatitis B or C will be obtained as required by individual institutions. If participants are found to have evidence of HIV or Hepatitis B or C, they will be excluded from the study but referred for appropriate counseling by specialists in these areas according to local regulations.

2.1. Clinical Sites

Informed consent obtained at a clinical site should follow all standard procedures. After the participant signs an Informed Consent Form/Assent Form, a copy should be provided to the participant while the original is stored at the clinical site. If there is a revision to the original IRB Approved Informed Consent signed by the participant the participant must be re-consented with the current IRB Approved Informed Consent. Participants will be re-consented annually for study participation. It should also be noted in the participant's medical/research chart that the participant consented to participation in the study.

Chapter 6. Summary of Data Forms

The following is a brief summary of each data form for this study. Further details are provided in the separate Manual of Operations for Forms. All study forms are to be completed by site study personnel (e.g. Trial/Study Coordinator, etc.)

Screening Form (RIT01)

This will be the first case report form completed when the participant comes into the clinic for the first time (following completion of the necessary Informed Consent documentation). This form is meant to be the first assessment of the participant's eligibility for the study. This form also gathers demographic information and a list of concomitant medications.

Pre-Randomization Exit Form (RIT01E)

This form is completed any time before randomization. This form serves the purpose of recording a participant's ineligibility or withdrawal from the study.

Baseline Medical History Form (RIT02)

This form collects information that will be used for all subsequent comparisons as the study progresses. The form is completed at the baseline visit before randomization and is meant to collect diabetes, disease, medical, and vaccination history, and concomitant medications.

Baseline Physical Exam Form (RIT03)

This form is completed during the baseline visit and collects the physical examination and Tanner staging done at baseline. The Tanner staging system is given in Appendix P.

Family History Form (RIT04)

The participant should be made aware of the information being collected on this form since it requires time and input from other family members to

complete. The participant may be provided a copy of the CRF to assist him/her in collecting their family medical history. The official form will then be completed at the next study visit, or it could be completed over the phone if desired. The Family History Form is meant to capture information on diabetes and other autoimmune conditions in the participant's mother, father, siblings, and children, if applicable.

Eligibility Form (RIT05)

This form is completed during the baseline visit before the participant is randomized. This form serves the purpose of reviewing the eligibility criteria one last time before the participant is entered into the study and randomized.

Randomization Form (RIT06)

This form is completed during the baseline visit immediately prior to randomization.

Study Drug Administration Form (RIT07)

This form is completed during the baseline and weeks 1, 2, and 3 visits. It is used to collect information on symptoms, acetaminophen and diphenhydramine administration, and intravenous infusion of study medication.

Dosing Vital Sign Monitoring Form (RIT08)

This form is completed during the baseline and weeks 1, 2, and 3 visits. It is used to collect vital signs during the intravenous infusion of study medication.

Diabetes Management Form (RIT09)

This form is completed during the baseline visit and most follow-up visits. It is used to collect glucose monitoring and insulin requirements from a 3-day diary. The form also gathers hypoglycemia history and contact with a diabetes health care provider.

Concomitant Medications Form (RIT10)

This form is completed during most follow-up visits to record any changes since the last visit in participant's concomitant medications.

Physical Exam Form (RIT11)

This form is completed during most follow-up visits to record the physical examination.

Follow-up Visit Form (RIT12)

This form is completed during most follow-up visits. It is used to collect information on pregnancy monitoring, adverse event assessment, and specimens to be drawn.

Tetanus Administration Form (RIT17)

This form is completed during the month 12 visit. It is used to collect information on the administration of the tetanus immunization.

Hepatitis A Administration Form (RIT18)

This form is completed during months 12 and 21. It is used to collect information on the administration of the hepatitis A immunization.

PhiX174 Administration Form (RIT19)

This form is completed during weeks 6, 12, 52, and 58. It is used to collect information on the administration of the PhiX174 immunization.

Neurologic Assessment Form (RIT22)

For enrolled participants following local IRB approval to resume enrollment, this form is completed during the physical exam at the first visit to the site. For participants enrolled after the implementation of neurologic assessments, this form is completed during the physical exam at screening/baseline prior to

randomization. For all participants, this form is completed at months 6, 12, 18, and 24. It is used to collect information on the outcome of the neurologic assessment.

Adverse Event Report Form (RIT13)

This form is to be completed for all adverse events that occur. A separate form is completed for each unique adverse event, or worsening of a previous adverse event. This form has conditional sections to be completed if the adverse event is deemed serious. For this form, the following information should be obtained: date of occurrence, general event information (type of event, intensity, relationship to study medication), serious event description, actions taken, and outcome.

Mortality Event Form (RIT13M)

If a study participant dies during the course of the study, the Mortality Event Report is completed regardless of the cause of death. Information is collected on the date of death, characteristics of the event, and the cause(s) of death. It is expected that a death certificate will be included with this form, or sent to the coordinating center once it is available.

Pregnancy Confirmation Form (RIT14)

This form is completed in the event that a study participant becomes pregnant at any point during the course of the study. The form is meant to capture information on the pregnancy including: expected delivery date, withdrawal of study medication, willingness to continue with follow-up visits, and pregnancy history. The form also inquires as to whether the participant has contacted her obstetric care provider regarding her participation in this study.

Pregnancy Outcome Report Form (RIT14R)

This form is completed at the conclusion of a pregnancy in a study participant. It is meant to capture information on the outcome of the pregnancy, most

importantly if the pregnancy resulted in a live birth, neonatal death, stillbirth, miscarriage, or an induced abortion, as well as the details of the outcome. If the pregnancy resulted in a live birth, information on the infant is collected.

Change of Status Form (RIT15)

This form is only completed in the event that a participant changes study status. This could involve the participant becoming inactive by not being able or willing to attend future follow-up visits. This form will also be completed if an inactive participant decides to return to active study participation by attending future follow-up visits. This form will collect information on the date of the change in status, as well as the reason for the change in status.

Missed Visit Form (RIT16)

This form is only completed if a participant misses a scheduled follow-up visit, and that visit was not rescheduled at any time before the next visit in the study sequence. This form is meant to capture information on the reason the visit was missed, as well as the whether the participant is expected to continue with future visits.

Permanent Participant Site Transfer Form (RIT20)

This form is only completed if a participant permanently transfers to another site. This form will collect the primary and secondary site numbers and the reason for the transfer.

Protocol Deviation Form (RIT21)

This form is completed for every protocol deviation that occurs. A separate form must be completed for every unique protocol deviation that occurs, even if the subject is the same. The form obtains specific information on the deviation that has occurred, including the date the deviation occurred, the specific deviation that has occurred, as well as the reason for the protocol deviation.

TrialNet MedWatch Form (RITSA)

This form is to be completed for all serious adverse events that occur. This form must be completed within **24 hours** of clinic notification that a serious adverse event has occurred in a study participant and faxed to **(301) 468-1676 or (866) 804-6058**. This form should be completed with as much information as is known at the time.

Chapter 7. Screening Visit(s)

1. Overview

New onset type 1 diabetes participants will be identified at or referred to the participating TrialNet sites. The family will be asked if they would be interested in participating in a research project. Those indicating interest are referred to one of the Investigators or Study Coordinators for a description of the study. TrialNet research personnel authorized to present the study to families have attended an approved IRB course and are registered by the IRB.
Essential components of the presentation are:

- the family is being invited to participate in research
- participation is voluntary
- participation may be ended at any time at the participant's request

TrialNet research personnel will provide the participant with a full description of the study, the inclusion and exclusion criteria, the procedures involved, the study groups and the randomization process, time commitments, and the schedule of visits.

The participant (and their parent/guardian in the case of adolescent participants) will then be required to complete a short, written Volunteer Understanding Quiz that is designed to measure the participant's understanding of the study, as well as what is being asked of him/her. This quiz must be completed prior to the Intervention Informed Consent Form being signed. If the Clinical Center is located in the United States, an explanation of the Health Insurance Portability and Accountability Act (HIPAA) should also be included as part of this discussion regardless of whether or not an institution has incorporated the Research Subject Authorization Form (RSAF) into the Informed Consent Forms. The participant should be provided with a copy of the signed Informed Consent Forms. It is also a legal requirement that the participant receive a copy of their signed RSAF (if

required), regardless of whether or not the authorization is a separate form or is incorporated into the Informed Consent Forms.

After a participant signs the Screening Informed Consent Form, the participant will receive a TrialNet Screening Identification Number, and the Screening Form (RIT01) will be completed. Blood will be taken to examine whether a participant is eligible for the Anti-CD20 Trial. Labs, the mixed meal tolerance test (MMTT), and other screening procedures will also be conducted.

2. Eligibility Criteria

Inclusion and exclusion criteria for the TrialNet Anti-CD20 Trial are listed below. The following inclusion and exclusion criteria should be strictly adhered to as described in the Anti-CD20 Protocol. Participants must meet all eligibility criteria prior to randomization, undergoing any baseline study procedures, or completing any study forms, other than the Screening Form (RIT01).

Inclusion Criteria:

The participant MUST:

- ***Be 8 to 45 years of age at the time of randomization***, this indicates that at the time of randomization the participant has passed his/her 8th birthday, but has not passed his/her 45th birthday.
- ***Be within 3-months (100 days) of diagnosis of type 1 diabetes*** based on ADA criteria ***at the time of randomization*.**

The current ADA criteria for diagnosing diabetes include the following:

1. Fasting (no caloric intake for at least 8 hours) plasma glucose is ≥ 126 mg/dl (7.0 mmol/L)

Or

2. Diabetes symptoms (i.e. polyuria, polydipsia, polyphagia, and/or weight loss) exist and casual (any time of day without regard to time since last meal) plasma glucose is ≥ 200 mg/dl (11.1 mmol/L)

Or

3. 2-hour plasma glucose is ≥ 200 mg/dl (11.1 mmol/L) during a 75 gram oral glucose tolerance test (OGTT)

Or

4. Unequivocal hyperglycemia with acute metabolic decompensation (e.g. ketoacidosis)

The first three criteria in any combination on two separate days are diagnostic. If criterion (4) is met, an OGTT is not recommended.

The date of diagnosis will be defined as follows:

- If the participant was symptomatic at diagnosis, the date of diagnosis will be the date of the **first** OGTT
- If the participant did not have symptoms at diagnosis, the date of diagnosis will be the date of the **second** (or confirmatory) OGTT

- ***Have either detectable anti-GAD, anti-ICA512/IA-2, insulin autoantibodies, or islet cell autoantibodies.*** If the participant has been taking insulin therapy for longer than 7 days, the presence of insulin autoantibodies alone is **not** sufficient. In this case the participant must also have evidence of detectable anti-GAD, anti-ICA512/IA-2, or islet cell autoantibodies. The reason for inclusion of these enrollment criteria is to avoid inclusion of participants with "type 1B diabetes mellitus", which may not involve the immunologic criteria measured by the assays that will be utilized. These antibodies will be measured by TrialNet Core Laboratories. See Section 2.4 for autoantibody retesting procedures, if initial tests are negative.
- ***Have stimulated C-peptide levels ≥0.2 pmol/ml*** measured during a mixed meal tolerance test (MMTT) conducted at least 3 weeks (**21 days**) from diagnosis of diabetes and within one month (**37 days**) of randomization.

- ***If female with reproductive potential, be willing to avoid pregnancy and have a negative pregnancy test.*** A urine pregnancy test will be conducted at Screening, Baseline, Weeks 1, 2, and 3, and Months 3, 6, 12, 18, and 24. Acceptable forms of birth control include, but are not limited to:
    - Abstinence
    - Barrier methods (condom, diaphragm, cervical cap, sponge, or spermicide)
    - Contraceptives (oral or implant)
    - Surgical methods (sterilization or intrauterine devices)
- ***Be at least one month (28 days) from last immunization received.***
    - **Influenza Vaccination:**
      Killed or inactivated Influenza vaccine may be given as indicated to potential research participants prior to enrollment. The vaccine must be given at least 1 month before baseline/randomization visit and should consist of the inactivated trivalent influenza (TIV) vaccine. It may also be given one year after the last infusion of study drug.
    - **Meningococcal Conjugate Vaccine (Menactra):**
      This vaccine is strongly recommended for all incoming college freshmen, particularly those who are going to live in college dormitories. For this scenario, we are recommending that research participants who will be attending college as freshmen receive the Menactra vaccine at least 1 month prior to baseline/randomization visit or one year following the last infusion of study drug. Potential research participants and their parents should be counseled regarding the potential impact of missing this vaccine. Investigators should consider suggesting that the research participant and/or their parents discuss the decision to enroll and its possible impact on immunization with Menactra with their primary care physician.

Administration of Menactra is currently recommended for children aged 11-12 years of age, though this is not an epidemiologically high risk population and thus timing is not critical. Accordingly, we would recommend that Menactra not be administered during or within the one month prior to baseline/randomization visit. It may be given one year after the last infusion of study drug.

- **Tdap (Tetanus-diptheria-acellular pertussis):**
  This vaccine was recently approved for adolescents and adults to provide protection against Bordetella pertussis. This vaccination is not felt to be time dependent. Participants should not receive this within one month prior to enrollment. It may be given two years after the last infusion of study medication.

No live viral vaccines should be administered from one month before to 12 months after the last study drug infusion in the trial.

- ***Be willing to comply with intensive diabetes management.*** For all participants, regardless of age, the goal of this management will be to maintain an HbA1c value of 7.0% or lower without significant hypoglycemia.
- ***Weigh at least 25 kg at study entry.*** This is to ensure that the participant is of sufficient body weight to allow for the blood volumes drawn for the study assessments.

Exclusion Criteria:

The participant MUST NOT:

- ***Be immunodeficient or have clinically significant chronic lymphopenia.***
- ***Have an active infection or positive purified protein derivative of tuberculin (PPD) test result.*** The PPD test is administered at the initial screening visit. A positive PPD indicates that the participant has been infected with Tuberculosis, and should be referred for appropriate counseling and treatment.
- ***Be currently pregnant or lactating, or anticipate getting pregnant.*** If the participant has any plans to become pregnant, or to attempt to become pregnant, during the course of the study she should be excluded from participation.
- ***Require chronic use of steroids, regardless of the type or route of administration (inhaled, topical, systemic, oral, etc.).*** Chronic use of steroids is defined as more than one-week of continuous use over the course of one-month. Acute use of steroids should not be considered grounds for exclusion, as long as the participant is not continuing to take the steroid medication at the time of screening.
- ***Require use of other immunosuppressive agents.***
- ***Have serologic evidence of current or past HIV, Hepatitis B, or Hepatitis C infection.*** Participants are screened for Human Immunodeficiency Virus, Hepatitis B virus, and Hepatitis C virus at the initial screening visit to determine if they are currently infected with these viruses. Note that if the participant is infected with HIV or Hepatitis, this information **must** be reported to the appropriate department of health.
- ***Have any complicating medical issues that interfere with study conduct or cause increased risk to include pre-existing cardiac disease, COPD, neurological, or blood count abnormalities (such as lymphopenia, leukopenia, or thrombocytopenia).*** If the screening CBC indicates an abnormal lymphocyte count, white blood cell count, or platelet

count, repeating the CBC and taking a good history are recommended. The PI should then decide if the counts are appropriate for participation and consult with the Protocol Chair and Medical Monitor as needed.

- ***Have a history of malignancies.***
- ***Be currently using non-insulin pharmaceuticals that affect glycemic control, such as metformin.*** If a participant is willing to stop therapy with these agents then they will be eligible for study participation following a two week (14 day) washout period.
- ***Be currently participating in another type 1 diabetes treatment study.***

The TrialNet Coordinating Center will be responsible for initially reviewing and adjudicating any instances where eligibility is unclear. If following this initial review eligibility is still unclear, the TrialNet Eligibility and Events Committee will review and adjudicate the situation.

2.1. Eligibility Issues

Eligibility issues should be submitted by completing an Eligibility and Deviation Review Form (Form ELIG), available in Appendix E and on the TrialNet Website, to the TrialNet Coordinating Center. The issue will be reviewed and a response provided to the site within an appropriate timeframe.

2.2. Intensive Diabetes Management

During the study, all participants will receive intensive management of their diabetes. The goal of treatment will be to keep the hemoglobin A1c level (HbA1c) as close to normal as possible without frequent occurrence of hypoglycemia. A goal would be an HbA1c of 7.0%.

The primary responsibility for diabetes management will be with the treating or referring physician, but additional support of the research team including a Certified Diabetes Educator (CDE) will be made available.

Participants will be expected to take a sufficient number of daily insulin injections or use insulin pump therapy to meet this goal, without causing severe hypoglycemic reactions. In general, glucose levels must be checked at least 4 times per day and records of the glucose levels should be communicated to the CDE every two weeks. After the CDE has reviewed these records, the CDE may contact the participant and the treating physician about adjustments in the insulin regimen, referral to a Registered Dietician, or other approaches that the CDE feels would improve the glucose control. Records of glucose logs and communication with the participant and treating physician will be kept as source documentation.

The general goal of glucose control is to target pre-prandial glucose levels of 90-130 mg/dl (plasma), post-prandial levels of <180 mg/dl, and bedtime levels of 110-150mg/dl. Participants who fail to achieve an HbA1c level according to the guidelines above will not be excluded from the study, but additional measures will be instituted to correct the glycemic control. The intent is not to eliminate a participant who is trying their best to achieve these aims.

In addition to Medical Monitor and DSMB oversight, regular monitoring of HbA1c levels will be done by the TrialNet Clinical Monitoring Group. Members of this group will work with clinical sites to review diabetes care for individual participants not attaining the study goal.

Any episodes of severe hypoglycemia (i.e. unconsciousness, seizure, or needing assistance of another individual to correct the hypoglycemia) will prompt a review of the cause of the episode and adjustment of insulin dosing/diet/exercise as deemed appropriate by the treating physician. All

episodes of severe hypoglycemia that require hospitalization and/or emergency care will be reviewed by the TrialNet Medical Monitor and reported to the Data Safety Monitoring Board (DSMB). If adhering to these goals of treatment results in any episodes of severe hypoglycemia, the goals of treatment may be relaxed to avoid a recurrent event. This will be decided on a case-by-case basis with recommendations of the DSMB and/or other monitoring committees as appropriate (Safety Monitoring Committee, Clinic Monitoring Committee).

2.3. Reading the PPD Test

The results of the PPD test need to be read within 48-72 hours of administering the test. The test must be read by a trained nurse or physician (either at the study site or at a site more convenient for the participant). The results of the test should be recorded in the participant's source documents. It may be helpful to call the participant as a reminder to have the test read in a timely manner and to call the site with the results. The Test Reading Guidelines and a Sample Participant Letter are given in Appendix F.

2.3.1. BCG Immunization

Tuberculin skin testing (TST) is required for all participants prior to enrollment in protocol. Previous Bacille Calmette-Guérin (BCG) immunization is not a contraindication to TST (Red Book 2006 Report of the Committee on Infectious Diseases, pg. 683).

Generally, interpretation of TST results in BCG recipients is the same as for people who have not received BCG vaccine. The size of the TST reaction attributable to BCG immunization depends on many factors, including age at BCG immunization, quality and strain of BCG vaccine used, number of doses

of BCG received, nutritional and immunologic status of the vaccine recipient and frequency of TST administration.

Evidence that increases the probability that a positive TST result is attributable to latent tuberculosis infection includes known contact with a person with contagious tuberculosis, a family history of tuberculosis disease, a long interval (>5 years) since neonatal immunization and a TST reaction >=15 mm.

2.3.2. Procedure

1) Place TST
   - 5 tuberculin units of purified protein derivative intradermally per local guidelines
   - Creation of a visible wheal 6-10 mm in diameter is crucial to accurate testing
2) Query participants regarding history of BCG vaccine. If there is a history of BCG vaccine, document the number of vaccinations and dates as available.

2.3.3. Interpretation of a Negative TST

Screening participants with a negative TST (<10 mm of induration) regardless of BCG vaccine history are eligible for participation.

2.3.4. Interpretation of a Positive TST

Screening participants with a positive TST defined by the area of induration >=10 mm, regardless of a previous history of BCG vaccine, are excluded from participation. Participants should be referred to primary care physician or infectious disease specialist for appropriate evaluation and management.

2.4. Repeating the BAA

If the initial screening antibody sample indicates that the participant is negative for all antibodies (or positive for mIAA only), the participant is eligible for repeat testing. The following procedure must be followed:

1. Contact participant and schedule re-draw. The scheduling of the re-draw, and time required for results to be reported, **must** fit within the eligibility windows for the study (100-days from date of diagnosis and 37 days from screening MMTT).
2. Complete a **new** specimen transmittal form (RIT99AA) and select "Screening" as the visit. Record the **new** draw date for question A1.
3. Apply appropriate barcode labels (BAA) to specimen transmittal form and vials containing the sample. **Note:** These barcode labels must be from a different block of labels than the labels that were included on the sample drawn previously!
4. Results from the new sample will be available within approximately 2 weeks from the date the sample was received by the laboratory. The results will be available on the TrialNet website in the eligibility report. **Note:** The eligibility report always displays the most recent results received.
5. Review results to determine eligibility. If the new results indicate that the participant is positive for any of the other three antibodies (GAD65, ICA512, ICA), in addition to mIAA, than this eligibility criterion has been met. If the new results indicate that the participant is still positive for mIAA only, than the participant has not met this eligibility criterion and is not eligible for a repeat sample.

2.5. Mixed Meal Tolerance Test

The mixed meal tolerance test (MMTT) will be scheduled within **7 days** after the Screening Visit and conducted at least 3 weeks (**21 days**) from diagnosis

of diabetes and within one month (**37 days**) of randomization. This test is meant to assess the potential participant's insulin production capability. In order for the results to be meaningful, it is important for the participant to follow certain dietary and lifestyle guidelines in the days preceding the test. A high carbohydrate diet must be followed for the three days leading up to the test. The participant is required to fast starting the night before the test, and is instructed to consume only water for at least 10 hours preceding the test. More detailed information on the mixed meal tolerance test can be found in Appendix G. This appendix includes detailed information on the procedure. This section also includes a detailed "Sample Menu" with recommended items to maintain the required high carbohydrate diet prior to the test.

The test takes approximately 4-hours to complete, and must be scheduled in the morning (i.e. must be started before 10 AM). It is important to carefully review the eligibility criteria with the participant before starting the test, since if certain criteria have been violated the test will need to be rescheduled for another date. For participants that live a great distance from the clinic, special arrangements to have the MMTT done the same day as the initial Screening Visit would be attempted if a second trip to the clinic would not be possible.

3. Assignment of the TrialNet Screening Number

Participants who are screened for enrollment will receive a TrialNet Screening Identification Number after they sign the Screening Informed Consent Form at the beginning of the screening visit. Participants who are randomized into the study will receive an additional identification number known as the Randomization Number.

The Screening ID consists of 5 digits. The first 4 digits will be a sequential number, and the last number will be a check digit. The Coordinating Center will supply each participating clinical site with a Screening Number Log

containing a range of numbers to be used for participants at that clinical site. For example, one site might be assigned the range from 0001 to 0300, while another is assigned 4001 to 4300. Each site will maintain a Screening Number Log to keep track of which Screening Numbers have been assigned to which participants. A blank Screening Number Log is given in Appendix H and assigned Screening Identification Numbers by site are given in Appendix B.

ANTI-CD20 TRIAL SCREENING VISIT(S) SUMMARY

NOTE: The participant **NEEDS TO BE FASTING** for this visit

1. Forms

A. Forms to be completed prior to the initiation of study procedures:

- TrialNet Screening Informed Consent
- Volunteer Understanding Quiz
- TrialNet Screening Assent (if applicable)
- Research Subject Authorization Form (if applicable)
- Local HIV Screening Consent (if applicable)

B. Case Report Forms:

- Screening Form (RIT01)

C. Specimen Transmittal Forms:

- CBC Results (RIT99CB)
- Chemistries (RIT99CH)
- HIV/Hep B/Hep C Screening (RIT99HV)
- HLA Determination (RIT99HL)
- Autoantibodies (RIT99AA)
- HbA1c (RIT99HB)
- 4-hour MMTT (RIT99M4)

2. Barcode Labels

- 4 Chemistries (CHEM)
- 4 HIV/Hep B/Hep C (SERO)
- 4 HLA Determination (HLA)
- 4 Autoantibodies (BAA)
- 4 HbA1c (HbA1c)
- 25 4-hour MMTT (MMT4)

3. Supplies

A. Blood Collection

- 1 x 2 ml EDTA lavender top tube
- 2 x 4 ml plain red top tubes
- 1 x 2.6 ml SSG clotting activator tube
- 12 x 1.2 ml EDTA lavender top tubes
- 11 x 1.2 ml gray top tubes
- 1 x 6 ml EDTA tube

B. Specimen Shipment

- 1 x 2 ml amber vial
- 1 x 4 ml cryovial
- 23 x 1.8 ml cryovials
- Collection tube for HLA

4. Activities to be completed

- PPD test
- Urine pregnancy test
- 4-hour MMTT

5. Labs to be drawn

- CBC with diff **ANALYSIS AT LOCAL LAB**
- Chemistries
- HIV/Hep B/Hep C
- Serum for Autoantibodies
- HbA1c
- 4-hour MMTT (glucose and C-peptide)
- HLA/DNA

6. Preparation for next visit

- Remind participant to bring immunization card to next visit
- Remind participant to bring blood glucose and insulin records to next visit

Chapter 8. Baseline Visit

1. Overview

At the baseline study visit, the lab results from the screening visit, the results from the MMTT, and the information from the screening and baseline data collection forms will be reviewed to assess whether a participant is eligible to be randomized and enrolled in the study. Participants who do not meet all of the inclusion criteria or have one of the exclusion criteria will be referred back to the attending diabetologist for standard education, treatment, and care. Participants not eligible for the Anti-CD20 Trial may also be offered the opportunity to participate in another TrialNet study, if one is available.

2. Neurologic Assessment

At the request of the DSMB, neurologic assessments will be performed as part of physical exams at regular intervals. Participants will undergo a neurologic assessment prior to randomization to look for evidence of underlying neurologic disease. Such participants will be excluded from participation to assure that any neurologic findings seen on follow-up are not associated with pre-existing disease. Follow-up neurologic assessments will also be performed at months 6, 12, 18, and 24.

3. Randomization

After participants sign the Informed Consent Forms, complete the screening visit(s) including the mixed meal tolerance test, meet all of the inclusion criteria and none of the exclusion criteria, and complete the baseline procedures, they will be randomized to one of the two study groups: active rituximab or placebo.

3.1. Randomization Method

Participants will be randomized in a 2:1 ratio of active treatment versus placebo. The randomization method will be stratified by TrialNet study site. This approach ensures that study site will not be a potential confounder.

The study will be double-masked, in that the participant and those involved in participant care at the clinics will be masked to the participant's group assignment. The TrialNet Central Pharmacy and the staff at The Coordinating Center will know to which treatment group each participant is assigned.

The Coordinating Center will generate a randomization schedule for the study sites. The Randomization Number will be a four-digit code, where each study participant's code is unique. The Randomization Number will in no way reflect the treatment group to which the participant has been assigned. After a participant is randomized, the study coordinator will contact the site pharmacist to obtain the study medication.

3.2. Randomization Procedure

The study participants will be randomized at the clinical sites at the Baseline visit or as close to it as possible, and they will receive a Randomization Number. When it is time to randomize a participant at the Baseline visit, the study personnel will log into the TrialNet website under their username, then click on Active Studies/Anti-CD20 (Rituximab)/Randomization. The randomization system will verify the study personnel, Screening ID, and eligibility criteria including lab results before issuing a Randomization Number. The Randomization Number will determine if a participant gets an active or placebo rituximab IV infusion.

3.3. Assigned Schedule of Assessments

All participants will follow the same schedule of assessments, except for the mechanistic specimens (flow cytometry, Frozen PBMC/Plasma, T cell Proliferation, Immunoblot, ELISpot, Tetramer, and RNA) and the phiX174 Immunization Course (optional). A schedule of assessments will be assigned for each participant at the time of randomization by the web randomization system. Each schedule will be based on that participant's age, weight, HLA results, and phiX174 Immunization Course enrollment. ALL of this information must be available at the time of randomization in order to successfully randomize the participant. The assigned schedule of assessments will be in printable form from the website and will need to be referred to throughout the study for that participant. A schedule of assessments is given in Appendix M to refer to prior to randomizing a participant.

3.4. Web Randomization System

Before logging into the TrialNet Web Randomization System, ensure the following:

- Participant meets **all** of the inclusion criteria (answered "Yes" to all questions in Section B of the Anti-CD20 Eligibility Form (**RIT05**)).
- Participant has **none** of the exclusion criteria (answered "No" to all questions in Section C of the Anti-CD20 Eligibility Form (**RIT05**)).
- Participant has reviewed and signed **all** consent forms (TrialNet Screening and Intervention Informed Consent Forms, and the local HIV screening consent form (if applicable)).
- Participant has completed all screening and baseline procedures (including a 4-hour MMTT).
- The Screening Form (**RIT01**), Baseline Medical History Form (**RIT02**), Baseline Physical Exam Form (**RIT03**), and Eligibility Form (**RIT05**) have all been completed for the participant.

If all of these criteria have been met, the participant is eligible for randomization and the TrialNet Web Randomization System should be accessed. The system is operable 24-hours per day, 7 days a week. Refer to Chapter 9 for instructions on using the Web Randomization System.

Before you login, make sure you have:

- Your (the user's) pass phrase to access the system.
- The participant's five-digit Anti-CD20 Screening ID.

If the web randomization system is down, contact The Coordinating Center immediately for manual randomization number assignment.

4. Initial Study Drug Administration

All participants will receive an IV infusion of either rituximab or placebo. The participant **does not** need to be fasting prior to this infusion. ***The initial treatment will be given at the end of the Baseline visit, which should take place within 100 days from the day of diagnosis and within 37 days of the screening MMTT.*** Following randomization, the local pharmacy at the TrialNet clinical site should be contacted and given the participant's Randomization Number. The local pharmacy will then prepare the IV infusion to be administered to the participant. The preparation of this IV infusion will be the same regardless of the whether the participant is receiving rituximab or placebo. Refer to Chapters 10 and 11 for study drug and administration information.

ANTI-CD20 TRIAL BASELINE VISIT SUMMARY

1. Forms

A. Forms to be completed prior to the initiation of study procedures:

- Volunteer Understanding Quiz (if not completed at Screening Visit) **PRIOR TO SIGNING INTERVENTION INFORMED CONSENT**
- TrialNet Intervention Informed Consent (if not completed at Screening Visit)
- TrialNet Intervention Assent (if applicable and not completed at Screening Visit)
- Research Subject Authorization Form (if applicable and not completed at Screening Visit)

B. Case Report Forms:

- Baseline Medical History Form (RIT02)
- Baseline Physical Exam Form (RIT03)
- Family History Form (RIT04)
- Diabetes Management Form (RIT09)
- Eligibility Form (RIT05)
- Randomization Form (RIT06)
- Study Drug Administration Form (RIT07)
- Dosing Vital Sign Monitoring Form (RIT08)

C. Specimen Transmittal Forms:

- CBC Results (RIT99CB)
- EBV/CMV PCR (RIT99PC)
- EBV/CMV Viral Serology (RIT99VS)
- Other Serology (RIT99SR)
- PK Analysis and HACA Levels (Covance to send)

2. Barcode Labels

- 4 EBV/CMV PCR (VIRVL)
- 4 EBV/CMV Viral Serology (VIRST)

- 4 Other Serology (SRLG)
- Covance to send barcodes for PK and HACA

3. Supplies

A. Blood Collection

- 2 x 2 ml EDTA tubes
- 2 x 3 ml plain red top tubes
- Covance to send supplies for PK and HACA

B. Specimen Shipment

- Collection tube for EBV/CMV PCR
- 2 x 1.8 ml cryovials
- Covance to send supplies for PK and HACA

4. Activities to be completed

- Medical history taken **PRIOR TO RANDOMIZATION**
- Physical examination including neurologic assessment **PRIOR TO RANDOMIZATION**
- Urine pregnancy test **PRIOR TO RANDOMIZATION**
- Randomization number obtained from web randomization system
- First infusion of rituximab or placebo

5. Labs to be drawn

- CBC with diff **ANALYSIS AT LOCAL LAB**
- EBV/CMV PCR
- EBV/CMV Viral Serology
- Other Serology
- PK and HACA

6. Mechanistic Specimens

- **REFER TO ASSIGNED SCHEDULE OF ASSESSMENTS AND VISIT CHECKLIST**

Chapter 9. TrialNet Web Randomization System

This chapter contains instructions on performing a mock randomization for certification and randomizing a participant into the Anti-CD20 Trial.

1. Performing a MOCK Randomization for Certification

1.1. Log In

- Provide the TNCC with your pass-phrase to access the system. The TNCC will notify you when your pass-phrase has been activated. *Note: the pass-phrase may be any length and consist of letters and/or numbers, include spaces, and is case sensitive.*

- Once your pass-phrase has been activated, log into the TrialNet Website under your username at: www.diabetestrialnet.org

- Click on Studies/Active Studies/Anti-CD20 (Rituximab)/Randomization

- Enter your pass-phrase and hit enter.

- Note that throughout the mock randomization, the banner at the top of the page should say “Mock Randomization”. If not, contact the TNCC.

1.2. Enter Screening ID

- Note that the mock randomization web pages are identical to the live randomization system, with the exception that you are assigned to the fictitious site number 9999 with corresponding fictitious screening IDs and lab results.

- The "Overview" page contains 3 columns of screening IDs for site number 9999.
    - The first column (Eligibility in progress) contains participants whose eligibility criteria are still being evaluated (i.e. required lab results have been received but the eligibility questions have not been answered).
    - The second column (Eligible, not randomized) contains participants who have met all of the eligibility requirements but have not been randomized.
    - The third column (Randomized) contains all randomized participants.

- For the purposes of the mock randomization, either click on one of the screening IDs listed in column one (Eligibility in progress) or enter one of these IDs into the space provided. *Note: if you choose to enter the screening ID, you must include the "-" (i.e. 9901-5).*

1.3. Confirm Eligibility and Randomize

- The "Subject Information" page contains a list of eligibility criteria for this study. Fictitious lab results have been automatically pre-filled.

- Identify:
    - whether the participant is enrolled in the PhiX immunization course (Question A.2) by clicking on either Yes or No,
    - what the participant's age and weight category are (Question A.3) using the drop down menu,
    - and whether the participant meets the eligibility requirements by clicking on either Yes or No for the remaining questions, in order to proceed.
    - *Note: all questions must be answered correctly in order to successfully randomize a participant and, therefore, become certified.*

- Once you have answered all of the eligibility questions, click on "Save my changes".

- The system will identify at the top of the page whether or not the participant is eligible. If the participant is ineligible, an * will appear on the left hand side of the page next to the violated eligibility criterion.

- If the participant is eligible and the "Save my changes" button is clicked, the participant will NOT be randomized and their screening ID will be moved to the second column (Eligible, not randomized) of the "Overview" page (refer to number 7 above). For the purposes of the mock randomization, click on "Randomize now".

- The eligibility criteria will be saved and the participant will be assigned a randomization number, which will appear at the top of the page. Once the participant is randomized, their screening ID will be moved to the third column (Randomized) of the "Overview" page (refer to number 7 above).

1.4. View Schedule of Assessments

- The participant will also be assigned a schedule of assessments based on their age, weight, and PhiX enrollment. To view the schedule, including visit target dates and allowable windows, click on "visit schedule". For the purposes of the mock randomization, click on "visit schedule" to complete the certification process.

- Note that once you have successfully completed the mock randomization, the word "certified" will appear in the upper right hand corner of the page under your name.

1.5. Log Out

- In order to access the live randomization system, log out of the system by clicking on "Log out" at the top of the page, and log back into the system. Note that when you are in the live randomization system, the banner at the top of the page will NOT say "Mock Randomization". *Note: if the system automatically prompts you for a password during the mock randomization, you have been logged out of the system. If you log back in, you will be in the live randomization system if you successfully completed the certification process. If you would like to repeat the mock randomization, please contact the TNCC.*

- Note that any page may be printed by clicking on "Print this page" at the top of the page.

2. Randomizing a Participant into the Anti-CD20 Study

2.1. Log In

- Log into the TrialNet Website under your username at: www.diabetestrialnet.org

- Click on Studies/Active Studies/Anti-CD20 (Rituximab)/Randomization

- Enter your pass-phrase and hit enter.

2.2. Enter Screening ID

- The "Overview" page contains 3 columns of screening IDs for your site. *Note: the valid screening IDs for your site are located on the Screening Number Log provided by the TNCC.*
    - The first column (Eligibility in progress) contains participants whose eligibility criteria are still being evaluated (i.e. required lab results have been received but the eligibility questions have not been answered).
    - The second column (Eligible, not randomized) contains participants who have met all of the eligibility requirements but have not been randomized.
    - The third column (Randomized) contains all randomized participants at your site.

- To randomize a new participant, either click on the screening ID of that participant listed in column one (Eligibility in progress) or enter the ID into the space provided. *Note: if you choose to enter the screening ID, you must include the "-" (i.e. 9901-5).* Note that if the screening ID of the participant you wish to randomize is not displayed in column one, the required lab results have not been received and you will not be allowed to randomize the participant. If this is incorrect, please contact the TNCC immediately.

- To randomize a participant who's eligibility criteria you have previously entered and saved, either click on the screening ID of that participant listed in column two (Eligible, not randomized) or enter the ID into the space provided.

- To view a previously randomized participant's eligibility criteria, randomization number, and/or schedule of assessments, either click on the screening ID of that participant listed in column three (Randomized) or enter the ID into the space provided.

2.3. Confirm Eligibility and Randomize

- The "Subject Information" page contains a list of eligibility criteria for this study. Note that this page is similar to the RIT05 Form, which should be completed prior to accessing the randomization system and serve as a guide for answering the eligibility questions. The required lab results that have been received will automatically be pre-filled.

- Identify:
    - whether the participant is enrolled in the PhiX immunization course (Question A.2) by clicking on either Yes or No,

- what the participant's age and weight category are (Question A.3) using the drop down menu,
- and whether the participant meets the eligibility requirements by clicking on either Yes or No for the remaining questions, in order to proceed.
- *Note: all questions must be answered correctly and all required lab results must be received in order to successfully randomize a participant. If any information is missing or violates an eligibility criterion you will NOT be allowed to proceed with randomization.*

- Once you have answered all of the eligibility questions, click on "Save my changes".

- The system will identify at the top of the page whether or not the participant is eligible. If the participant is ineligible, an * will appear on the left hand side of the page next to the violated eligibility criterion.

- If the participant is eligible and the "Save my changes" button is clicked, the participant will NOT be randomized and their screening ID will be moved to the second column (Eligible, not randomized) of the "Overview" page (refer to number 4 above). Click on "Randomize now" to randomize the participant.

- The eligibility criteria will be saved and the participant will be assigned a randomization number, which will appear at the top of the page. This number must be recorded on the RIT06 Form. Once the participant is randomized, their screening ID will be moved to the third column (Randomized) of the "Overview" page (refer to number 4 above).

2.4. View Schedule of Assessments

- The participant will also be assigned a schedule of assessments based on their age, weight, HLA results, and PhiX enrollment. To view the schedule, including visit target dates and allowable windows, click on "visit schedule". The schedule should be printed and inserted into the participant binder for reference throughout the study. Note that any page may be printed by clicking on "Print this page" at the top of the page.

2.5. Log Out

- Log out of the system by clicking on "Log out" at the top of the page when finished.

Chapter 10. Study Drug and Vaccines

This chapter provides descriptions of the study drug and vaccines being used in the Anti-CD20 Trial as well as shipping, ordering, returning, and accountability information for these products. The study drug and vaccines will be provided by EMINENT Services Corporation.

1. EMINENT Services Corporation

EMINENT Services Corporation is Good Manufacturing Practice certified and serves as the central distributor for TrialNet studies. EMINENT receives, stores, packages, labels, and ships drug products and clinical supplies, as necessary, for studies, and processes and destroys returned drug.

Accurate records are maintained of all product labeling, packaging and shipment tracking. Products are tracked by EMINENT to their final destination, whether it is the participant, back to the warehouse, or product destruction.

EMINENT hours are Monday through Friday, 8:00 AM to 5:00 PM Eastern Time (North America). A pharmacist can be reached during these normal business hours at phone number: (240) 629-1972. For all other emergencies during the non-business hours, weekends, and holidays, sites may contact EMINENT using the clinical emergency hot-line **(888) 321-4364 or (240) 629-1972 (Option 8)**.

Contact Information:

Type 1 Diabetes TrialNet – Anti-CD20 Trial (0052)
c/o EMINENT Services Corporation
7495 New Technology Way
Frederick, MD 21703-9401

Tel: (240) 629-1972
Fax: (240) 629-3298
E-mail: service@emiserv.com

2. Study Drug

All participants will receive an IV infusion of rituximab or placebo at four visits during the study. The first IV infusion will occur at the Baseline visit AS SOON AFTER RANDOMIZATION AS POSSIBLE or on the same day the participant is randomized. The second IV infusion will occur at the Week 1 visit, the third IV infusion will occur at the Week 2 visit, and the fourth IV infusion will occur at the Week 3 visit. Details of this study medication are given below.

2.1. Packaging and Shipment Information

Rituximab/placebo is supplied as 100 mg and 500 mg of sterile, preservative-free, single-use vials. Single unit 100 mg carton contains one 10 mL vial of rituximab/placebo (10 mg/mL). Single unit 500 mg carton contains one 50 mL vial of rituximab/placebo (10 mg/mL). EMINENT will ship an initial supply of rituximab/placebo sufficient for three participants, four infusions each, to clinical site pharmacies whose sites have been authorized by The Coordinating Center to begin protocol enrollment.

2.2. Description of Drug

Rituximab/placebo solutions for infusion are stable at 2 to 8 °C (36 to 46 °F) for 24 hours and at room temperature for an additional 12 hours. Do not use beyond expiration date stamped on carton. No incompatibilities between rituximab/placebo and polyvinylchloride or polyethylene bags have been observed. Rituximab/placebo vials should be protected from direct sunlight.

2.3. Ordering Study Drug

Note that the initial supply of study medication must be on-site **before** the date the first participant is randomized and enrolled into the study. Medication is ordered based on Randomization Number, so it can be ordered before a particular participant is assigned the Randomization Number! The initial supply of rituximab/placebo will be arranged by The Coordinating Center and all subsequent supplies must be ordered by the clinical site pharmacy. Study drug inventory must be monitored closely to ensure that an adequate supply is on site before the participant's visit. For ordering a subsequent supply of rituximab/placebo, the guidelines are as follows.

To order study drug, please complete an
Agent Request Form - Rituximab and FAX to (240) 629-3298.

Instructions: Type or clearly print all information. Complete all sections except for the box labeled **For EMINENT Use Only**. Sign the form in the space provided. All requests received by 2:00 PM Eastern Time (North America) weekdays will be shipped to arrive the 2nd business day by 4:30 PM Eastern Time. Requests pertaining to refrigerated drug products will be shipped by overnight service to arrive by the next business day by 10:00 AM Easter Time. If study drug is needed overnight, check "Yes" in the "Overnight"

field. If an overnight shipment is needed for Saturday and clinic personnel will be available to receive it, check "Yes" in the "Saturday" field.

Instructions for completing the *Agent Request Form - Rituximab* appear below. A master copy of the *Agent Request Form - Rituximab* is located in Appendix I and on the TrialNet Website. This standard form should be photocopied and used for ordering agents from EMINENT Services Corporation (EMINENT).

2.3.1 Instructions for Completing an Agent Request Form - Rituximab

1. When completing an *Agent Request Form - Rituximab*, please type or clearly print all requested information. Data that remains the same from order to order, such as the clinical investigator's name, phone number, and shipping address, should be typed on the original form prior to photocopying.

2. Enter the site number, clinical investigator's name, and other required information in the top, middle section of the form.

3. Each line of the order should contain only one item. Complete each line as follows:
   a. Protocol number (pre-printed Protocol TN05)
   b. Current inventory (at the site)
   c. Agent name, strength, and dosage form (unit pre-filled)
   d. Quantity required

   The following study drug can be ordered for the Anti-CD20 Trial using the Agent Request Form - Rituximab:
   Rituximab/placebo 10 mg, 10 mL vials
   Rituximab/placebo 10 mg, 50 mL vials

4. ONLY the designated clinical site pharmacy contact may sign and date the order form.

5. The shipping address must be typed. Do not use a rubber stamp, since this usually does not yield a sharp image and does not photocopy or transmit well by FAX.

6. The completed *Agent Request Form – Rituximab* may be FAXED to (240) 629-3298 or sent via express courier to the EMINENT Services Corporation.

7. Retain a copy of the order; when study supplies arrive, you may want to verify what was received against what was ordered.

Upon the participant's first visit to the site for an infusion, the dose of rituximab/placebo will be calculated based on that participant's BSA (refer to Section 4 of Chapter 11 for more information). **The same BSA is used for all subsequent infusions.** The site will complete the Pharmacy Drug Request Form indicating the dose needed for that participant and provide it in the physician orders to their local pharmacy. The clinical site pharmacy will then prepare the study medication for that participant.

Instructions for completing the *Pharmacy Drug Request Form* appear below. A master copy of the *Pharmacy Drug Request Form* is located in Appendix I and on the TrialNet Website.

2.3.2. Instructions for Completing a Pharmacy Drug Request Form

1. When completing a *Pharmacy Drug Request Form*, please type or clearly print all requested information.

2. Complete the follow sections:
    a. Site #
    b. Participant Name
    c. Screening ID
    d. Participant Letters
    e. Randomization #
    f. Randomization Date
    g. Initial Request or Subsequent Request (check ONE)
    h. Participant Height in **cm**
    i. Participant Weight in **kg**
    j. Participant Body Surface Area (BSA)
    k. Rituximab/placebo dose in mg for randomized participant (BSA x 375 = Dose)
    l. Requested by (signature of individual AUTHORIZED to order medication)
    m. Date (date of request)

3. ONLY individuals authorized by the clinical investigator may sign and date the order form.

4. The completed *Pharmacy Drug Request Form* should be sent to the clinical site pharmacy.

5. The clinical site pharmacy will then prepare the study medication for that participant.

2.4. Receipt and Storage

When study medication is received at the clinical site pharmacy from EMINENT, IT IS IMPORTANT THAT IT BE INSPECTED AS SOON AS POSSIBLE. Carefully check items received against the packing slip, noting

container sizes, quantities, and lot numbers. EMINENT should be notified IMMEDIATELY if there are any discrepancies.

Place study drug in an appropriate storage area. This area must be kept separate from routine stock. This storage area should have limited access. Note that rituximab/placebo is to be stored at refrigerated temperature (2-8 °C).

2.5. Accountability

In order to comply with FDA regulations, each site is required to keep a record of receipts and dispositions of all study drug received from EMINENT. This can be accomplished by using the *Randomization and Drug Delivery Forms* provided to the clinical site pharmacy in the Confidential Binder supplied by EMINENT.

These forms are designed for maintaining perpetual inventories. Each time study drug is dispensed to a participant an appropriate entry should be made on the *Randomization and Drug Delivery Form* for that protocol, participant, and study drug.

FDA regulations require that drug accountability records be retained until two years after the Investigational New Drug (IND) application or the study is closed. These records shall be made available, upon request, for inspection and copying by a properly authorized employee, representative, or monitor of the FDA. The *Randomization and Drug Delivery Forms* will be kept in the Confidential Binder provided by EMINENT.

2.6. Returning Study Medication

Study medication shall be returned to EMINENT for the following reasons:

- The study is completed or terminated,
- The drug has expired,
- The drug has been stored improperly or was received damaged, and/or
- The drug's return was requested by the EMINENT pharmacist.

A master copy of the *Agent Return Form* is located in Appendix I and on the TrialNet Website. This is a standard form that should be photocopied and used to return all study products of this protocol.

2.6.1. Instructions for Returning Study Supplies to EMINENT

1. Use a separate *Agent Return Form* for each site.
2. Complete all sections of the form, except the right-hand section with the heading FOR EMINENT USE ONLY (shaded area).
3. Print or type site address on the form.
5. Quantity: Enter the total number of dosage units being returned.
6. Sign and date the form. Please include the site phone number.
8. Enclose ONLY those items that EMINENT provided.
9. Include the completed *Agent Return Form* in the package. Make a copy of the completed form for your records.
10. *Pack the materials so that they will not break during transit!* Ship all items at room temperature, unless otherwise instructed.
11. The EMINENT address is located in the upper right of the *Agent Return Form*.

2.7. Unmasking Treatment Group Assignment

In the event that the treatment group assignment of a participant needs to be unmasked, The Coordinating Center should be contacted.

3. Hepatitis A, Diphtheria/Tetanus, and Bacteriophage PhiX174 Vaccines

3.1. Packaging and Shipment Information

Hepatitis A Vaccine (HAVRIX®) is supplied as 720 EL.U. /0.5 mL pre-filled disposable *Tip-Lok* syringes without needles for pediatric and adolescent participants, and as 1440 EL.U. /1.0 mL pre-filled disposable *Tip-Lok* syringes without needles for adult participants.

Diphtheria Tetanus Toxoids Adsorbed (DECAVAC™) Vaccine is supplied in unit dose preservative-free 0.5 mL pre-filled Luer-Lok™ latex-free syringes (without needles), which contain a trace amount of thimerosal from the manufacturing process.

Bacteriophage PhiX174 Vaccine is supplied as 0.8 ml single use vials for pediatric participants up to 40 kg, 1.8 ml single use vials for adult participants less than 90 kg, and 2.8 ml single use vials for adult participants greater than 90 kg with a concentration of 1×10^{11} PFU/ml. Dose of PhiX174 is dependent on weight and calculated as follows: 0.022 ml/kg body weight.

The Coordinating Center will NOT arrange an initial shipment of immunizations for the clinical sites. Sites must order their own supply of immunizations from EMINENT. A Pharmacy Vaccine Notification Form, for recording the target dates for each participant's immunizations, is located in Appendix I and on the TrialNet Website. This Form will be completed by the site based on the participant's Visit Schedule assigned at randomization. It

will assist the clinical site pharmacy in completing the Agent Request Form in a timely manner to ensure that vaccine is on site before the date it is needed.

3.2. Description of Vaccines

Hepatitis A vaccine is supplied as a sterile suspension for intramuscular administration. The vaccine is ready for use without reconstitution; it must be shaken before administration.

Diphtheria tetanus vaccine is indicated for active immunization of persons 7 years of age and older. The syringe should be shaken well before administration intramuscularly.

Bacteriophage PhiX174 is an investigational drug and is given intravenously.

3.3. Ordering Vaccine

All initial and subsequent supplies of vaccines must be ordered from EMINENT. EMINENT will ship the vaccines to the clinical site pharmacy. The guidelines are as follows:

To order vaccine, please complete an Agent Request Form and FAX to (240) 629-3298.

Instructions: Type or clearly print all information. Complete all sections except for the box labeled **For EMINENT Use Only**. Sign the form in the space provided. All requests received by 2:00 PM Eastern Time (North America) weekdays will be shipped to arrive the 2nd business day by 4:30 PM Eastern Time. Requests pertaining to refrigerated drug products will be shipped by overnight service to arrive by the next business day by 10:00 AM Easter Time. If vaccine is needed overnight, check “Yes” in the “Overnight”

field. If an overnight shipment is needed for Saturday and clinic personnel will be available to receive it, check "Yes" in the "Saturday" field.

Instructions for completing the *Agent Request Form* appear below. A master copy of the *Agent Request Form* is located in Appendix I and on the TrialNet Website. This standard form should be photocopied and used for ordering agents from EMINENT Services Corporation (EMINENT).

3.3.1. Instructions for Completing an Agent Request Form

1. When completing an *Agent Request Form*, please type or clearly print all requested information. Data that remains the same from order to order, such as the clinical investigator's name, phone number, and shipping address, should be typed on the original form prior to photocopying.

2. Enter the site number, clinical investigator's name, and other required information in the top, middle section of the form.

3. Each line of the order should contain only one item. Complete each line as follows:
   a. Protocol number (pre-printed Protocol TN05)
   b. Current inventory (at the site)
   c. Agent name, strength, and dosage form (unit pre-filled)
   d. Quantity required

   The following vaccines can be ordered for the Anti-CD20 Trial using the Agent Request Form:

   Bacteriophage PhiX174, 0.8 ml vials
   Bacteriophage PhiX174, 1.8 ml vials
   Bacteriophage PhiX174, 2.8 ml vials
   Hepatitis A (Havrix®), 0.5 ml pre-filled syringes

Hepatitis A (Havrix®), 1.0 ml pre-filled syringes
Diphtheria Tetanus Toxoids Adsorbed (DECAVAC™), 0.5 ml pre-filled syringes

4. ONLY individuals authorized by the clinical investigator may sign and date the order form.

5. The shipping address must be typed. Do not use a rubber stamp, since this usually does not yield a sharp image and does not photocopy or transmit well by FAX.

6. The completed *Agent Request Form* may be FAXED to (240) 629-3298 or sent via express courier to the EMINENT Services Corporation.

7. Retain a copy of the order; when study supplies arrive, you may want to verify what was received against what was ordered.

3.4. Receipt and Storage

When a vaccine order is received from EMINENT, it is important that it be inspected as soon as possible. Carefully check items received against the packing slip, noting container sizes, quantities, and lot numbers. EMINENT should be notified immediately if there are any discrepancies.

Place vaccine in an appropriate storage area. This area must be kept separate from routine stock. This storage area should have limited access.

- Note that hepatitis A vaccine is to be stored at refrigerated temperature (2-8 °C).
- Note that diphtheria tetanus vaccine is to be stored at refrigerated temperature (2-8 ºC).

- Note that Bacteriophage PhiX174 vaccine is stable for 10 years or more if stored at –70 or –80 °C, for 7 days without noticeable change if stored at –20 °C, and for no more than 2 days if stored at refrigerated temperature (2-8 ºC). Once it has been thawed it may NOT be refrozen.

3.5. Accountability

In order to comply with FDA regulations, each site is required to keep a record of receipts and dispositions of all vaccines received from EMINENT. This can be accomplished by using the *Investigational Drug Accountability Record* or an equivalent computerized record. Master copies of the *Investigational Drug Accountability Records* for all vaccines listed above are located in Appendix I and on the TrialNet Website. Retain these forms as originals and photocopy for future use.

These forms are designed for maintaining perpetual inventories. Each time a vaccine is dispensed to a participant, or received and/or returned to EMINENT, an appropriate entry should be made on the *Investigational Drug Accountability Record* for that vaccine and protocol. The inventory balance documented on this form should match the actual vaccine inventory on-hand at all times. It is suggested that a regular schedule be established for a physical inventory, the results of which should be noted on the *Investigational Drug Accountability Record*. When the recorded balance and the actual inventory are not equal, ascertain the reason and notify the EMINENT staff pharmacist via memo by FAX or mail. Attach a copy of the memo to the back of the *Investigational Drug Accountability Record* and retain.

FDA regulations require that drug accountability records be retained until two years after the Investigational New Drug (IND) application or the study is closed. These records shall be made available, upon request, for inspection

and copying by a properly authorized employee, representative, or monitor of the FDA. The *Investigational Drug Accountability Records* will be kept in the Documents binder provided by The TrialNet Coordinating Center.

3.6. Returning Vaccine

Vaccine must be returned to EMINENT for the following reasons:

- The study is completed or terminated,
- The vaccine has expired,
- The vaccine has been stored improperly or was received damaged, and/or
- The vaccine's return was requested by the EMINENT pharmacist.

A master copy of the *Agent Return Form* is located in Appendix I and on the TrialNet Website. This is a standard form that should be photocopied and used to return all study products of this protocol.

3.6.1. Instructions for Returning Study Supplies to EMINENT

1. Use a separate *Agent Return Form* for each site.
2. Complete all sections of the form, except the right-hand section with the heading FOR EMINENT USE ONLY (shaded area).
3. Print or type site address on the form.
5. Quantity: Enter the total number of dosage units being returned.
6. Sign and date the form. Please include the site phone number.
8. Enclose ONLY those items that EMINENT provided.
9. Include the completed *Agent Return Form* in the package. Make a copy of the completed form for your records.
10. *Pack the materials so that they will not break during transit!* Ship all items at room temperature, unless otherwise instructed.
11. The EMINENT address is located in the upper right of the Agent Return Form.

Chapter 11. Study Drug Administration

This chapter includes study drug handling, drug dosing, administration, and infusion monitoring.

1. Tools for Study Team

Be sure that the pharmacy and infusion team is thoroughly prepared before the participant is seen. Know who in your institution is familiar with administration of this drug.

There are three tools that have been developed for guidance. A sample set of physician orders for the infusion is given in Appendix L. A quick reference worksheet is given in Appendix J. A key points quiz that can be used as a teaching tool is provided in Appendix K.

2. Preparation for Administration

This should be done by qualified pharmaceutical personnel only. Use appropriate aseptic technique. Withdraw the necessary amount of rituximab/placebo and dilute to a final concentration of **4 mg/ml** into an infusion bag containing either 0.9% Sodium Chloride USP or 5% Dextrose in Water USP. Gently invert the bag to mix the solution. Discard any portion left in the vial. Parental drug products should be inspected visually for particulate matter discoloration prior to administration.

3. Preparation of Participant

- Since transient hypotension may occur during the infusion, assure that the participant has not taken antihypertensive medications within the previous 12 hours.

- Check to be sure that the urine pregnancy test was negative (if appropriate).
- Place peripheral IV line. Obtain pre-infusion blood samples.
- To attenuate infusion-related events, pre-medicate the participant 30 min before start of infusion with acetaminophen (adults and children over age 12: 650-1000 mg PO every 4-6 hours, not to exceed 4000 mg per day, children under age 12: recommended dose 10-15 mg/kg body weight PO every 4-6 hours, not to exceed 5 doses (50-75 mg/kg) per day)
- To attenuate infusion-related events, pre-medicate the participant 30 min before start of infusion with diphenhydramine (adults: 25-50 mg PO every 4-6 hours, children over age 12: 12.5-25 mg ORALLY every 4-6 hours, maximum dose: 150 mg/day)
- Since diphenhydramine may be sedating, may use non-sedating antihistamine instead, particularly if the participant will be driving home after the infusion.

4. Calculation of Drug Dose

- Determine participant's weight in kg and height in cm.
- Calculate their Body Surface Area (BSA) or refer to nomogram.
  BSA (m^2) = square root of [(ht in cm x wt in kg)/3600]
- Calculate total dose, Dose = 375/m^2. Therefore, Dose = 375 x BSA.
- Rounding should be kept to a MINIMUM. Keep the dose AS CLOSE AS POSSIBLE to the actual dose calculated. To calculate the dose, round to the nearest milligram (e.g. 1.5 = 2.0 and 1.4 = 1.0).

5. Administration of Drug

Precaution: Anaphylaxis is possible. A crash cart must be available.
DO NOT ADMINISTER AS AN INTRAVENOUS PUSH OR BOLUS.

The rituximab solution for the first infusion should be administered intravenously at an initial rate of 50 mg/hr. Since the drug is at a 4 mg/ml concentration, this would be an infusion rate of 12.5 cc/hr. Rituximab should not be mixed or diluted with other drugs. If hypersensitivity or infusion-related events do not occur, escalate the infusion rate in 50 mg/hr increments every 30 minutes, to a maximum of 400 mg/hr. If hypersensitivity or an infusion related event develops, the infusion should be temporarily slowed or interrupted (see below for drug dosing changes).

If there were no problems with the first infusion or prior infusion, the rituximab for subsequent infusions should be started at 100 mg/hr (25 cc/hr). The rate can increase in 100 mg/hr increments every 30 minutes to a maximum of 400 mg/hr. If there were infusion related problems during the first infusion or prior infusion, start at 50 mg/hr and increase as described for first infusion.

6. Dose Modification

If mild hypersensitivity or infusion related events occur, the infusion should be temporarily slowed or interrupted. Retreatment with acetaminophen and diphenhydramine (or equivalent) can occur every 3-4 hours. Mild hypotension is to be treated with saline infusion and bronchospasms with bronchodilators. If mild symptoms improve, the infusion can be resumed at one-half the previous rate and increased by 50 mg/hr every 30 minutes as tolerated.

If moderate or severe hypersensitivity or infusion reactions occur (i.e. those requiring pressor support for hypotension or epinephrine for bronchospasm), the infusion should be STOPPED permanently. Pressor support, epinephrine,

and other measures should be instituted as appropriate. The infusion should NOT be restarted. In addition, the participant should NOT receive any subsequent doses.

7. Monitoring

At minimum, vital signs including blood pressure, heart rate, respiratory rate, and temperature are to be done prior to the start of infusion, every 15 minutes for the first hour, and then every 30 minutes until maximum dose. At maximum dose, vital signs are measured every hour. More frequent vital sign measurements should be done according to institutional procedures and/or clinical judgment.

At the end of the infusion, keep IV line patent for approximately 60 minutes and determine vital signs before discharging the participant.

8. Instructions to Participant at Discharge

The participant is to contact the study team for any problems including skin rash, hives, and shortness of breath.

Chapter 12. Subsequent Treatment and Follow-up Visits

1. Overview

After the Baseline visit, participants will return for 3 more treatment visits each a week apart for a total of 4 treatment visits. Following the last treatment visit, participants will return to the clinic approximately every 3 months for up to 2 years. It is anticipated that a participant would have approximately 15 outpatient visits after the baseline visit over the 2-year study period.

At each clinic visit, participants will see their diabetologists, CDE, etc. to review blood sugar control and hypoglycemic episodes. Each clinical site will have a Certified Diabetes Educator to assist participants in their diabetes care. In addition to diabetes care, they will review issues related to development of potential side effects of immunosuppression (infections). Routine clinical laboratory tests will be performed (such as CBC with differential, chemistries, C-peptide, glucose, HbA1c, CMV serology, EBV serology, etc.). Autoantibody analysis will be performed at TrialNet central laboratories for all study centers. C-peptide measurements will also be examined by a TrialNet central laboratory.

Laboratory specimens collected at the local clinical sites will be forwarded to appropriate laboratories for analysis. These specimens will be labeled by a study assigned specimen number, the date of collection, and three letters of the participant's choice.

Ideally, all blood draws would be done at the study site. However, if it is inconvenient for a participant to have the blood drawn at the study site, the blood draw may be done at the participant's primary care physician's office. The study site will provide instructions to the physician for how to process and ship the specimen.

If allowable by blood drawing guidelines and with the participant's permission, samples will also be collected and stored at the NIDDK Repository for later testing. Blood for DNA testing will be stored when blood is drawn for HLA determination. T-cells and blood for RNA testing will be stored. Serum will also be stored for autoantibody isotype and proteomic analysis when blood is drawn for autoantibody testing. Samples will be available for use by investigators within or outside of TrialNet for research related to the development and treatment of type 1 diabetes or related diseases. The utilization of these samples will be subject to NIDDK policies and procedures.

At every visit except Week 10, and Months 5 and 13, the sexual activity of female participants of reproductive age will be re-assessed. If a participant who was previously sexually inactive becomes sexually active, she will be counseled about the need to use a reliable form of birth control. Female participants will also be required to undergo urine pregnancy tests at the following visits: Weeks 1, 2, and 3, and Months 3, 6, 12, 18, and 24.

2. Scheduling Participant Visits

All visits should be scheduled Monday through Wednesday due to inadequate weekend coverage at the laboratories. Visits on Thursday, Friday, Saturday or Sunday are discouraged, but allowable on a case-by-case basis, for matters of convenience. These restrictions are in place to ensure that any specimens drawn during a visit can be shipped to the laboratory on the same day. Specimens can only be shipped to the laboratory Monday through Wednesday, and not on the day preceding a federal US holiday. There are no exceptions. When scheduling a participant's visit, take into consideration holidays surrounding that visit. No blood samples are to be shipped the day before a holiday. In the event of an extenuating circumstance, contact the TNCC.

3. Visit Windows

All subsequent TREATMENT visits after baseline should be ***at least 96 hours (4 days) from the start of the previous infusion*** and ***within 2 days on either side of the targeted date*** to be permissible. For visits in which a response to the phiX174 immunization is being determined, the visit window should be within 2 days on either side of the targeted date (Weeks 6, 7, 8, 10, 12, 13, 14, 16, 52, 53 54, 56, 58, 59, 60, and 62). For follow-up visits through month 3, a 7-day window on either side of the targeted date is permissible, except for participants enrolled in the phiX174 Immunization Course (follow the above requirements). For follow-up visits after month 3, a 14-day window on either side of the targeted date is permissible, except Month 13 in which 2-days prior to the targeted date and 7-10 days after the targeted date are permissible.

For example, at month 21, a 14-day window on either side of the targeted date is permissible. Therefore, if a visit needs to be rescheduled, every effort should be made to do so within the 14-day window period surrounding the original visit date. As an example, assume a participant was scheduled to have his/her month 21 visit on Monday, May 15. The window of acceptable dates for this visit extends 14 days before and after May 15. Therefore, for the visit to be considered in the allowable window, the participant must visit the clinic sometime between May 1 and May 29.

Below are the permissible visit windows by visit for participants enrolled in the phiX174 Immunization Course:

Visit	Type	Window
Screening	Screening	MMTT within 7 days of Screening and at least 21 days from date of diagnosis
Baseline	Infusion	Within 100 days of date if diagnosis and 37 days of Screening MMTT
Week 1	Infusion	+/- 2 days, at least 96 hours from previous infusion
Week 2	Infusion	+/- 2 days, at least 96 hours from previous infusion
Week 3	Infusion	+/- 2 days, at least 96 hours from previous infusion
Week 5	Regular Follow-up	+/- 2 days
Week 6	phiX ONLY	+/- 2 days
Week 7	phiX ONLY	+/- 2 days
Week 8	phiX ONLY	+/- 2 days
Week 10	Regular Follow-up and phiX	+/- 2 days
Week 12 (Month 3)	Regular Follow-up and phiX	+/- 2 days
Week 13	phiX ONLY	+/- 2 days
Week 14	phiX ONLY	+/- 2 days
Week 16	phiX ONLY	+/- 2 days
Week 19 (Month 5)	Regular Follow-up	+/- 14 days
Week 26 (Month 6)	Regular Follow-up	+/- 14 days
Week 39 (Month 9)	Regular Follow-up	+/- 14 days
Week 52 (Month 12)	Regular Follow-up and phiX	+/- 2 days
Week 53	phiX ONLY	+/- 2 days
Week 54	phiX ONLY	+/- 2 days
Week 56 (Month 13)	Tetanus, Hep A and phiX	+/- 2 days
Week 58	phiX ONLY	+/- 2 days
Week 59	phiX ONLY	+/- 2 days
Week 60	phiX ONLY	+/- 2 days
Week 62	phiX ONLY	+/- 2 days
Week 65 (Month 15)	Regular Follow-up	+/- 14 days
Week 78 (Month 18)	Regular Follow-up	+/- 14 days
Week 91 (Month 21)	Regular Follow-up	+/- 14 days
Week 104 (Month 24)	Hep A	+/- 14 days

Below are the permissible visit windows by visit for participants NOT enrolled in the phiX174 Immunization Course:

Visit	Type	Window
Screening	Screening	MMTT within 7 days of Screening and at least 21 days from date of diagnosis
Baseline	Infusion	Within 100 days of date if diagnosis and 37 days of Screening MMTT
Week 1	Infusion	+/- 2 days, at least 96 hours from previous infusion
Week 2	Infusion	+/- 2 days, at least 96 hours from previous infusion
Week 3	Infusion	+/- 2 days, at least 96 hours from previous infusion
Week 5	Regular Follow-up	+/- 7 days
Week 10	Regular Follow-up	+/- 7 days
Week 12 (Month 3)	Regular Follow-up	+/- 7 days
Week 19 (Month 5)	Regular Follow-up	+/- 14 days
Week 26 (Month 6)	Regular Follow-up	+/- 14 days
Week 39 (Month 9)	Regular Follow-up	+/- 14 days
Week 52 (Month 12)	Regular Follow-up	+/- 14 days
Week 56 (Month 13)	Tetanus and Hep A	- 2 days + 7-10 days
Week 65 (Month 15)	Regular Follow-up	+/- 14 days
Week 78 (Month 18)	Regular Follow-up	+/- 14 days
Week 91 (Month 21)	Regular Follow-up	+/- 14 days
Week 104 (Month 24)	Hep A	+/- 14 days

The Visit Schedule assigned at randomization provides the target visit date and allowable window around the date for all subsequent visits.

4. Missed Visits

If a participant misses a scheduled visit, every effort should be made to reschedule the visit within the permissible window period surrounding the original visit date. If the visit is rescheduled within this timeframe, no further actions need to be taken, and the visit should go on as planned. If the visit is not rescheduled within this window, **every effort should be made to bring**

the participant into the clinic as soon as possible. This is important to continue to closely monitor the health of the participant. If the participant comes into the clinic beyond the allowable visit window, the applicable Follow-up Forms (**RIT09, RIT10, RIT11,** and **RIT12**) should be completed. A special comment should be placed on the forms indicating that the visit occurred outside of the allowable visit window. The only time a visit should be considered "missed" is if the participant is unable to come into the clinic at any time prior to the subsequent visit in the study sequence.

Example 1: A participant is scheduled to have his Month 9 visit on January 1. The participant is supposed to then have his Month 12 visit on April 1. The participant misses the visit on January 1 and is unable to come into the clinic at any time before April 1. In this situation the Month 9 visit is considered missed, and the Missed Visit Form (RIT16) needs to be completed to document this information. The participant then continues with the Month 12 visit on April 1 as scheduled.

Example 2: A participant is scheduled to have his Month 9 visit on January 1. Following the study schedule, the participant is then expected to have his Month 12 visit on April 1. The participant misses the visit on January 1 and the earliest date the participant can reschedule his visit for is March 20. The participant should be encouraged to come in on that date to complete the Month 9 visit. The applicable Follow-up Forms (**RIT09, RIT10, RIT11,** and **RIT12**) should be completed as usual, with a special comment on the form to indicate that this visit occurred outside of the visit window. The participant should then proceed with the Month 12 visit on April 1 as scheduled.

These two examples make two important points:

1. If a participant misses a scheduled clinic visit, he/she may be allowed to make up that visit at any point up until the date of next scheduled clinic visit. Once that date is reached, the participant should proceed with the

next scheduled visit, and the prior visit is considered officially missed triggering completion of the Missed Visit Form (**RIT16**).

2. The visit schedule is determined based on the **date the participant was randomized into the study**. Therefore, it is possible to establish the exact date and corresponding window period for every visit the participant will make from randomization until the end of the study.

Every effort should be made to contact participants who fail to attend their follow-up visits in order to ensure that they are in satisfactory health and to encourage them to continue with future study follow-up visits. This will entail, at a minimum, three telephone contact attempts and two written attempts with return receipt requests. Research staff at each of the sites is responsible for keeping participant contact information up to date at every study visit.

5. Changing Study Status

This study will be conducted according to the intent-to-treat principle. This means that once randomized into the study a participant will remain in the assigned treatment group until he or she either dies or withdraws consent for further participation. If a participant does not receive all four infusions of study drug, it does not automatically entail withdrawal from the study: this could occur for a number of reasons, some of which are outlined below. Withdrawal from the study and its scheduled assessments should only occur if the participant dies or withdraws consent.

A participant may elect not to receive, or be unable to continue with, or be withdrawn from receiving all four infusions of study drug at the discretion of the Principal Investigator under the following circumstances:

- Adverse effect of immunosuppression, such as leukopenia and opportunistic infections
- Pregnancy
- A need to start on another immunosuppressive medication – such as systemic steroids.
- Missed 2 or more consecutive visits

If a participant withdraws or is withdrawn from receiving all four infusions of study drug for any reason, the participant will be encouraged to continue with all scheduled follow-up visits. Study personnel will make every effort to keep participants in the study even if the participant does not receive all four infusions of study medication. If a participant fails to attend a visit, site study personnel will contact the participant to reschedule and encourage him/her to come back for follow-up evaluations.

5.1. Inactive Status

In the event that a participant is unable or unwilling to continue making future visits then the participant is declared inactive. Every effort should be made to encourage all participants to continue making follow-up visits. If a participant is declared inactive, the Change of Status Form (**RIT15**) must be completed.

5.2. Reactivation into the Study

In some circumstances, a participant may enter inactive status and be unwilling or unable to return to the study clinic for future visits. It is hoped that at a later date this participant may decide to once again resume active participation in the study. If the participant desires to return to the clinic for future follow-up visits, he/she should be allowed and encouraged to do so. The participant should be allowed to return to active participation regardless of the length of the inactivity period, as long as the study is still active. If a

participant who is inactive decides to become reactivated, the Change of Status Form (**RIT15**) must be completed.

This form is completed for *every* change of status that occurs, even if the participant is the same. Therefore, if a participant becomes inactive and then reactivates at a later date, two separate forms should be completed. Completion of this form allows The Coordinating Center and the clinical site to know that a participant is no longer actively participating in the study and, therefore, not to expect any forms or laboratory results. Conversely, if a participant is becoming active again following a period of inactivity, the completion of this form allows The Coordinating Center and the clinical site to know that a participant is once again participating in the study and to begin expecting forms and laboratory results from that participant according to the study schedule.

6. Visit Schedule and Activities

This section describes in detail the activities that will occur at each visit after baseline. Participants should be asked if they have experienced any adverse events since the last scheduled clinic visit. If any events have occurred, the Adverse Event Report Form (RIT13) should be completed to record the details of these events. Participants should also be asked about any major hypoglycemic events that have occurred since the last scheduled clinic visit.

ANTI-CD20 TRIAL WEEK 1 VISIT SUMMARY

1. Forms

A. Case Report Forms:

- Concomitant Medications Form (RIT10)
- Physical Exam Form (RIT11)
- Follow-up Visit Form (RIT12)
- Study Drug Administration Form (RIT07)
- Dosing Vital Sign Monitoring Form (RIT08)
- Adverse Events Report Form (RIT13) **IF NECESSARY**

B. Specimen Transmittal Forms:

- CBC Results (RIT99CB)

2. Supplies

A. Blood Collection

- 1 x 2 ml EDTA tube

3. Activities to be completed

- Adverse events assessed
- Physical examination
- Urine pregnancy test
- Second infusion of rituximab or placebo

4. Labs to be drawn

- CBC with diff **ANALYSIS AT LOCAL LAB**

5. Preparation for next visit

- Remind participant to bring blood glucose and insulin records to next visit

ANTI-CD20 TRIAL WEEK 2 VISIT SUMMARY

1. Forms

A. Case Report Forms:

- Diabetes Management Form (RIT09)
- Concomitant Medications Form (RIT10)
- Physical Exam Form (RIT11)
- Follow-up Visit Form (RIT12)
- Study Drug Administration Form (RIT07)
- Dosing Vital Sign Monitoring Form (RIT08)
- Adverse Events Report Form (RIT13) **IF NECESSARY**

B. Specimen Transmittal Forms:

- CBC Results (RIT99CB)
- EBV/CMV PCR (RIT99PC)
- EBV/CMV Viral Serology (RIT99VS)

2. Barcode Labels

- 4 EBV/CMV PCR (VIRVL)
- 4 EBV/CMV Viral Serology (VIRST)

3. Supplies

A. Blood Collection

- 2 x 2 ml EDTA tubes
- 1 x 3 ml plain red top tube

B. Specimen Shipment

- Collection tube for EBV/CMV PCR
- 1 x 1.8 ml cryovial

4. Activities to be completed

- Adverse events assessed

- Physical examination
- Urine pregnancy test
- Third infusion of rituximab or placebo

5. Labs to be drawn

- CBC with diff **ANALYSIS AT LOCAL LAB**
- EBV/CMV PCR
- EBV/CMV Viral Serology

ANTI-CD20 TRIAL WEEK 3 VISIT SUMMARY

1. Forms

A. Case Report Forms:

- Concomitant Medications Form (RIT10)
- Physical Exam Form (RIT11)
- Follow-up Visit Form (RIT12)
- Study Drug Administration Form (RIT07)
- Dosing Vital Sign Monitoring Form (RIT08)
- Adverse Events Report Form (RIT13) **IF NECESSARY**

B. Specimen Transmittal Forms:

- CBC Results (RIT99CB)

2. Supplies

A. Blood Collection

- 1 x 2 ml EDTA tube

3. Activities to be completed

- Adverse events assessed
- Physical examination
- Urine pregnancy test
- Fourth (last) infusion of rituximab or placebo

4. Labs to be drawn

- CBC with diff **ANALYSIS AT LOCAL LAB**

5. Preparation for next visit

- Remind participant to bring blood glucose and insulin records to next visit

ANTI-CD20 TRIAL WEEK 5 VISIT SUMMARY

1. Forms

A. Case Report Forms:

- Diabetes Management Form (RIT09)
- Concomitant Medications Form (RIT10)
- Physical Exam Form (RIT11)
- Follow-up Visit Form (RIT12)
- Adverse Events Report Form (RIT13) **IF NECESSARY**

B. Specimen Transmittal Forms:

- CBC Results (RIT99CB)
- Autoantibodies (RIT99AA)
- EBV/CMV PCR (RIT99PC)
- EBV/CMV Viral Serology (RIT99VS)

2. Barcode Labels

- 4 Autoantibodies (BAA)
- 4 EBV/CMV PCR (VIRVL)
- 4 EBV/CMV Viral Serology (VIRST)

3. Supplies

A. Blood Collection

- 2 x 2 ml EDTA tubes
- 1 x 2.6 ml SSG clotting activator tube
- 1 x 3 ml plain red top tube

B. Specimen Shipment

- Collection tube for EBV/CMV PCR
- 2 x 1.8 ml cryovials

4. Activities to be completed

- Adverse events assessed

- Physical examination

5. Labs to be drawn

- CBC with diff **ANALYSIS AT LOCAL LAB**
- Autoantibodies
- EBV/CMV PCR
- EBV/CMV Viral Serology

6. Mechanistic Specimens

- **REFER TO ASSIGNED SCHEDULE OF ASSESSMENTS AND VISIT CHECKLIST**

ANTI-CD20 TRIAL WEEK 10 VISIT SUMMARY

NOTE: The participant may complete this visit at a site other than the study site

1. Forms

A. Case Report Forms:

- Adverse Events Report Form (RIT13) **IF NECESSARY**

B. Specimen Transmittal Forms:

- CBC Results (RIT99CB)
- PhiX174 Serology (RIT99PX) **IF APPLICABLE**

2. Barcode Labels

- 4 PhiX174 Serology (PHIX) **IF APPLICABLE**

3. Supplies

A. Blood Collection

- 1 x 2 ml EDTA tube
- 1 x 4 ml plain red top tube **IF APPLICABLE**

B. Specimen Shipment

- 1 x 1.8 ml cryovial **IF APPLICABLE**

4. Activities to be completed

- Adverse events assessed

5. Labs to be drawn

- CBC with diff **ANALYSIS AT LOCAL LAB**
- PhiX174 Serology **IF APPLICABLE**

6. Preparation for next visit

- Remind participant to bring blood glucose and insulin records to next visit
- Instruct participant about preparation for MMTT

ANTI-CD20 TRIAL MONTH 3 VISIT SUMMARY

NOTE: The participant **NEEDS TO BE FASTING** for this visit

1. Forms

A. Case Report Forms:

- Diabetes Management Form (RIT09)
- Concomitant Medications Form (RIT10)
- Physical Exam Form (RIT11)
- Follow-up Visit Form (RIT12)
- Adverse Event Report Form (RIT13) **IF NECESSARY**
- PhiX174 Administration Form (RIT19) **IF APPLICABLE**

B. Specimen Transmittal Forms:

- CBC Results (RIT99CB)
- Chemistries (RIT99CH)
- Autoantibodies (RIT99AA)
- HbA1c (RIT99HB)
- 2-hour MMTT (RIT99M2)
- EBV/CMV PCR (RIT99PC)
- EBV/CMV Viral Serology (RIT99VS)
- PhiX174 Serology (RIT99PX) **IF APPLICABLE**

2. Barcode Labels

- 4 Chemistries (CHEM)
- 4 Autoantibodies (BAA)
- 4 HbA1c (HbA1c)
- 17 2-hour MMTT (MMT2)
- 4 EBV/CMV PCR (VIRVL)
- 4 EBV/CMV Viral Serology (VIRST)
- 5 PhiX174 Serology (PHIX) **IF APPLICABLE**

3. Supplies

A. Blood Collection

- 2 x 2 ml EDTA tubes
- 1 x 4 ml plain red top tube
- 1 x 2.6 ml SSG clotting activator tube
- 8 x 1.2 ml EDTA tubes
- 7 x 1.2 ml gray top tubes
- 1 x 3 ml plain red top tube
- 2 x 4 ml plain red top tubes **IF APPLICABLE**

B. Specimen Shipment

- 1 x 2 ml amber vial
- 16 x 1.8 ml cryovials
- Collection tube for EBV/CMV PCR
- 2 x 1.8 ml cryovials **IF APPLICABLE**

4. Activities to be completed

- Adverse events assessed
- Physical examination
- Urine pregnancy test
- 2-hour MMTT
- PhiX174 intravenous immunization **IF APPLICABLE**

5. Labs to be drawn

- CBC with diff **ANALYSIS AT LOCAL LAB**
- Chemistries
- Serum for Autoantibodies
- HbA1c
- 2-hour MMTT (glucose and C-peptide)
- EBV/CMV PCR
- EBV/CMV Viral Serology
- PhiX174 Pre-Immunization Serology **IF APPLICABLE**

- PhiX174 15-Minute Post-Immunization Specimen **IF APPLICABLE**

6. Mechanistic Specimens

- **REFER TO ASSIGNED SCHEDULE OF ASSESSMENTS AND VISIT CHECKLIST**

ANTI-CD20 TRIAL MONTH 5 VISIT SUMMARY

NOTE: The participant may complete this visit at a site other than the study site

1. Forms

A. Case Report Forms:

- Adverse Events Report Form (RIT13) **IF NECESSARY**

B. Specimen Transmittal Forms:

- EBV/CMV PCR (RIT99PC)
- EBV/CMV Viral Serology (RIT99VS)

2. Barcode Labels

- 4 EBV/CMV PCR (VIRVL)
- 4 EBV/CMV Viral Serology (VIRST)

3. Supplies

A. Blood Collection

- 1 x 2 ml EDTA tube
- 1 x 3 ml plain red top tube

B. Specimen Shipment

- Collection tube for EBV/CMV PCR
- 1 x 1.8 ml cryovial

4. Activities to be completed

- Adverse events assessed

5. Labs to be drawn

- EBV/CMV PCR
- EBV/CMV Viral Serology

6. Preparation for next visit

- Remind participant to bring blood glucose and insulin records to next visit
- Instruct participant about preparation for MMTT

ANTI-CD20 TRIAL MONTH 6 VISIT SUMMARY

NOTE: The participant **NEEDS TO BE FASTING** for this visit

1. Forms

A. Case Report Forms:

- Diabetes Management Form (RIT09)
- Concomitant Medications Form (RIT10)
- Physical Exam Form (RIT11)
- Follow-up Visit Form (RIT12)
- Adverse Event Report Form (RIT13) **IF NECESSARY**

B. Specimen Transmittal Forms:

- CBC Results (RIT99CB)
- Chemistries (RIT99CH)
- Autoantibodies (RIT99AA)
- HbA1c (RIT99HB)
- 2-hour MMTT (RIT99M2)
- EBV/CMV PCR (RIT99PC)
- EBV/CMV Viral Serology (RIT99VS)
- Other Serology (RIT99SR)
- PK Analysis and HACA Levels (Covance to send)

2. Barcode Labels

- 4 Chemistries (CHEM)
- 4 Autoantibodies (BAA)
- 4 HbA1c (HbA1c)
- 17 2-hour MMTT (MMT2)
- 4 EBV/CMV PCR (VIRVL)
- 4 EBV/CMV Viral Serology (VIRST)
- 4 Other Serology (SRLG)
- Covance to send barcodes for PK and HACA

3. Supplies

A. Blood Collection

- 2 x 2 ml EDTA tubes
- 1 x 4 ml plain red top tube
- 1 x 2.6 ml SSG clotting activator tube
- 8 x 1.2 ml EDTA tubes
- 7 x 1.2 ml gray top tubes
- 2 x 3 ml plain red top tubes
- Covance to send supplies for PK and HACA

B. Specimen Shipment

- 1 x 2 ml amber vial
- 17 x 1.8 ml cryovials
- Collection tube for EBV/CMV PCR
- Covance to send supplies for PK and HACA

4. Activities to be completed

- Adverse events assessed
- Physical examination including neurologic assessment
- Urine pregnancy test
- 2-hour MMTT

5. Labs to be drawn

- CBC with diff **ANALYSIS AT LOCAL LAB**
- Chemistries
- Serum for Autoantibodies
- HbA1c
- 2-hour MMTT (glucose and C-peptide)
- EBV/CMV PCR
- EBV/CMV Viral Serology
- Other Serology
- PK and HACA

6. Preparation for next visit

- Remind participant to bring blood glucose and insulin records to next visit

7. Mechanistic Specimens

- **REFER TO ASSIGNED SCHEDULE OF ASSESSMENTS AND VISIT CHECKLIST**

ANTI-CD20 TRIAL MONTH 9 VISIT SUMMARY

1. Forms

A. Case Report Forms:

- Diabetes Management Form (RIT09)
- Concomitant Medications Form (RIT10)
- Physical Exam Form (RIT11)
- Follow-up Visit Form (RIT12)
- Adverse Event Report Form (RIT13) **IF NECESSARY**

B. Specimen Transmittal Forms:

- CBC Results (RIT99CB)
- Autoantibodies (RIT99AA)
- HbA1c (RIT99HB)
- EBV/CMV PCR (RIT99PC)
- EBV/CMV Viral Serology (RIT99VS)
- PK Analysis and HACA Levels (Covance to send)

2. Barcode Labels

- 4 Autoantibodies (BAA)
- 4 HbA1c (HbA1c)
- 4 EBV/CMV PCR (VIRVL)
- 4 EBV/CMV Viral Serology (VIRST)
- Covance to send barcodes for PK and HACA

3. Supplies

A. Blood Collection

- 2 x 2 ml EDTA tubes
- 1 x 2.6 ml SSG clotting activator tube
- 1 x 1.2 ml EDTA tube
- 1 x 3 ml plain red top tube
- Covance to send supplies for PK and HACA

B. Specimen Shipment

- 2 x 1.8 ml cryovials
- Collection tube for EBV/CMV PCR
- Covance to send supplies for PK and HACA

4. Activities to be completed

- Adverse events assessed
- Physical examination

5. Labs to be drawn

- CBC with diff **ANALYSIS AT LOCAL LAB**
- Serum for Autoantibodies
- HbA1c
- EBV/CMV PCR
- EBV/CMV Viral Serology
- PK and HACA

6. Preparation for next visit

- Remind participant to bring blood glucose and insulin records to next visit
- Instruct participant about preparation for MMTT

7. Mechanistic Specimens

- **REFER TO ASSIGNED SCHEDULE OF ASSESSMENTS AND VISIT CHECKLIST**

ANTI-CD20 TRIAL MONTH 12 VISIT SUMMARY

NOTE: The participant **NEEDS TO BE FASTING** for this visit

1. Forms

A. Case Report Forms:

- Diabetes Management Form (RIT09)
- Concomitant Medications Form (RIT10)
- Physical Exam Form (RIT11)
- Follow-up Visit Form (RIT12)
- Tetanus Administration Form (RIT17)
- Hepatitis A Administration Form (RIT18)
- Adverse Event Report Form (RIT13) **IF NECESSARY**
- PhiX174 Administration Form (RIT19) **IF APPLICABLE**

B. Specimen Transmittal Forms:

- CBC Results (RIT99CB)
- Chemistries (RIT99CH)
- Autoantibodies (RIT99AA)
- HbA1c (RIT99HB)
- 4-hour MMTT (RIT99M4)
- Hepatitis A and Tetanus Serology (RIT99HT)
- PhiX174 Serology (RIT99PX) **IF APPLICABLE**

2. Barcode Labels

- 4 Chemistries (CHEM)
- 4 Autoantibodies (BAA)
- 4 HbA1c (HbA1c)
- 25 4-hour MMTT (MMT4)
- 4 Hepatitis A and Tetanus Serology (HEPT)
- 5 PhiX174 Serology (PHIX) **IF APPLICABLE**

3. Supplies

A. Blood Collection

- 1 x 2 ml EDTA tube
- 1 x 4 ml plain red top tube
- 1 x 2.6 ml SSG clotting activator tube
- 12 x 1.2 ml EDTA tubes
- 11 x 1.2 ml gray top tubes
- 1 x 3 ml plain red top tube
- 2 x 4 ml plain red top tubes **IF APPLICABLE**

B. Specimen Shipment

- 1 x 2 ml amber vial
- 24 x 1.8 ml cryovials
- 2 x 1.8 ml cryovials **IF APPLICABLE**

4. Activities to be completed

- Adverse events assessed
- Physical examination including neurologic assessment
- Urine pregnancy test
- 4-hour MMTT
- Tetanus intramuscular immunization
- Hepatitis A intramuscular immunization
- PhiX174 intravenous immunization **IF APPLICABLE**

5. Labs to be drawn

- CBC with diff **ANALYSIS AT LOCAL LAB**
- Chemistries
- Serum for Autoantibodies
- HbA1c
- 4-hour MMTT (glucose and C-peptide)
- Hepatitis A and Tetanus Pre-Immunization Serology
- PhiX174 Pre-Immunization Serology **IF APPLICABLE**

- PhiX174 15-Minute Post-Immunization Specimen **IF APPLICABLE**

6. Mechanistic Specimens

- **REFER TO ASSIGNED SCHEDULE OF ASSESSMENTS AND VISIT CHECKLIST**

ANTI-CD20 TRIAL MONTH 13 VISIT SUMMARY

NOTE: The participant may complete this visit at a site other than the study site

1. Forms

A. Specimen Transmittal Forms:

- EBV/CMV PCR (RIT99PC)
- EBV/CMV Viral Serology (RIT99VS)
- Other Serology (RIT99SR)
- Hepatitis A and Tetanus Serology (RIT99HT)
- PhiX174 Serology (RIT99PX) **IF APPLICABLE**

2. Barcode Labels

- 4 EBV/CMV PCR (VIRVL)
- 4 EBV/CMV Viral Serology (VIRST)
- 4 Other Serology (SRLG)
- 4 Hepatitis A and Tetanus Serology (HEPT)
- 4 PhiX174 Serology (PHIX) **IF APPLICABLE**

3. Supplies

A. Blood Collection

- 1 x 2 ml EDTA tube
- 3 x 3 ml plain red top tubes
- 1 x 4 ml plain red top tube **IF APPLICABLE**

B. Specimen Shipment

- Collection tube for EBV/CMV PCR
- 3 x 1.8 ml cryovials
- 1 x 1.8 ml cryovial **IF APPLICABLE**

4 Labs to be drawn

- EBV/CMV PCR
- EBV/CMV Viral Serology
- Other Serology

- Hepatitis A and Tetanus Serology
- PhiX174 Serology **IF APPLICABLE**

5. Preparation for next visit

- Remind participant to bring blood glucose and insulin records to next visit

6. Mechanistic Specimens

- **REFER TO ASSIGNED SCHEDULE OF ASSESSMENTS AND VISIT CHECKLIST**

ANTI-CD20 TRIAL MONTH 15 VISIT SUMMARY

1. Forms

A. Case Report Forms:

- Diabetes Management Form (RIT09)
- Concomitant Medications Form (RIT10)
- Physical Exam Form (RIT11)
- Follow-up Visit Form (RIT12)
- Adverse Events Report Form (RIT13) **IF NECESSARY**

B. Specimen Transmittal Forms:

- CBC Results (RIT99CB)
- HbA1c (RIT99HB)
- EBV/CMV PCR (RIT99PC)
- EBV/CMV Viral Serology (RIT99VS)

2. Barcode Labels

- 4 HbA1c (HbA1c)
- 4 EBV/CMV PCR (VIRVL)
- 4 EBV/CMV Viral Serology (VIRST)

3. Supplies

A. Blood Collection

- 2 x 2 ml EDTA tubes
- 1 x 1.2 ml EDTA tube
- 1 x 3 ml plain red top tube

B. Specimen Shipment

- Collection tube for EBV/CMV PCR
- 1 x 1.8 ml cryovial

4. Activities to be completed

- Adverse events assessed

- Physical examination

5. Labs to be drawn

- CBC with diff **ANALYSIS AT LOCAL LAB**
- HbA1c
- EBV/CMV PCR
- EBV/CMV Viral Serology

6. Preparation for next visit

- Remind participant to bring blood glucose and insulin records to next visit
- Instruct participant about preparation for MMTT

ANTI-CD20 TRIAL MONTH 18 VISIT SUMMARY

NOTE: The participant **NEEDS TO BE FASTING** for this visit

1. Forms

A. Case Report Forms:

- Diabetes Management Form (RIT09)
- Concomitant Medications Form (RIT10)
- Physical Exam Form (RIT11)
- Follow-up Visit Form (RIT12)
- Adverse Event Report Form (RIT13) **IF NECESSARY**

B. Specimen Transmittal Forms:

- CBC Results (RIT99CB)
- Autoantibodies (RIT99AA)
- HbA1c (RIT99HB)
- 2-hour MMTT (RIT99M2)
- EBV/CMV PCR (RIT99PC)
- EBV/CMV Viral Serology (RIT99VS)
- Other Serology (RIT99SR)

2. Barcode Labels

- 4 Autoantibodies (BAA)
- 4 HbA1c (HbA1c)
- 17 2-hour MMTT (MMT2)
- 4 EBV/CMV PCR (VIRVL)
- 4 EBV/CMV Viral Serology (VIRST)
- 4 Other Serology (SRLG)

3. Supplies

A. Blood Collection

- 2 x 2 ml EDTA tubes
- 1 x 2.6 ml SSG clotting activator tube
- 8 x 1.2 ml EDTA tubes

- 7 x 1.2 ml gray top tubes
- 2 x 3 ml plain red top tubes

B. Specimen Shipment

- 17 x 1.8 ml cryovials
- Collection tube for EBV/CMV PCR

4. Activities to be completed

- Adverse events assessed
- Physical examination including neurologic assessment
- Urine pregnancy test
- 2-hour MMTT

5. Labs to be drawn

- CBC with diff **ANALYSIS AT LOCAL LAB**
- Serum for Autoantibodies
- HbA1c
- 2-hour MMTT (glucose and C-peptide)
- EBV/CMV PCR
- EBV/CMV Viral Serology
- Other Serology

6. Preparation for next visit

- Remind participant to bring blood glucose and insulin records to next visit

7. Mechanistic Specimens

- **REFER TO ASSIGNED SCHEDULE OF ASSESSMENTS AND VISIT CHECKLIST**

ANTI-CD20 TRIAL MONTH 21 VISIT SUMMARY

1. Forms

A. Case Report Forms:

- Diabetes Management Form (RIT09)
- Concomitant Medications Form (RIT10)
- Physical Exam Form (RIT11)
- Follow-up Visit Form (RIT12)
- Hepatitis A Administration Form (RIT18)
- Adverse Event Report Form (RIT13) **IF NECESSARY**

B. Specimen Transmittal Forms:

- CBC Results (RIT99CB)
- HbA1c (RIT99HB)
- EBV/CMV PCR (RIT99PC)
- EBV/CMV Viral Serology (RIT99VS)
- Hepatitis A and Tetanus Serology (RIT99HT)

2. Barcode Labels

- 4 HbA1c (HbA1c)
- 4 EBV/CMV PCR (VIRVL)
- 4 EBV/CMV Viral Serology (VIRST)
- 4 Hepatitis A and Tetanus Serology (HEPT)

3. Supplies

A. Blood Collection

- 2 x 2 ml EDTA tubes
- 1 x 1.2 ml EDTA tube
- 2 x 3 ml plain red top tubes

B. Specimen Shipment

- 2 x 1.8 ml cryovials

- Collection tube for EBV/CMV PCR

4. Activities to be completed

- Adverse events assessed
- Physical examination
- Hepatitis A intramuscular immunization

5. Labs to be drawn

- CBC with diff **ANALYSIS AT LOCAL LAB**
- HbA1c
- EBV/CMV PCR
- EBV/CMV Viral Serology
- Hepatitis A Pre-Immunization Serology

6. Preparation for next visit

- Remind participant to bring blood glucose and insulin records to next visit
- Instruct participant about preparation for MMTT

7. Mechanistic Specimens

- **REFER TO ASSIGNED SCHEDULE OF ASSESSMENTS AND VISIT CHECKLIST**

ANTI-CD20 TRIAL MONTH 24 VISIT SUMMARY

NOTE: The participant **NEEDS TO BE FASTING** for this visit

1. Forms

A. Case Report Forms:

- Diabetes Management Form (RIT09)
- Concomitant Medications Form (RIT10)
- Physical Exam Form (RIT11)
- Follow-up Visit Form (RIT12)
- Adverse Event Report Form (RIT13) **IF NECESSARY**

B. Specimen Transmittal Forms:

- CBC Results (RIT99CB)
- Chemistries (RIT99CH)
- Autoantibodies (RIT99AA)
- HbA1c (RIT99HB)
- 4-hour MMTT (RIT99M4)
- EBV/CMV PCR (RIT99PC)
- EBV/CMV Viral Serology (RIT99VS)
- Hepatitis A and Tetanus Serology (RIT99HT)
- Other Serology (RIT99SR)

2. Barcode Labels

- 4 Chemistries (CHEM)
- 4 Autoantibodies (BAA)
- 4 HbA1c (HbA1c)
- 25 4-hour MMTT (MMT4)
- 4 EBV/CMV PCR (VIRVL)
- 4 EBV/CMV Viral Serology (VIRST)
- 4 Hepatitis A and Tetanus Serology (HEPT)
- 4 Other Serology (SRLG)

3. Supplies

A. Blood Collection

- 2 x 2 ml EDTA tubes
- 1 x 4 ml plain red top tube
- 1 x 2.6 ml SSG clotting activator tube
- 12 x 1.2 ml EDTA tubes
- 11 x 1.2 ml gray top tubes
- 3 x 3 ml plain red top tubes

B. Specimen Shipment

- 1 x 2 ml amber vial
- 26 x 1.8 ml cryovials
- Collection tube for EBV/CMV PCR

4. Activities to be completed

- Adverse events assessed
- Physical examination including neurologic assessment
- Urine pregnancy test
- 4-hour MMTT

5. Labs to be drawn

- CBC with diff **ANALYSIS AT LOCAL LAB**
- Chemistries
- Serum for Autoantibodies
- HbA1c
- 4-hour MMTT (glucose and C-peptide)
- EBV/CMV PCR
- EBV/CMV Viral Serology
- Hepatitis A Serology
- Other Serology

6. Preparation for next visit (IF NECESSARY)

- Remind participant to bring blood glucose and insulin records to next visit
- Instruct participant about preparation for MMTT

7. Mechanistic Specimens

- **REFER TO ASSIGNED SCHEDULE OF ASSESSMENTS AND VISIT CHECKLIST**

Chapter 13. Immunizations

1. Overview

An important aspect of this study is the planned immunizations with phiX174, tetanus, and hepatitis A. These immunizations serve two purposes. The first purpose is mechanistic by determining if the infusion of rituximab can cause tolerance (a non-responsive condition) to an antigen given while the participant's B cells are gone. The second purpose is for safety by determining the ability of the immune system to recover and to respond to reimmunizations and future new immunizations. In order to do this, three immunizations were chosen, the first is not required but strongly desired for all participants in the study, whereas the second two are required for all participants.

2. Bacteriophage phiX174

Bacteriophage phiX174 is a T cell dependent antigen to which people would have not been exposed outside of a research study (neoantigen). This bacteriophage, a virus that only grows in the bacteria E. coli, does not infect human cells and, with the possible exception of mild and rare fever, no toxic effects have been observed. Bacteriophage phiX174 has been used to immunize both pediatric and adult participants with primary immunodeficiency disorders including participants with immune disorders and participants treated with various immunosuppressive drugs including rituximab. Measuring the immune response to phiX174 allows the assessment of various aspects of the immune system: amplification, immunologic memory, and isotype switching. Following primary immunization, the resulting immune response consists of only IgM antibody with the titer peaking at 2 weeks. After a secondary immunization, given six weeks later, the resulting antibody titer peaks earlier (1 week) and is substantially higher (approximately 10-fold) and

consists of both IgM (50%) and IgG (50%). Because of this well described pattern and timing of response, the timing of the immunizations and blood samples is critical in order that comparison to normals and between participants can be done. It is expected that the response will be very weak in participants who get rituximab and relatively normal in the controls. The response seen at the 1-year immunization is to be tested.

2.1. Supplies and Storage Requirements

PhiX174 is supplied in 0.8 ml single use vials for pediatric participants up to 40 kg, 1.8 ml single use vials for adult participants less than 90 kg, and 2.8 ml single use vials for adult participants greater than 90 kg. PhiX174 is stable for 10 years or more if stored at –70 or –80 °C, for 7 days without noticeable change if stored at –20 °C, and for no more than 2 days if stored at refrigerated temperature (2-8 ºC). Any phiX174 stored improperly should be returned to EMINENT.

2.2. Administration Guidelines

The PhiX174 Immunization Course consists of 2 intravenous immunizations administered 6 weeks apart. Additional serology specimens are drawn at other weeks (*Weeks 7, 8, 10, 13, 14, 16, 53, 54, 56, 59, 60, and 62*). Participants consenting to participate will undergo 2 courses.

Participants enrolled in phiX174 may combine the week 5 and week 6 visits. Week 5 may be moved to week 6 but week 6 may NOT be moved to week 5. Sites should contact participants via telephone at week 5. This will not be considered a protocol deviation since visit windows (other than infusion visits) are not specified in the protocol.

There are two types of specimens that will be collected for the phiX174 evaluation. One type of specimen is serology, which tests the immune response. There are no special considerations for these samples.

The other type of specimen is measuring the phiX174 viral particles to ensure the participant received the active material. Since the phiX174 itself may adhere to the infusion tubing, it is strongly preferred that this sample, obtained 15 minutes after the immunization, be obtained through a separate venipuncture site and NOT drawn through the IV. However, if it is necessary to draw through the existing IV site, disconnect the tubing used to infuse the phiX174, run saline by flush or drip through the IV catheter, and draw the specimen through a new set of lines.

After the participant has arrived and agreed to receive the immunization, the vial may be removed from the freezer and thawed either in a water bath or by warming it in the hands for 2-5 minutes. PhiX174 should be thawed just prior to administration and should not remain at room temperature for longer than 8 hours. Once it has been thawed it may NOT be refrozen.

Dose preparation: The dose of phiX174 is dependent on weight and calculated as: **0.022 ml/kg** body weight. No reconstituting or mixing of the vaccine is required.

Once the phiX174 has been thawed, the calculated dose is withdrawn from the appropriately-sized vial and administered as an intravenous push. The administration should take about 30 seconds. A Pharmacy Vaccine Request Form is provided in Appendix I and on the TrialNet Website.

Any remaining phiX174 solution should be discarded into a large quantity of 10% bleach/water (at least 10 times the amount of phiX174 being discarded).

All empty vials should be disposed of into "sharps" containers, which will be autoclaved.

The first course begins 3 weeks after the last rituximab infusion at Week 6.

- The first immunization is administered at Week 6. A pre-immunization serology specimen is drawn prior to the immunization.
- A different vein and different needle are used to collect the 15-minute post-immunization specimen drawn 15 minutes after the immunization.
- The second immunization is administered at Week 12. A pre-immunization serology specimen is drawn prior to the immunization.
- A different vein and different needle are used to collect the 15-minute post-immunization specimen drawn 15 minutes after the immunization.

The second course begins at Week 52.

- The first immunization is administered at Week 52. A pre-immunization serology specimen is drawn prior to the immunization.
- A different vein and different needle are used to collect the 15-minute post-immunization specimen drawn 15 minutes after the immunization.
- The second immunization is administered at Week 58. A pre-immunization serology specimen is drawn prior to the immunization.
- A different vein and different needle are used to collect the 15-minute post-immunization specimen drawn 15 minutes after the immunization.

There is a possibility of a fever immediately after the injection. This can be treated with anti-pyretics. There have been no delayed side effects noted.

3. Diphtheria Tetanus

The participants in this study will have had a previous series of tetanus shots as part of normal medical care. At one year after the rituximab, the participants will get a tetanus booster. This is called a recall antigen and will

test the immune responsiveness to an antigen to which the participant has already responded. A blood sample for tetanus is obtained at the start of the study, at the end of first year, and one month after a tetanus shot. If the level of tetanus antibody in the blood has fallen over the year, it would suggest that participants getting rituximab would need to have a booster shot. The response after the booster would ensure that such a response is possible. For this reason, this shot is part of the safety aspects of the study. A Pharmacy Vaccine Request Form is provided in Appendix I and on the TrialNet Website.

The shot is given intramuscularly. There may be pain and soreness at the site of the injection and possibly a low-grade fever that could develop 24 to 72 hours after the immunization. These could be treated with anti-pyretics and mild analgesics (e.g. acetaminophen and aspirin).

4. Hepatitis A immunization

Although hepatitis A is now being recommended for all children, this is not always done. If a participant were immunized with a full course of hepatitis A prior to the study, this would exclude them from subsequent hepatitis A immunizations as part of the study. Participants who have received a partial course of hepatitis A may complete the course at month 21. Participants who have not been immunized with hepatitis A will be given a hepatitis A immunization at month 12. A blood sample for hepatitis A antibody will be obtained one month later. Since the normal course of hepatitis A immunization includes a booster, this will be performed to complete the prophylaxis at month 21 with an antibody titer obtained at 2 years. A Pharmacy Vaccine Request Form is provided in Appendix I and on the TrialNet Website.

The two hepatitis A shots are given intramuscularly. There may be pain and soreness at the site of the injection and possibly a low-grade fever that could

develop 24 to 72 hours after the immunization. These could be treated with anti-pyretics and mild analgesics (e.g. acetaminophen and aspirin).

Chapter 14. Reports

This chapter contains descriptions of the reports implemented in the Anti-CD20 Trial. Examples of these reports are provided in Appendix O.

1. Eligibility Report

The Eligibility Report is a one-page summary of key laboratory results and other participant information related to study eligibility. It is available on the TrialNet Website under the Clinical Site's page and is generated when the Screening Form (RIT01), Specimen Transmittal Forms, and screening lab results have been received by the TNCC.

2. Lab Results

Lab results are available on the TrialNet Website under the Clinical Site's page. The file is a PDF and the results are organized by Screening ID Number and Visit Number. The normal ranges for the lab tests are also included. Contact the Protocol RA with questions.

3. Schedule of Assessments Assigned by the TrialNet Web Randomization System

The Web Randomization System assigns a Schedule of Assessments specific for the participant being randomized based on age, weight, HLA results, and phiX174 participation. This is a participant-specific schedule. It provides a detailed schedule of assessments by visit, with target dates and allowable visit windows based on the participant's date of randomization for the participant's follow-up in the study.

Chapter 15. Follow-up After 24 Months

1. Overview

Participants will be followed for up to two years after the initial treatment. Additional follow-up for up to two years will continue for those who have persistence of beta cell function at two years and/or detectable immunologic effects of treatment by descriptive analysis until the disappearance of detectable beta cell function or resolution of immunologic changes. Those participants will be asked to return to the study site every 6 months for up to another 2 years. Participants will be given a 2-hour Mixed Meal Tolerance Test, and a physical examination will be completed. The following tests will also be conducted at each of these 6-month visits: CBC with differential, autoantibodies, HbA1c, other serology and flow cytometry. Stored samples (RNA and B and T-cells) will also be collected.

ANTI-CD20 TRIAL MONTHS 30, 36, 42, AND 48 VISIT SUMMARY

NOTE: The participant **NEEDS TO BE FASTING** for this visit

1. Forms

A. Case Report Forms:

- Diabetes Management Form (RIT09)
- Concomitant Medications Form (RIT10)
- Physical Exam Form (RIT11)
- Follow-up Visit Form (RIT12)
- Adverse Event Report Form (RIT13) **IF NECESSARY**

B. Specimen Transmittal Forms:

- CBC Results (RIT99CB)
- Autoantibodies (RIT99AA)
- HbA1c (RIT99HB)
- 2-hour MMTT (RIT99M2)
- Other Serology (RIT99SR)

2. Barcode Labels

- 4 Autoantibodies (BAA)
- 4 HbA1c (HbA1c)
- 17 2-hour MMTT (MMT2)
- 4 Other Serology (SRLG)

3. Supplies

A. Blood Collection

- 1 x 2 ml EDTA tube
- 1 x 2.6 ml SSG clotting activator tube
- 8 x 1.2 ml EDTA tubes
- 7 x 1.2 ml gray top tubes
- 1 x 3 ml plain red top tube

B. Specimen Shipment

- 16 x 1.8 ml cryovials

4. Activities to be completed

- Adverse events assessed
- Physical examination
- 2-hour MMTT

5. Labs to be drawn

- CBC with diff **ANALYSIS AT LOCAL LAB**
- Serum for Autoantibodies
- HbA1c
- 2-hour MMTT (glucose and C-peptide)
- Other Serology

6. Preparation for next visit (IF NECESSARY)

- Remind participant to bring blood glucose and insulin records to next visit
- Instruct participant about preparation for MMTT

Chapter 16. Monitoring for Infection

1. Overview

As with any immunosuppressant agent, treatment with Rituximab requires careful monitoring and assessments for infection. Four weeks of Rituximab therapy will result in a diminished number of B cells within a few weeks of therapy until 6-12 months later. This is not likely to be reflected in a decrease in the number of total WBC that is obtained for clinical care since the percentage of affected cells make up such a small proportion of total WBC. Thus, monitoring for infections is dependent upon carefully questioning of participants about symptoms and alertness by clinical personnel for signs of infection. To accomplish this, participants are to be questioned at each visit and reminded to report any health issues between visits.

2. Infections

Infections can be bacterial or viral. Clinically significant bacterial infections associated with rituximab use include pneumonia. Viral infections of particular concern in immunosuppressed participants include EBV and CMV. Either new exposure to these virus or re-activation of these viruses in individuals who have been previously infected can theoretically occur. While blood samples will be drawn at regular intervals for the purpose of assessing viral status, these samples will only be run if there is a clinical indication or as a retrospective analysis. Thus, if there is a subject with signs or symptoms that would lead to suspicion of EBV or CMV, testing can be done on these stored samples as well as those obtained during the acute situation.

3. Immunoglobulin Levels

While typically there is no change in serum IgG level in participants who have received rituximab as part of clinical trials, there have been a few reported cases of hypogammaglobulinemia, particularly in the setting for treatment of lymphoma. In the rheumatoid arthritis study, hypogammaglobulinemia has not been reported. Because of this concern, serum IgG levels will be obtained before and during the trial. In the unlikely event of hypogammaglobulinemia, treatment will be performed as per standard of care. Serum IgG levels less than 500 mg/dl would be considered an adverse event (normal 1000 mg/dl by age 6 years). A level of less than 300 mg/dl would be considered a serious adverse event and would be an indication for treatment.

3.1. Treatment

Treatment will be IV Ig 100 mg/kg monthly. The first dose would be 200 mg/kg. A sample for IgG concentration would be obtained prior to infusion to determine the need for future treatments as it is expected that IgG levels would recover spontaneously as B-cell counts recover. For participants with IgG levels between 300 and 500 mg/dl, treatment would only be given in the presence of documented viral or bacterial infection. If infection were with CMV or EBV, then CMV Hyperimmune Globulin would be the preferred preparation of IV Ig.

4. Contact Information

While primary responsibility for monitoring, diagnosis, and treatment of infectious disease remains with the clinical investigator, consultation with TrialNet Anti-CD20 Trial Chair, Mark Pescovitz, MD, PhD, and TrialNet infectious disease consultants Adrianna Weinberg, MD, Michael Green, MD,

and Lindsey Baden, MD, is readily available. In addition, the Anti-CD20 Study Group will have regular phone calls to discuss clinical situations that have occurred at the sites.

5. Algorithm for Approach to Ill Participants

5.1. Introduction

This algorithm has been designed to provide guidance to the evaluation and management of participants who develop infectious syndromes during the first year of participation in the Anti-CD20 (rituximab) Trial. It should be noted that the precise impact of rituximab on the competency of the immune system in those participants who receive active treatment is not known. Hypogammaglobulinemia (low antibody levels) has been reported following use of this agent in organ transplant recipients, although it was rarely seen in multiple large studies of rituximab as treatment for lymphoma. *Therefore, the possibility that a research participant presenting with an infectious syndrome could be functionally hypogammaglobulinemic must be considered.*

In general, the types of infections associated with hypogammaglobulinemia include increased frequency of sinorespiratory diseases (e.g. otitis media, sinusitis and pneumonia). In addition, participants with more marked hypogammaglobulinemia are at increased risk of developing invasive infection (i.e. bacteremia) caused by encapsulated bacteria (e.g. Streptococcus pneumoniae). Participants with hypogammaglobulinemia are also potentially at risk for development of viral infections. A syndrome of chronic enterovirus meningitis has been reported in patients with hypogammaglobulinemia. It is worth noting that these patients do not experience a fulminant enteroviral infection, but rather present with chronic symptoms.

In approaching an ill research participant, it is strongly recommended that participants initially be seen by their primary care providers who are presumably experienced in the assessment of children and young adults presenting with acute illness. *Clinical judgment, tempered by knowledge of the potential existence of underlying an acquired immune deficiency related to participation in this study, is absolutely central to the management of these participants.*

5.2. Recommendations

The following recommendations are provided as a general guide to the evaluation and management of participants who develop an infectious illness during this study. Fever is defined as ≥ 101.3°F oral or ≥ 101.0°F x 3 times in a 24 hour time period.

Afebrile illness within the first 12 months following infusion of study drug, but after infusions completed:

- Absence of fever is likely associated with mild clinical illness
- Evaluation by primary physician optional based upon severity and duration of clinical symptoms
- Study personnel should be informed as part of routine study surveillance
- TrialNet ID Group available for consultation as needed

Febrile illness within first 12 months following infusion of study drug (but after infusions completed):

- Fever for ≤ 3 days
    - Participant should inform Clinical Site Study Personnel
        - Review most recent immunoglobulin studies
        - Inform evaluating physician if immunoglobulin studies abnormal

- Participant should be seen by local/primary physician
  - Performance of history & physical examination
- Management is as per local/primary physician
  - Physician should be aware of and consider possibility that participant could be "immunosuppressed"
  - Awareness of presence or absence of hypogammaglobulinemia
- TrialNet ID Group available for consultation

- Fever for > 3 days
  - Participant should inform Clinical Site Study Personnel
    - Review most recent immunoglobulin studies
    - Inform evaluating physician if immunoglobulin studies abnormal
  - Participant should be seen by local/primary physician
    - Performance of history & physical examination
  - Local/primary physician *encouraged* to make etiologic diagnosis
    - Physician should be aware of and consider possibility that subject could be "immunosuppressed"
    - Awareness of presence or absence of hypogammaglobulinemia
      - Review of most recently available results of surveillance quantitative immune globulins (QIG) measurement obtained for study protocol
      - Consider measurement of QIGs as part of evaluation of current febrile illness if prolong or severe
    - Consideration of consultation with local Infectious Diseases specialist particularly if participant is ill appearing
  - TrialNet ID Group available for input and guidance
    - Notification within 1-3 days recommended

- Site specific treatment as appropriate based on clinical signs and symptoms
- General Recommendations for Etiologic Evaluation of participants with fever > 3 days and presenting with Respiratory Tract symptoms
  - Consideration of seasonal pathogens (e.g. influenza, respiratory syncytial virus, Streptococcus pyogenes)
    - Effort to differentiate between upper and lower respiratory tract involvement
    - Clinical evaluation (e.g. auscultation) in all participants
    - Radiologic evaluation (e.g. chest radiograph) for those with evidence of lower respiratory tract infection on exam
  - Performance of diagnostic microbiologic tests where appropriate
    - Consider evaluation of nasal specimen for respiratory viruses
    - Recommend obtaining throat culture for Streptococcus pyogenes in participants whose sore throat as a major component of presenting illness
    - Potential evaluation of sputum for those participants with productive coughs who can produce a sputum sample

Febrile illness during the infusion period (first month)

- Fever present within 24 hours prior to scheduled infusion
  - Defer infusion until fever resolved for at least 24 hours
- Afebrile illness
  - Decisions regarding going forward with study infusion per site and study P.I.

Chapter 17. Assessment and Management of Adverse Events

ALL adverse events (Grade 1 and greater), whether observed by the investigator, reported by the participant, or from other means, will be recorded on the Adverse Event Report Form (RIT13) and graded according to the NCI CTCAE. Refer to the Safety Monitoring and Adverse Event Reporting Binder distributed in January 2007 for more information.

Chapter 18. Protocol Adherence

This section contains specific information about participant protocol adherence requirements. This includes information on expected adherence to the visit schedule as well as the study medication. The section also includes the details of specific protocol deviations and actions that need to be taken to address these deviations.

1. Protocol Deviations

This section provides specific examples of protocol deviations and what actions need to be taken to address these deviations. Every effort should be made to ensure that no protocol deviations occur. If any deviations do occur, the Protocol Deviation Form (RIT21) needs to be completed.

1.1. Examples of Protocol Deviations

The following list (not meant to be exhaustive) provides examples of other protocol deviations that would require the completion of the Protocol Deviation Form (RIT21):

- Participant does not undergo rituximab/placebo IV infusion.
- Participant undergoes rituximab/placebo IV infusion outside the protocol-specified window.
- Participant is randomized outside of 3-month (100 days) since diagnosis window or screening MMTT window (37 days).
- Baseline procedure/sample collection schedule specified in the protocol is not followed.

Chapter 19. Form Completion

1. Form Shipment

All study forms completed at the participating clinical centers will be mailed to the Coordinating Center for entry into the official database. These forms should be mailed using the FedEx PassKey System to the following address:

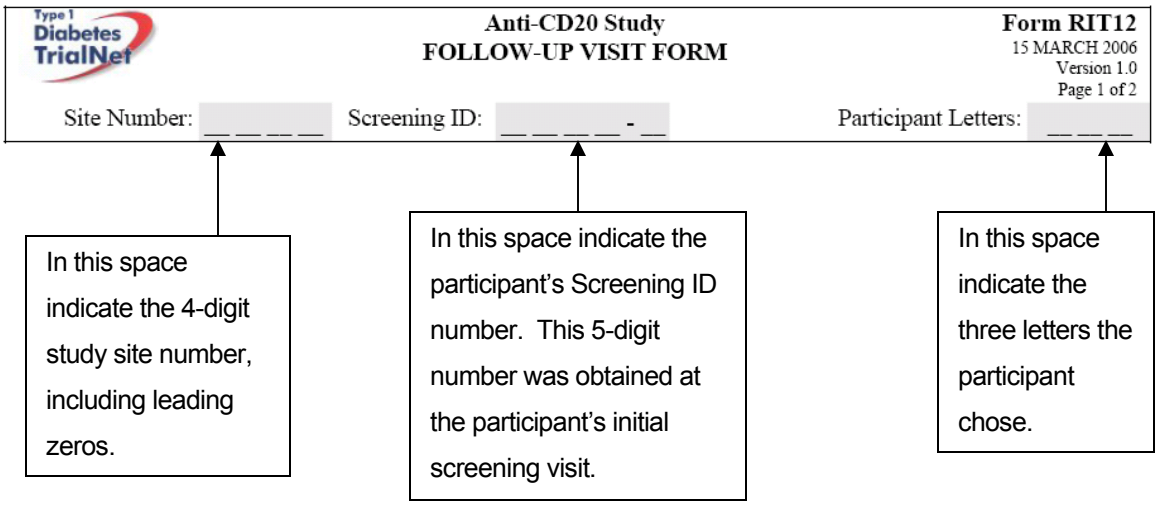
Type 1 Diabetes TrialNet Coordinating Center
The GWU Biostatistics Center
6110 Executive Blvd., Suite 500
Rockville, MD 20852
Contact: FORMS Seshu Pakalapti
Phone: (301) 881-9260
Fax: (301) 881-0179

A mailing list should be included with **ALL** shipments of forms. This should be an annotated list of all the forms included in the mailing. Once a particular mailing is received by the Coordinating Center, this mailing list is checked to ensure that all the indicated forms are included. Each of the received forms is stamped with the date of receipt at the Coordinating Center.

2. Instructions for Completing Forms

2.1. Completing the Header

The following header appears at the top of all pages of every form:



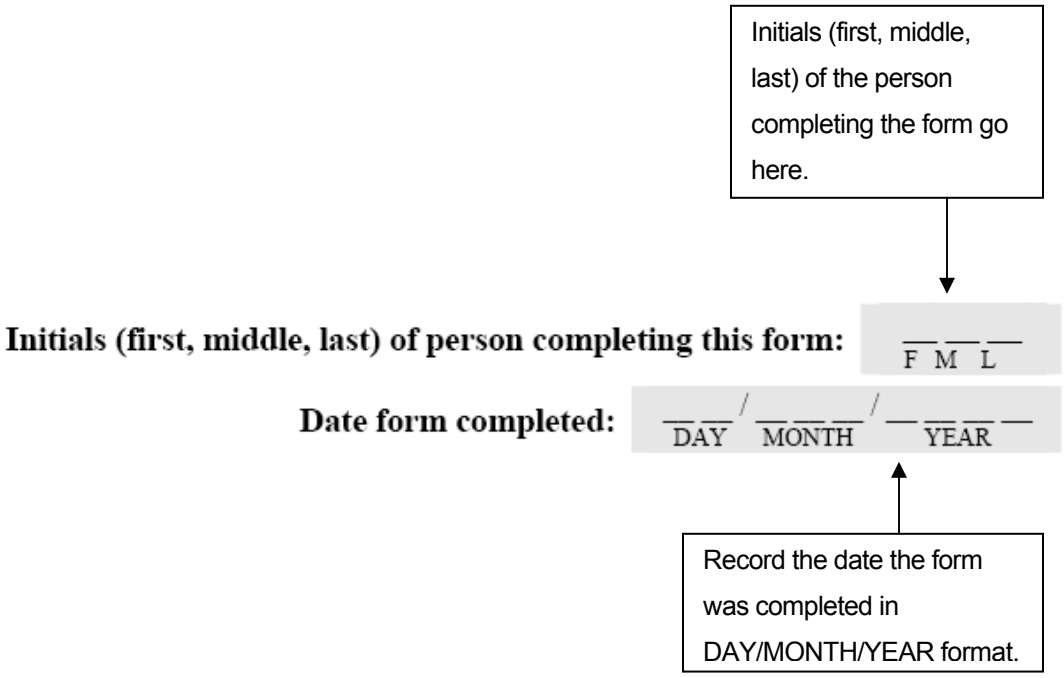
The banner needs to be completed with the information indicated above on *every* page of *every* form. The following are the site numbers for the site's participating in this study:

Clinic Name	Site Number
University of Florida	0001
CHLA	0004
Stanford University	0005
University of Miami	0006
Barbara Davis Center	0007
Joslin Diabetes Center	0008
University of Minnesota	0009
Benaroya Research Institute	0010
UCSF	0011
University of Texas	0012
Hospital for Sick Children – Toronto	0013
University of Pittsburgh	0014
Columbia University	0015
Indiana University	0016
San Raffaele Hospital	0017

Walter and Eliza Hall Institute	0019
University of Rochester	0252
University of Maryland	0210

2.2. Completing the Footer

The following footer appears on the bottom of the last page of all case report forms used in this study:



The initials of the person completing each form should be placed in the box indicated on the last page of every form. These initials will be recorded and used if the TNCC needs to contact the clinic with any questions about the completion of the form.

2.3. Yes/No Fields

Questions requiring a yes or no response will be indicated with a Y for Yes and an N for No. Circle the correct letter to indicate the response to the question.

2.4. Date Fields

Dates should be recorded using the DAY/MONTH/YEAR format, which utilizes two-digits for the date, three-characters for the month, and four-digits for the year. Indicate the month as three-characters (e.g., Sep for September). Indicate the year as four-digits (e.g., 1972 instead of 72).

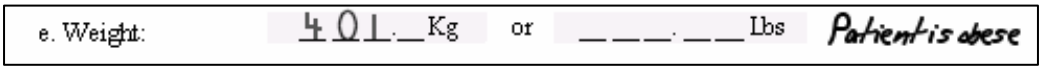
2.5. Checkbox Fields

Fields offering several options using a checkbox format should be carefully reviewed to make sure the appropriate number of responses are selected. Some checkbox fields only allow one response while other checkbox fields allow more than one response. When recording the response, enter a √ or an X into the box.

2.6. The Use of Comments

Additional comments can be attached to **any** gray field on **any** of the data collection forms. These comments are used when the Research Assistant is reviewing the edits. If the comment included on the form sufficiently explains the edit, then the issue will be considered resolved, and the edit will not be forwarded to the clinical center. The comments are also used during the analysis of the data collection forms.

Anyone completing the data collection forms is strongly encouraged to enter comments for any piece of information that would be considered to be helpful in the interpretation of the data. An example of the potential utility of a comment is illustrated below:



Without the comment, this value for the participant's weight would have generated an edit that would have been reviewed by the Research Assistant and then sent on to the clinic for clarification. The presence of the comment lets the Research Assistant know that the person completing the form was aware of the extreme value, and therefore an edit does not need to be sent to the clinic for further verification.

2.7. Missing Value Codes

There are two predefined codes that can be used when completing any of the data collection forms for this or any TrialNet Study. These codes are used to offer an explanation for a missing value to a particular question on the form. If the answer to a particular question is not available when the form is being completed, one of these codes can be entered. This will tell the official database the reason for the missing value. The two codes are as follows:

"*" – The asterisk indicates that the value is permanently missing and will not be available at any future time. The official database will then know not to send any edits regarding this missing value.

"?" - The question mark indicates that the value is currently unavailable (at the time this form is being completed), but is being checked and will be available in the future. The official database then knows to generate an edit regarding this missing value to remind the clinic that it was being checked on.

These two codes can be entered anywhere in the gray shaded response area for a particular question.

The following footer appears on the bottom of every page of every case report form as a reminder of these missing value codes:

On all questions write "?" if the desired information is currently unavailable, but is being checked and will be known in future updates. Write "" if the desired information is permanently unavailable (i.e. will not be known in any future updates).*

3. The Edit System

The edit system is built into the official study database. This system is designed to automatically capture such potential errors in form completion as: missing values, unexpected values, missing value codes, and information on a study form that is either inconsistent or out of range. The edits are generated and then sent to the clinic for resolution.

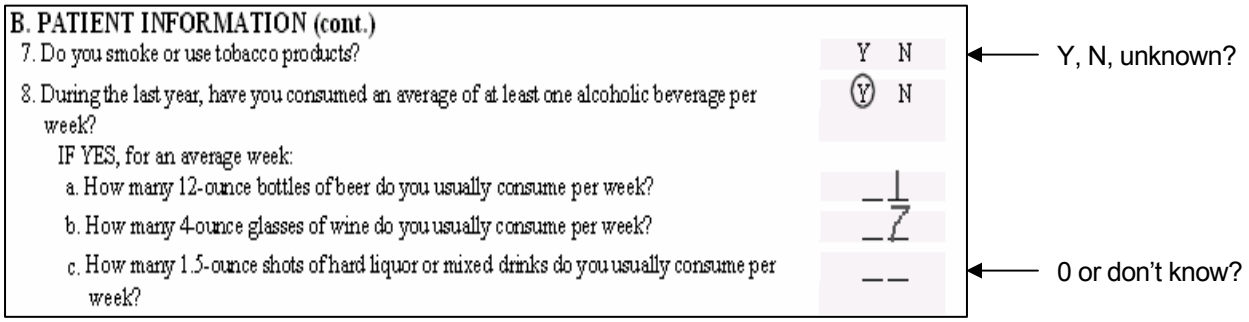
The clinic completes the edit forms and mails them back to the Coordinating Center. The Coordinating Center reviews the response received and, if deemed adequate, updates the official study database. If the response does not completely resolve the issue that generated the edit, a follow-up edit will be sent to the clinic for further clarification. If an edit is sent to the clinic and no response is received within 4 weeks, a follow-up edit will automatically be generated by the study database, reviewed by the study Research Assistant, and sent to the clinic for resolution. Edits will continue to be sent until a correction is received back from the site and the corrected information is entered into the database.

3.1. Types of Edits

There are many different edits that can be generated, but all of them tend to fall into four broad categories.

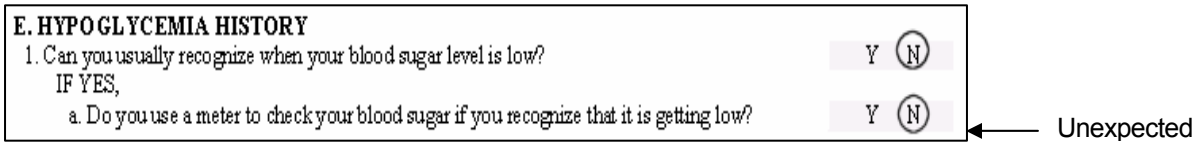
Missing Values

A particular question on a form was not answered. Illustrated by the following example:



Unexpected Answers

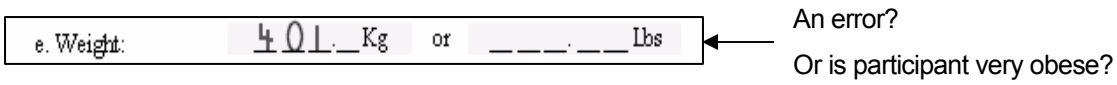
A particular question was unexpectedly answered; say for example that a conditional sub-question was answered despite the condition for answering the question not being met. This is demonstrated in the following example:



Range Checks

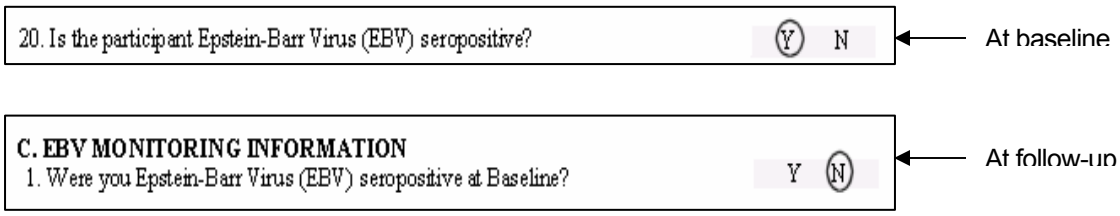
The answer for a particular question is out of a predefined range of "normal". This means the value indicated was either very high or very low. The answer provided may be correct, but the edit is generated to be sure that an error has

not occurred due to the peculiarity of the value recorded. This is illustrated in the following example:



Consistency Checks

The answer for a particular question on one form is not consistent with the answer given on a previous form for the same participant. This is illustrated in the following example:



3.2. Common Problems with Form Completion

There are several common problems that are encountered when completing the data collection forms that need to be kept in mind.

Skip Patterns

These are sections that are labeled “IF YES” (or similar language). These sections should **only** be completed when the condition applies, and should be **left blank** otherwise.

Dates

There are two problems that are commonly encountered when dates are entered on the data collection forms. The first is called the “January problem”.

This problem is encountered during the early part of the New Year, when a common mistake is to write the previous year. This will cause the official database to generate an edit, since the date written on the form is clearly out of the expected range.

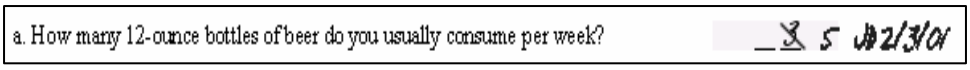
The second problem commonly encountered with dates involves the switching of the month and day elements. To avoid this problem, keep in mind that all dates written on the data collection forms should be in the format: **DAY/MONTH/YEAR** and the month should be in character format NOT numeric.

4. Corrections to Data Forms

When a correction needs to be made to a data collection form, the following guidelines need to be followed:

- Corrections need to be made in **black** ballpoint pen
- The original value needs to be crossed out with a single line so that the original entry remains legible
- The correction should be made on the right of and immediately adjacent to the original entry
- The change should be dated and confirmed with initials

The proper way to make a correction to a data collection form is demonstrated below:



Chapter 20. T Cell Assays

1. Cytokine ELISpot Lab

This laboratory will serve as the central laboratory for identifying and enumerating cytokine secreting peptide-reactive T cells from specimens collected at clinical sites. Interluken-10 production will also be monitored by Cytokine ELISpot, which may demonstrate the presence of cells with a regulatory phenotype.

1.1. Brief Description of Cytokine ELISpot Assay

The Cytokine ELISpot assay provides information about the profiles of cytokines that are released from a single T cell following antigen stimulation. This provides a qualitative and quantitative measure of T cell response to activation. The assay provides simultaneous analysis of post inflammatory production of interferon-gamma and of interleukin-10 production, which may allow optimal discrimination of cells with a regulatory phenotype. The reported data will include the number of antigen reactive T cells expressing individual cytokines.

1.2. Collection of Serum for Cytokine ELISpot Testing

This section describes in detail the collection and shipping of serum for Cytokine ELISpot tests. Cytokine ELISpot testing is performed at various time points throughout the study based on the participant's age, weight, and HLA results. Please refer to the Schedule of Assessments assigned at randomization for the collection schedule.

1.3. Clinic Preparation (Supplies and Labeling of Tubes and Forms)

Prior to the start of testing, label and prepare supplies and forms. For children 25-40kg, **two** green topped 10ml blood collection tubes and **one** green topped 6ml blood collection tube will be collected. For all other participants, **three** green topped 10ml blood collection tubes will be collected. Samples will be shipped with the Cytokine ELISpot Specimen Transmittal Form (RIT99EL) to the Barbara Davis Center in Denver, Colorado.

Required Supplies for Collection of Serum for Cytokine ELISpot	
Item	**Quantity**
Collection tube, green top, 10 ml	3 *(depending on age and weight)*
Collection tube, green top, 6 ml	1 *(depending on age and weight)*
Alcohol swabs	2
Needles, Butterfly, 21 G	1
Needle Holder, Single Use	1
Biohazard plastic bag & Tyvek outer envelope	1
Gel packs	2
Bubble wrap sleeve	1
Absorbent Pad	1
Ambient shipper	1
Label, UN3373 Clinical Specimen	1

- Label each green topped tube with the appropriate **barcode label** indicating specimen type (ELSPT) from an **unused** ELSPT barcode label group. Write the date of draw (e.g. 05/Sep/2005) and the participant letters with an alcohol-proof pen. Pre-printed barcode labels will be provided by the TrialNet Coordinating Center.
- Apply barcode labels vertically.
- Attach matching barcode labels from the **same barcode group** to <u>each page</u> of the Cytokine ELISpot Specimen Transmittal Form (RIT99EL)

1.4. Blood Collection Procedures

- Draw blood into the labeled tubes.
- Invert each tube gently **six-eight** times and place upright in a tube rack.
- Allow blood to clot for 20- 30 minutes at room temperature (65-75° F).
- Samples for Cytokine ELISpot are shipped as whole blood Priority Overnight Monday - Wednesday. (Refer to shipping procedures below)
- Place collection tubes in the bubble wrap sleeve.

To Prepare Samples for Shipment:

- Samples may be shipped at room temperature **Monday**-**Wednesday** (except days before U.S. federal holidays)
- Place the bubble wrap sleeve in a biohazard plastic bag with an absorbent pad and seal.
- Place the yellow copy of the Cytokine ELISpot Specimen Transmittal Form (RIT99EL) into the outside sleeve of the bag.
- Place the bag into a Tyvek outer envelope and seal.
- Place one gel pack left at room temperature on the bottom of the ambient shipper.
- Place the sealed Tyvek envelope on top of the gel pack.
- Place the second gel pack on top of the Tyvek envelope and seal the shipper.
- Affix a Diamond UN 3373 Clinical Specimen label on the outside of the box.
- Write “Clinical Specimens” above the diamond shaped label.

Shipment of All Cytokine ELISpot Samples:

Prepare and print a pre-paid FedEx airbill to ship all samples **Priority Overnight** to:

Rebecca Wagner- Gottlieb Lab
UCHSC, BDC
1775 N. Ursula
Room 4201U
Aurora, CO 80010
Phone (303) 724-6804

2. Cellular Immunoblot Lab

This laboratory will serve as the central laboratory for measuring study participant's T cell proliferative responses to a pool of antigen prepared from human islet cells.

2.1. Brief Description of Cellular Immunoblot Assay

The Cellular Immunoblot assay measures the proliferative response of T cell to a pool of antigens from human islet cells that are separated, blotted to nitrocellulose, and cultured. Responses to a consistent set of antigens are tested against the molecular weight protein regions indicated by each blot section. The reported data provides a quantitative manner as the number of positive blot sections and the intensity of the response to each blot section as stimulation indices.

2.2. Collection of Serum for Cellular Immunoblot Testing

This section describes in detail the collection and shipping of serum for Cellular Immunoblot tests. Cellular Immunoblot testing is performed at various time points throughout the study based on the participant's age, weight, and HLA results. Please refer to the Schedule of Assessments assigned at randomization for the collection schedule.

2.3. Clinic Preparation (Supplies and Labeling of Tubes and Forms)

Prior to the start of testing, label and prepare supplies and forms. For children 25-40kg, **two** green topped 10ml blood collection tubes and **one** green topped 6ml blood collection tube will be collected. For all other participants, **three** green topped 10ml blood collection tubes will be collected. Samples will be shipped with the Cellular Immunoblot Specimen Transmittal Form (RIT99CI) to the VA Medical Center in Seattle, Washington.

Required Supplies for Collection of Serum for Cellular Immunoblot	
Item	**Quantity**
Collection tube, green top, 10 ml	3 *(depending on age and weight)*
Collection tube, green top, 6 ml	1 *(depending on age and weight)*
Alcohol swabs	2
Needles, Butterfly, 21 G	1
Needle Holder, Single Use	1
Biohazard plastic bag & Tyvek outer envelope	1
Gel packs	2
Bubble wrap sleeve	1
Absorbent Pad	1
Ambient shipper	1
Label, UN3373 Clinical Specimen	1

- Label each green topped tube with the appropriate **barcode label** indicating specimen type (BLOT) from an **unused** BLOT barcode label group. Write the date of draw (e.g. 05/Sep/2005) and the participant letters with an alcohol-proof pen. Pre-printed barcode labels will be provided by the TrialNet Coordinating Center.
- Apply barcode labels vertically.
- Attach matching barcode labels from the **same barcode group** to <u>each page</u> of the Cellular Immunoblot Specimen Transmittal Form (RIT99CI)

2.4. Blood Collection Procedures

- Draw blood into the labeled tubes.
- Invert each tube gently **six- eight** times and place upright in a tube rack.
- Allow blood to clot for 20- 30 minutes at room temperature (65-75° F).
- Samples for Cellular Immunoblot are shipped as whole blood Priority Overnight Monday- Wednesday. (Refer to shipping procedures below)
- Place collection tubes in the bubble wrap sleeve.

To Prepare Samples for Shipment:

- Samples may be shipped at room temperature **Monday-Wednesday** (except days before U.S. federal holidays)
- Place the bubble wrap sleeve in a biohazard plastic bag with an absorbent pad and seal.
- Place the yellow copy of the Cellular Immunoblot Specimen Transmittal Form (RIT99CI) into the outside sleeve of the bag.
- Place the bag into a Tyvek outer envelope and seal.
- Place one gel pack left at room temperature on the bottom of the ambient shipper.
- Place the sealed Tyvek envelope on top of the gel pack.
- Place the second gel pack on top of the Tyvek envelope and seal the shipper.
- Affix a Diamond UN 3373 Clinical Specimen label on the outside of the Clinical Pak.
- Write “Clinical Specimens” above the diamond shaped label.

Shipment of all Cellular Immunoblot Samples:

Prepare and print a pre-paid FedEx airbill to ship all samples **Priority Overnight** to:

Dr. Barbara Brooks-Worrell
VA Medical Center
1660 S. Columbia Way,
Building 1, Room 609
Seattle, WA 98108
Phone (206) 764-2696 or 206-764-2616

3. Tetramer Laboratory

This laboratory will perform a MHC Class II/Peptide Tetramer study specific for HLA-DR4 and DR3 alleles, and GAD65 and proinsulin peptides. These peptide tetramers will identify the presence of effector CD4+ T cells specific for a given complex of peptide fragments of antigen (GAD65) bound to self MHC molecules (HLA-DR4)

3.1. Brief Description of Tetramer Assay

In the Tetramer assay, soluble flurochrome tagged MHC class II tetramers loaded with the peptides representing epitopes form GAD65 and proinsulin are used to enumerate antigen specific CD4+ T cells by flow cytometry. The assay provides a quantitative measurement of the frequency and staining intensity of the tetramer binding cells as well as the number of epitopes recognized.

3.2. Collection of Serum for Tetramer Testing

This section describes in detail the collection and shipping of serum for Tetramer tests. Tetramer testing is performed at various time points throughout the study based on the participant's age, weight, and HLA results. Please refer to the Schedule of Assessments assigned at randomization for the collection schedule.

3.3. Clinic Preparation (Supplies and Labeling of Tubes and Forms)

Prior to the start of testing, label and prepare supplies and forms. For children 25-40kg, **two** green topped 10ml blood collection tubes and **one** green topped 6ml blood collection tube will be collected. For children 41-65kg, **three** green topped 10ml blood collection tubes will be collected. For children >65kg and adults, **two** green topped 10ml blood collection tubes will be collected. Samples will be shipped with the Tetramer Specimen Transmittal Form (RIT99TM) to the Clinical Core Lab in Seattle, WA.

Required Supplies for Collection of Serum for Tetramer	
Item	**Quantity**
Collection tube, green top, 10 ml	3 *(depending on age and weight)*
Collection tube, green top, 6 ml	1 *(depending on age and weight)*
Alcohol swabs	2
Needles, Butterfly, 21 G	1
Needle Holder, Single Use	1
Biohazard plastic bag & Tyvek outer envelope	1
Gel packs	2
Bubble wrap sleeve	1
Absorbent Pad	1
Ambient shipper	1
Label, UN3373 Clinical Specimen	1

- Label each green topped tube with the appropriate **barcode label** indicating specimen type (TETRA) from an **unused** TETRA barcode label group. Write the date of draw (e.g. 05/Sep/2005) and the participant letters with an alcohol-proof pen. Pre-printed barcode labels will be provided by the TrialNet Coordinating Center.
- Apply barcode labels vertically.
- Attach matching barcode labels from the **same barcode group** to <u>each page</u> of the Tetramer Specimen Transmittal Form (RIT99TM)

3.4. Blood Collection Procedures

- Draw blood into the labeled tubes.
- Invert the tube gently **six-eight** times and place upright in tube rack.
- Allow blood to clot for 20- 30 minutes at room temperature (65-75° F).
- Samples for Tetramer are shipped as whole blood Priority Overnight Monday- Wednesday. (Refer to shipping procedures below)
- Place collection tubes in the bubble wrap sleeve.

To Prepare Samples for Shipment:

- Samples may be shipped at room temperature **Monday-Wednesday** (except days before U.S. federal holidays)
- Place the bubble wrap sleeve in a biohazard plastic bag with an absorbent pad and seal.
- Place the yellow copy of the Tetramer Specimen Transmittal Form (RIT99TM) into the outside sleeve of the bag.
- Place the bag into a Tyvek outer envelope and seal.
- Place one gel pack left at room temperature on the bottom of the ambient shipper.
- Place the sealed Tyvek envelope on top of the gel pack.
- Place the second gel pack on top of the Tyvek envelope and seal the shipper.
- Affix a Diamond UN 3373 Clinical Specimen label on the outside of the Clinical Pak.
- Write “Clinical Specimens” above the diamond shaped label.

Shipment of all Tetramer Samples:

Prepare and print a pre-paid FedEx airbill to ship all samples **Priority Overnight** to:

Clinical Core Lab
Benoroya Research Institute
1201 Ninth Ave.
Seattle, WA 98101
Phone (206) 341-1986

4. Toronto T Cell Proliferation Laboratory

This laboratory will serve as the central laboratory to perform the *Toronto Assay* using a pilot of antigens (GAD65, Insulin B, inactive Tetanus Toxin) and a subset of the lab's established and tested autoantigens, as the cell yields allow to test reactivity of the T cell population.

4.1. Brief Description of T Cell Proliferation Assay

The Toronto T Cell Proliferation assay measures T cell proliferative responses to a panel of autoantigenic peptides by examining ^{3}H-Thymidine incorporation by peripheral blood mononuclear cells following a primary *in vitro* culture in the presence of soluble, purified type 1 diabetes-relevant antigens (Miyazaki et al. 1995). This procedure can detect functional as well as anergic T cell pools. The assay will report data in a qualitative manner.

4.2. Collection of Serum for Toronto T Cell Proliferation Testing

This section describes in detail the collection and shipping of serum for Toronto T Cell Proliferation tests. Toronto T Cell Proliferation testing is performed at baseline, months 6, 12, 13, 18, and 24.

4.3. Clinic Preparation (Supplies and Labeling of Tubes and Forms)

Prior to the start of testing, label and prepare supplies and forms. For children 25-65kg, **one** green topped 6ml blood collection tube will be collected. For

children >65kg and adults, **one** green topped 10ml blood collection tube will be collected (**one** additional green topped 6 ml blood collection tube will be collected at baseline, months 6, 13, and 18 ONLY). Samples will be shipped with the Toronto T Cell Proliferation Specimen Transmittal Form (RIT99TP) to the Hospital for Sick Children in Toronto, ON.

Required Supplies for Collection of Serum for Tetramer	
Item	**Quantity**
Collection tube, green top, 10 ml	1 *(depending on age and weight)*
Collection tube, green top, 6 ml	1 *(depending on age and weight)*
Alcohol swabs	2
Needles, Butterfly, 21 G	1
Needle Holder, Single Use	1
Biohazard plastic bag	1
Mailer, 3 Tube Styrofoam	1
Mailer Sleeve, 3 Tube	1
Absorbent Pads	1
FedEX Large Clinical Pak	1
Label, UN3373 Clinical Specimen	1

- Label each green topped tube with the appropriate **barcode label** indicating specimen type (PROL) from an **unused** PROL barcode label group. Write the date of draw (e.g. 05/Sep/2005) and the participant letters with an alcohol-proof pen. Pre-printed barcode labels will be provided by the TrialNet Coordinating Center.
- Apply barcode labels vertically.
- Attach matching barcode labels from the **same barcode group** to <u>each page</u> of the Toronto T Cell Proliferation Specimen Transmittal Form (RIT99TP)

4.4. Blood Collection Procedures

- Draw blood into the labeled tube.

- Invert the tube gently **six-eight** times and place upright in a tube rack.
- Allow blood to clot for 20- 30 minutes at room temperature (65-75° F).
- Samples for Toronto T Cell Proliferation are shipped as whole blood Priority Overnight Monday- Wednesday. (Refer to shipping procedures below)
- Place the collection tube in the 3 tube Styrofoam holder with an absorbent pad. Tape the holder securely closed.

To Prepare Samples for Shipment:

- Samples may be shipped at room temperature **Monday**-**Wednesday** (except days before U.S. and Canadian federal holidays)
- Place the 3 tube Styrofoam holder in the cardboard 3 tube mailer sleeve.
- Place the mailer in a biohazard plastic bag and seal.
- Place the yellow copy of the T Cell Proliferation Specimen Transmittal Form (RIT99TP) into the outside sleeve of the bag.
- Place the bag into a large FedEx Clinical Pak to ship at room temperature.
- Affix a Diamond UN 3373 Clinical Specimen label on the outside of the Clinical Pak.
- Write “Clinical Specimens” above the diamond shaped label.

Shipment of all Toronto T Cell Proliferation Samples:

Prepare and print a pre-paid FedEx airbill to ship all samples **Priority Overnight** to:

Dr. H. Michael Dosch
The Hospital for Sick Children
IIIR Program
555 University Ave.
Toronto, ON
Canada M5G 1X8
Phone (416) 813-6260

Chapter 21. Laboratory Procedures

1. Local Laboratory

All laboratory result forms must be reviewed and signed by the Principal Investigator (PI) at each site. Any abnormal laboratory values will need to be reviewed and signed by the PI at each site. If PI confirms that the participant's lab results are abnormal, the abnormal labs will be redrawn. An Investigator comment is required for all clinically significant laboratory results.

1.1. CBC with Differential

The following parameters will be measured:

- Red blood cell count
- Hemoglobin
- Hematocrit
- Red blood cell indices
  - Mean corpuscular volume (MCV)
  - Mean corpuscular hemoglobin (MCH)
  - Mean corpuscular hemoglobin concentration (MCHC)
- White blood cell count (WBC) and differential count
  - Neutrophils
  - Lymphocytes
  - Monocytes
  - Eosinophils
  - Basophils
- Platelet count

This sample will be drawn into a 2ml EDTA tube at almost every study visit. This test will be analyzed at each site's local lab, so a different sized EDTA tube might be used for collection. The CBC will primarily be used to monitor

for leukopenia and neutropenia, as well as monitor participant's hemoglobin and hematocrit.

The results of the CBC will be transmitted to the TrialNet Coordinating Center by completing the Results Transmittal Form (RIT99CB). This form should be completed with the results of the CBC and mailed to the TrialNet Coordinating Center.

2. Shipping Locations and Addresses for Blood Specimens

Specimens not analyzed at the local laboratory will be shipped to one of the following for analysis:

- TrialNet Core Laboratory;
- Mechanistic T Cell Laboratory; or
- Contracted Laboratory.

Samples collected for storage will be sent to the National Institute of Diabetes, Digestive and Kidney Diseases (NIDDK).

2.1. Core Beta Cell Function Laboratory

Address:	Jerry Palmer Lab Specimen Processing Northwest Lipid Research Laboratories 401 Queen Anne Avenue North Seattle, WA 98109-4517
Phone:	(206) 685-3327
Samples:	• C-peptides and glucoses (MMTT) • HbA1c

2.2. Core Biochemistry Laboratory

Address:	Santica Marcovina Lab Specimen Processing Northwest Lipid Research Laboratories 401 Queen Anne Avenue North Seattle, WA 98109-4517
Phone:	(206) 685-3327
Samples:	• Chemistries • HIV, Hep B, Hep C

2.3. Core HLA/DNA Laboratory

Address:	Attn: Sunanda Babu UCHSC at Fitzsimons 1775 N. Ursula Street, M20-4201 C Aurora, CO 80045
Phone:	(303) 724-6806
Samples:	• HLA Determination

2.4. Core Autoantibody Laboratory

Address:	Liping Yu University of Colorado Barbara Davis Center 1775 N. Ursula Street, M20-4201 E Aurora, CO 80045
Phone:	(303) 724-6809
Samples:	• Autoantibody (ICA-512, GAD, IAA)

2.5. Core Viral Laboratory

Address:	Adriana Weinberg Lab University of Colorado Hospital Clinical Lab – LOB room 253 12401 E. 17th Ave. Aurora, CO 80045
Phone:	(720) 848-4401
Samples:	• Serology • Tetanus Ab Testing • Hep A Ab Testing • EBV/CMV PCR (analysis of viral load) • Viral Serology (EBV serology, CMV serology)

2.6. Core ICA Laboratory (Gainesville)

Address:	TrialNet Core Screening Laboratory (UFDRL) Bill Winter University of Florida 4800 SW 35th Drive Gainesville, FL 32608
Phone:	(352) 265-9900
Samples:	• Autoantibody (ICA)

2.7. Mechanistic T Cell Lab: Cytokine ELISpot Laboratory

Address:	Rebecca Wagner – Gottlieb Lab UCHSC, BDC 1775 North Ursula Street, Room 4201U Aurora, CO 80045
Phone:	(303) 724-6804
Samples:	• Cytokine ELISpot

2.8. Mechanistic T Cell Lab: Immunoblot Lab

Address:	Dr. Barbara Brooks-Worrell VA Medical Center 1660 S. Columbia Way, Bldg 1, Rm 609 Seattle, WA 98108
Phone:	(206) 764-2696 or (206) 764-2616
Samples:	• Immunoblot

2.9. Mechanistic T Cell Lab: Tetramer Lab

Address:	Helena Reijonen, PhD Clinical Core Lab Benaroya Research Institute 1201 Ninth Ave. Seattle, WA 98101
Phone:	(206) 341-1986
Samples:	• Peptide Tetramers

2.10. Mechanistic T Cell Lab: T Cell Proliferation Lab

Address:	H. Michael Dosch, MD The Hospital for Sick Children Attn: Roy Cheung 555 University Ave., Elm Wing, Room 10128 Toronto, ON Canada M5G 1X8
Phone:	(416) 813-6260
Samples:	• T Cell Proliferation

2.11. Contracted Lab: Molecular Diagnostics Laboratory (University of Washington School of Medicine)

Address:	Hans D. Ochs, MD Attn: Marge Young, Dept. of Pediatrics University of Washington School of Medicine 307 Westlake Ave N, Ste. 300 (MS CW) Seattle, WA 98109
Phone:	(206) 987-7450
Samples:	• PhiX174 (phage) antibody titering

2.12. Contracted Lab: Covance Laboratory

Address:	Director: Michael Hirsch, MD Covance Laboratories Inc. 3635 Concorde Parkway, Suite 100 Chantilly, VA 20151
Phone:	(703) 245- 2200 ext. 5478
Samples:	• PK/HACA

2.13. Contracted Lab: Flow Cytometry Lab

Address:	Paul Wallace Lab Roswell Park Cancer Institute Elm and Carlton Streets Buffalo, New York, 14263
Phone:	(877) 275-7724
Samples:	• Flow Cytometry

2.14. Contracted Lab: University of Alabama

Address:	University of Alabama at Birmingham Division of Clinical Immunology and Rheumatology SIBR 276 1825 University Boulevard Birmingham, Alabama 35294
Phone:	(205) 996- 4478
Samples:	• FcR Genotyping

2.15. NIDDK Repository (stored samples)

Address:	Director: Heather Higgins NIDDK Repository Fisher BioServices 20301 Century Blvd. Building 6, Suite 400 Germantown, MD 20874
Phone:	(240) 686- 4702
Samples:	• RNA • Residual DNA • Plasma/PBMC/Frozen B Cells

3. Collecting, Processing/Preparing and Shipping Blood Specimens

3.1. Samples Analyzed at Local Laboratory

CBC with Differential: This blood collection will be performed at Screening, Baseline, Weeks 1, 2, 3, 5, 10, and Months 3, 6, 9, 12, 15, 18, 21, 24. The specimens will be analyzed at your local laboratory. Specimens will be shipped as whole blood. Please contact your local lab to determine what type

of tube you should collect the specimen into, and for instructions on how to process and ship the sample.

Urine Pregnancy Test: To be collected as a random urine pregnancy test completed by Research Nurse (per supplied First Response test kit instructions). This will be performed at Screening, Baseline, and Weeks 1, 2, 3, and Months 3, 6, 12, 18, 24.

3.2. Samples Analyzed by TrialNet Laboratories

Chemistries: To be collected in a 4 ml plain red top tube at Screening, Months 3, 6, 12, 24. Allow blood to clot in tube for 15-30 minutes at room temperature (65-75°F), centrifuge for 10-15 minutes. Transfer 1 ml of serum into a pre-labeled 2 ml amber vial. Screw the top on tightly to prevent leakage. Refrigerate or keep on ice until shipping. Ship on cold packs by FedEx Priority Overnight to the TrialNet Core Biochemistry Laboratory.

International Sites would follow the same procedure as domestic sites for specimen collection. Extracted frozen serum would be shipped on dry ice to the TrialNet Core Biochemistry Laboratory.

The chemistry panel will include:

- Sodium	- Albumin
- Potassium	- Globulin
- Chloride	- Alkaline phosphatase (ALP)
- Urea	- Aspartate aminotransferase (AST)
- Creatinine	- Alanine aminotransferase (ALT)
- Calcium	- Gamma glutamyl transferase (GGT)
- Phosphorus	- Lactate dehydrogenase (LDH)
- Glucose	- Cholesterol
- Total and direct bilirubin	- Uric acid
- Total proteins	- Bicarbonate

HIV, Hepatitis B and C: To be collected only at Screening in a 4 ml plain red top tube. Rotate tube gently and place upright in tube rack. Allow blood to clot in tube for 15-30 minutes at room temperature (65-75°F), and then centrifuge the sample for 10-15 minutes. Transfer serum into a 4 ml cryovial. Screw top on tightly to prevent leakage. Freeze specimen at –20° C until ready to ship. Ship on dry ice by FedEx Priority Overnight to the TrialNet Core Biochemistry Laboratory.

International Sites would follow the same procedure as domestic sites for specimen collection. Extracted frozen serum would be shipped on dry ice to the TrialNet Core Biochemistry Laboratory.

Serum for Autoantibodies: 2 ml of blood will be collected in a 2.6 ml serum separation gel (SSG) clotting activator tube at Screening, Week 5, Months 3, 6, 9, 12, 18, & 24. Invert tube gently 5 times and place upright in tube rack. Allow blood to clot in tube for 20-30 minutes at room temperature (65-75°F), then centrifuge for 15 minutes. Transfer serum into a 1.8 ml cryovial. Screw top on tightly to prevent leakage. Freeze specimen at –20° C until ready to ship. Ship the cryovial Priority Overnight on **dry ice** to the TrialNet Core ICA Lab. Frozen serum shipped on dry ice is preferred (frozen serum is stable indefinitely).

To ship unfrozen samples (the SAME DAY): Place tubes into a Styrofoam tube holder with an absorbent pad. Place the tube holder into cardboard sleeve and then into a biohazard Ziploc bag. Place the middle copy of the STF into the outside pocket of the bag. Place the bag into a FedEx Diagnostics Specimen Envelope and ship to address provided. Samples should be drawn **Monday-Wednesday** only (except days before U.S. federal holidays) and shipped the same day at room temperature.

International Sites would follow the same procedure as domestic sites for specimen collection (only frozen specimens). Extracted frozen serum would be shipped on dry ice to the TrialNet Core ICA Laboratory.

Rituximab PK Analysis and HACA Levels: Baseline, Months 6 & 9. To be collected in a 4 ml red top tube. Allow blood to clot in tube for 30 minutes. Centrifuge the sample at 2000 rpm for 10–15 minutes. Transfer 1.0 –1.5 ml of serum into the pre-labeled 1.8 ml cryovials. Freeze and store samples upright at or below –20°C as soon as possible. Ship batched specimens on dry ice FedEx Priority Overnight to the Covance Laboratory on a quarterly basis. Quarters end March 31, June 30, September 30, and December 31. Refer to instructions supplied by Covance for more information.

International Sites would follow the same procedure as domestic sites for specimen collection and storage. Batched frozen specimens should be shipped quarterly on dry ice to the Covance Laboratory.

HbA1c: To be collected in a 1.2 ml lavender top EDTA blood collection tube at Screening, Month 3, 6, 9, 12, 15, 18, 21, & 24. Gently invert the tube 6-8 times to mix the sample. **DO NOT CENTRIFUGE!** Keep tube on ice or in a refrigerator until shipping. The sample should be shipped on cold packs as whole blood by FedEx Priority Overnight to the TrialNet Core Beta Cell Function Laboratory (Seattle, WA). **Note:** The HbA1c sample will remain stable for up to four days after being drawn, if kept refrigerated and shipped on cold packs.

International Sites would follow the same procedure as domestic sites for specimen collection. Extracted frozen serum would be shipped on dry ice to the TrialNet Core Beta Cell Function Laboratory.

MMTT (2 and 4 Hour)

C-peptide: Directions for collecting at Screening, Months 12 & 24: First, pre-label cryovials by snapping the lavender cover onto the lid of the eleven 1.8 ml cryovials. Next, draw eleven 1.2 ml C-peptide samples into 1.2 ml lavender top (EDTA) monovette tubes. Immediately invert each tube gently about 6-8 times to mix the sample, avoid jarring or shaking and then place the sample on ice or in a refrigerator. Centrifuge for 15 minutes in a chilled centrifuge within one hour of collection. Transfer plasma to appropriate pre-labeled 1.8 ml cryovials. Screw tops on tightly to prevent leakage. Ship on **dry ice** by FedEx Priority Overnight to the TrialNet Core Beta Cell Function Laboratory. **Note:** If not shipping immediately overnight, freeze at -20°C.
Directions for collecting at Months 3, 6, & 18: First, pre-label cryovials by snapping the lavender cover onto the lid of the seven 1.8 ml cryovials. Next, draw seven 1.2 ml C-peptide samples into 1.2 ml lavender top (EDTA) monovette tubes. Immediately invert each tube gently about 6-8 times to mix the sample, avoid jarring or shaking and then place the sample on ice or in a refrigerator. Centrifuge for 15 minutes in a chilled centrifuge within 1 hour after drawing specimen. Transfer plasma to appropriate 1.8 ml cryovials. Screw tops on tightly to prevent leakage. Ship on dry ice by FedEx Priority Overnight to the TrialNet Core Beta Cell Function Laboratory. **Note:** If not shipping immediately overnight, freeze at -20°C.

Glucose: Directions for collecting at Screening, Months 12 & 24: First, pre-label cryovials by snapping the gray cover onto the lid of the eleven 1.8 ml cryovials. Next, draw eleven 1.2 ml Glucose samples into 1.2 ml gray top plastic tubes. Immediately invert each tube gently about 6-8 times to mix the sample, avoid jarring or shaking, and then place the sample on ice or in a refrigerator. Centrifuge for 15 minutes in a chilled centrifuge within one hour of collection. Transfer plasma to appropriate 1.8 ml cryovials. Screw tops on

tightly to prevent leakage. Ship on dry ice by FedEx Priority Overnight to the TrialNet Core Beta Cell Function Laboratory. **Note:** If not shipping immediately overnight, freeze at -20°C.

Directions for collecting at Months 3, 6, & 18: First, pre-label cryovials by snapping the gray cover onto the lid of the seven 1.8 ml cryovials. Next, draw seven 1.2 ml Glucose samples into 1.2 ml gray top plastic tubes. Immediately invert each tube gently about 6-8 times to mix the sample, avoid jarring or shaking, and then place the sample on ice or in a refrigerator. Centrifuge for 15 minutes in a chilled centrifuge within one hour of collection. Transfer plasma to appropriate 1.8 ml cryovials. Screw tops on tightly to prevent leakage. Ship on dry ice by FedEx Priority Overnight to the TrialNet Core Beta Cell Function Laboratory. **Note:** If not shipping immediately overnight, freeze at -20°C.

International Sites would follow the same procedure as domestic sites for specimen collection. Extracted frozen serum would be shipped on dry ice to the TrialNet Core Beta Cell Function Laboratory.

HLA Determination, FcR Genotyping: At Screening, 5ml of blood will be collected in a 6 ml lavender-top EDTA tube. Gently invert the tube 8-10 times. This sample should be kept at room temperature. **DO NOT CENTRIFUGE!** The sample should be shipped as whole blood, within **24 hours** by FedEx Priority Overnight to the TrialNet Core HLA/DNA Laboratory. The HLA lab will ship an aliquot of DNA to the FcR lab at UAB for FcR genotyping. **Note:** HLA results are required to determine the appropriate schedule of assessments assigned at randomization.

International Sites**:** Draw 5 ml blood sample in appropriately labeled EDTA tube (see above). Centrifuge the tube at 2500 rpm for 30 minutes at 4°C in a tabletop centrifuge. Remove the upper plasma layer carefully. The grayish white layer above the red cells is the buffy coat containing the white blood cells and is collected with a wide mouthed transfer pipette and transferred into

a 15 ml tube. Freeze samples immediately at -20° C or ship on dry ice the same day or freeze at –20°C until specimen can be shipped. Ship sample on **dry ice** to the TrialNet Core HLA/DNA Laboratory.

EBV/CMV PCR (Viral Load): 2 ml will be collected in a 2 ml EDTA tube (lavender top) at Baseline, Weeks 2 and 5, Months 3, 5, 6, 9, 13, 15, 18, 21 and 24. Gently invert the tube 8-10 times to mix the sample and then place on ice or in a refrigerator until shipping. **DO NOT CENTRIFUGE!** The tube should be shipped as whole blood in the EDTA tube on cold packs by FedEx Priority Overnight to the TrialNet Core Viral Laboratory. **Note:** The blood sample for PCR analysis will remain stable for up to four days after being drawn, if kept refrigerated and shipped on cold packs.
International Sites: Draw 5 ml blood sample in appropriately labeled EDTA tube. DO NOT CENTRIFUGE. Place lavender top collection tube into pre-labeled 15 ml polypropylene tube. Freeze samples immediately on dry ice or freeze at –20°C until specimen can be shipped. Ship sample on **dry ice** to the TrialNet Core Viral Laboratory.

EBV/CMV Viral Serology: 2 ml will be collected in a 3ml red top tube at Baseline, Weeks 2 and 5, Months 3, 5, 6, 9,13, 15, 18, 21 and 24. Rotate the tube gently and place upright in tube rack. Allow blood to clot in tube for 15-30 minutes at room temperature (65-75°F) then centrifuge for 10-15 minutes. Transfer serum into a 1.8 ml cryovial. Screw top on tightly. Place specimen on ice or in refrigerator until shipping. Ship with cold packs by FedEx Priority Overnight to the TrialNet Core Viral Laboratory. **Note:** The serum will remain stable for up to 48 hours, if kept refrigerated and shipped on cold packs. If not shipping immediately overnight, freeze serum and ship on dry ice.
International Sites would follow the same procedure as domestic sites for specimen collection. Extracted frozen serum would be shipped on dry ice to TrialNet Core Viral Laboratory.

Other Serology: 2 ml of blood will be collected into a 3 ml red-top tube at Baseline, Months 6, 13, 18, & 24. Rotate tube gently and place upright in tube rack. Allow blood to clot for 15-30 minutes at room temperature (65-75°F). Centrifuge sample for 10-15 minutes. Transfer serum into pre-labeled 1.8 ml cryovial. Screw top on tightly. Freeze at –20°C. Place samples upright in a 3" partitioned freezer storage box. Ship on **dry ice** by FedEx to TrialNet Core Viral Laboratory.

International Sites would follow the same procedure as domestic sites for specimen collection. Extracted frozen serum would be shipped on dry ice to TrialNet Core Viral Laboratory.

Hepatitis A/Tetanus Serology: 2 ml of blood will be drawn into the 3 ml red top tube. Rotate the tube gently and place upright in tube rack. Allow blood to clot for 15-30 minutes at room temperature (65-75°F). Centrifuge sample for 10-15 minutes. Transfer serum into pre-labeled 1.8 ml cryovial. Screw the top on tightly. Freeze at –20°C. Place samples upright in a 3" partitioned freezer storage box. Ship on **dry ice** by FedEx Priority Overnight to TrialNet Core Viral Laboratory.

International Sites would follow the same procedure as domestic sites for specimen collection. Extracted frozen serum would be shipped on **dry ice** by FedEx Priority Overnight to TrialNet Core Viral Laboratory.

PhiX174 Specimens: A pre-immunization serology specimen and a 15-minute post-immunization specimen will be obtained at Weeks 6, 12, 52, & 58. Draw blood into 4 ml red top tubes. Rotate tubes gently and place upright in tube rack. Allow bloods to clot for a minimum of 30 minutes at room temperature (65-75°F) or specimen can be refrigerated overnight (4°C). Centrifuge samples for 10-15 minutes. Transfer serum into matching pre-labeled 1.8 ml cryovials. Screw the tops on tightly. Freeze at –20°C. Place samples upright in a 3" partitioned freezer storage box. Ship daily or one full course of specimens batched on **dry ice** by FedEx Priority Overnight to the

Molecular Diagnostics Laboratory (University of Washington School of Medicine).

A serology specimen will be obtained at Weeks 7, 8, 10, 13, 14, 16, 53, 54, 56, 59, 60, & 62. Draw blood into 4 ml red top tube. Rotate tube gently and place upright in tube rack. Allow blood to clot for a minimum of 30 minutes at room temperature (65-75°F) or specimen can be refrigerated overnight (4°C). Centrifuge samples for 10-15 minutes. Transfer serum into matching pre-labeled 1.8 ml cryovial. Screw the top on tightly. Freeze at –20°C. Place sample upright in a 3" partitioned freezer storage box. Ship daily or one full course of specimens batched on **dry ice** by FedEx Priority Overnight to the Molecular Diagnostics Laboratory (University of Washington School of Medicine).

NOTE: If serology specimens are collected at a site other than the study site (i.e. Weeks 7, 8, 13, 14, 16, 53, 54, 59, 60, and 62), the collection should be completed as previously directed. Serum should be shipped overnight on **dry ice** to the study site with a copy of the STF. If immediate separation of serum is not possible, the whole blood specimen may be shipped overnight on cold packs to the study site. The study site will complete the original STF and ship specimens daily, or one full course of specimens batched, to the lab on dry ice.

Refer to the Schedule of Assessments assigned at randomization for the visit schedule for the following specimen collections.

Flow Cytometry: Refer to ITN Manual of Laboratory Operations.

Frozen PBMC/Plasma: Refer to ITN Manual of Laboratory Operations.

T cell Proliferation: For this procedure, only collect blood **Monday–Wednesday** if possible. Blood collection will be performed at Baseline,

Months 6, 12, 13, 18, and 24. If the participant is a child 25-65 kg, collect 6 ml of blood into one 6 ml green-topped collection tube. If the participant is a child over 65 kg or an adult, collect 10 ml of blood into one 10 ml green-topped collection tube, and at Baseline, Months 6, 13, and 18, collect an additional 6 ml of blood into one 6 ml green-topped tube. Once blood is drawn, the samples should be inverted 3-4 times. The samples should be kept at **room temperature** and shipped on the **same day** of the blood draw. Ship by FedEx Priority Overnight to Dr. H. Michael Dosch, The Hospital for Sick Children (Toronto, ON).

NOTE: The collection schedule for the following 3 assays will vary based on the participant's weight, age, and HLA results. Once an assay or multiple assays have been assigned, the participant will only be tested for those assays, their schedule will not change.

Cytokine ELISpot: If the participant is a child 25-40 kg, collect 25 ml of blood into two 10 ml green-topped blood collection tubes, and one 6 ml green-topped blood collection tube. If the participant is a child over 40 kg or an adult, collect 30 ml of blood into three10 ml green-topped blood collection tubes. The collection schedule will depend on the age, weight, and HLA results of the participant. Please refer to the Schedule of Assessments assigned at randomization for more information. Immediately and gently invert the tube 8-10 times. DO NOT CENTRIFUGE. Keep tubes at **room temperature**. Ship as whole blood Priority Overnight Monday-Wednesday. Samples must be shipped on the **same day** of the blood draw. Ship the samples at room temperature, by FedEx Priority Overnight to the Cytokine ELISpot Laboratory. Samples should only be collected **Monday–Wednesday** if possible.

Immunoblot: If the participant is a child 25-40 kg, collect 25 ml of blood into two 10 ml green-topped blood collection tubes and one 6 ml green-topped blood collection tube. If the participant is a child over 40 kg or an adult, collect

30 ml of blood into three 10 ml green-topped blood collection tubes. The collection schedule will depend on the age, weight, and HLA results of the participant. Please refer to the Schedule of Assessments assigned at randomization for more information. Immediately and gently invert the tube 8-10 times. DO NOT CENTRIFUGE. Keep tubes at **room temperature**. Ship as whole blood priority overnight Monday-Wednesday. Samples must be shipped on the **same day** of the blood draw. Ship the samples at room temperature, by FedEx Priority Overnight to the Immunoblot Laboratory. Samples should only be collected **Monday–Wednesday** if possible.

Tetramer: If the participant is a child 25-40 kg, collect 25 ml of blood into two 10 ml green-topped blood collection tubes and one 6 ml green-topped blood collection tube. If the participant is a child 41-65 kg, collect 30 ml of blood into three 10 ml green-topped blood collection tubes. If the participant is a child over 65 kg or an adult, collect 20 ml of blood into two 10 ml green-topped blood collection tubes. The collection schedule will depend on the age, weight, and HLA results of the participant. Please refer to the Schedule of Assessments assigned at randomization for more information. Immediately and gently invert the tube 8-10 times. DO NOT CENTRIFUGE. Keep tubes at **room temperature**. Ship as whole blood priority overnight Monday-Wednesday. Samples must be shipped on the **same day** of the blood draw. Ship the samples at room temperature, by FedEx Priority Overnight to the Tetramer Laboratory. Samples should only be collected **Monday – Wednesday** if possible.

3.3. Samples for Storage at NIDDK Repository (ITN will coordinate collection and shipping of these specimens)

Frozen PBMC/Plasma: Refer to ITN Manual of Laboratory Operations.

RNA: Refer to ITN Manual of Laboratory Operations.

DNA: DNA will be extracted from the whole blood shipped to the Core HLA/DNA Laboratory at the Screening visit. Aliquots of the DNA will be frozen, batched, and sent to the NIDDK Repository for storage.

Plasma: Plasma remaining following ficoll separation will be collected, processed, and stored under the coordination of ITN. Refer to ITN Manual of Laboratory Operations.

4. Total Blood Volumes Drawn

The minimum amount of blood drawn at any visit is 2 ml, which occurs at Weeks 1 and 3. The maximum amount of blood drawn at any visit is 73.1 ml (child 25- 40 kg) at Month 12 and 24; 118.0 ml (child 41- 65 kg) at Months 12, 18, & 24; and 196.0 and 196.1 (adult or child >65 kg) at Months 12 and 24 respectively.

5. Specimen Transmittal Forms

Specimen transmittal forms must accompany all samples sent to the laboratory for analysis. These forms capture information on the participant the sample was collected from, as well as the date and visit at which the sample was collected. For tests such as the MMTT, the specimen transmittal form captures information on each of the different blood draws that are part of this test. The transmittal forms all have space for the appropriate barcode labels to be attached, as well as to record the contact information of the person who shipped the samples. The following page provides a sample specimen transmittal form and highlights its important features.

The specimen transmittal forms are printed on 3-sheet NCR paper. Always remember that:

- The **white copy** should always be sent to the TrialNet Coordinating Center.
- The **yellow copy** should always be sent to the laboratory with the samples for analysis.
- The **pink copy** should be kept at the clinic.

5.1. Sample Specimen Transmittal Form (HbA1c)

The **header** is used to record information on the participant the sample is collected from.

Type 1 Diabetes TrialNet

Anti-CD20 Study
HEMOGLOBIN A1C (HbA1c) SPECIMEN TRANSMITTAL FORM

Form RIT99HB
15 MARCH 2006
Version 1.0
Page 1 of 1

Site Number: Screening ID: - Participant Letters:

A. COLLECTION INFORMATION

- Label one 1.2 ml lavender top blood collection tube with a subject identifier. Do not use a barcode for this tube.
- Label one 7 ml shipment tube with the appropriate barcode label indicating specimen type (HbA1c) from an **unused** HbA1c barcode label sheet. Write in three letters of the participant's choice and the date of draw (e.g. 05/Sep/2005) with an alcohol-proof pen. Apply barcode label vertically.
- Attach matching barcode labels from the **same barcode sheet** to each page of this Specimen Transmittal Form in **Section B**.
- Draw blood into 1.2 ml lavender top tube and gently invert the tube **6-8** times to mix the sample. **DO NOT CENTRIFUGE.**
- Transfer lavender top collection tube into the pre-labeled 7 ml shipment tube. Snap the top on firmly.
- Keep tube **on ice or in refrigerator.** Ship as **whole blood.**

Section A always contains instructions for collecting the blood samples.

Section A also captures information on when the specimen was collected and for which visit.

1. Date specimen collected *(e.g. 05/Sep/2005):* __ __ / __ __ __ / __ __ __ __ (DAY / MONTH / YEAR)

2. Study visit: *(check one)*

☐ 1	Screening	☐ 17	Month 9	☐ 27	Month 18	☐ 30	Month 30	☐ 33	Month 48
☐ 11	Month 3	☐ 18	Month 12	☐ 28	Month 21	☐ 31	Month 36	☐ 99	Other
☐ 16	Month 6	☐ 26	Month 15	☐ 29	Month 24	☐ 32	Month 42		

B. SPECIMEN INFORMATION

1. Place **HbA1c** Barcode Label Here:

A barcode label from the **same sheet** as the labels put on the specimen tubes must be attached here.

C. SHIPPING INFORMATION

- Place the 7 ml shipment tube of whole blood into a styrofoam tube holder with an absorbent pad.
- Place the styrofoam tube holder into a cardboard sleeve and then into a biohazard Ziploc bag.
- Place the yellow copy of this completed form in the outside sleeve of the bag.
- Ship sample with **cold packs** in a styrofoam shipping container. Tape outer box securely closed.
- Affix the following label to the outside of the box: "Diagnostic Specimens"
- Prepare and print a pre-paid airbill to FedEx all samples **Priority Overnight** to: Northwest Lipid Research Laboratories, 401 Queen Anne Avenue North, Seattle, WA 98109-4517, Phone: (206) 685-3327
- **Ship specimens Monday-Thursday only** (except days before a U.S. federal holiday).

Section C always contains shipping instructions for the samples.

Section C also captures information on the person who shipped the samples.

1. Shipped by Name: _____ 2. Phone #: _____

3. Date Shipped: __ __ / __ __ __ / __ __ __ __ (DAY / MONTH / YEAR)

4. Comments: _____

On all questions write "?" if the desired information is currently unavailable, but is being checked and will be known in future updates. Write "" if the desired information is permanently unavailable (i.e. will not be known in any future updates).*

White Copy – Send to TrialNet Coordinating Center **Yellow Copy** – Place in outside sleeve of the biohazard Ziploc bag
Pink Copy – Retain at site

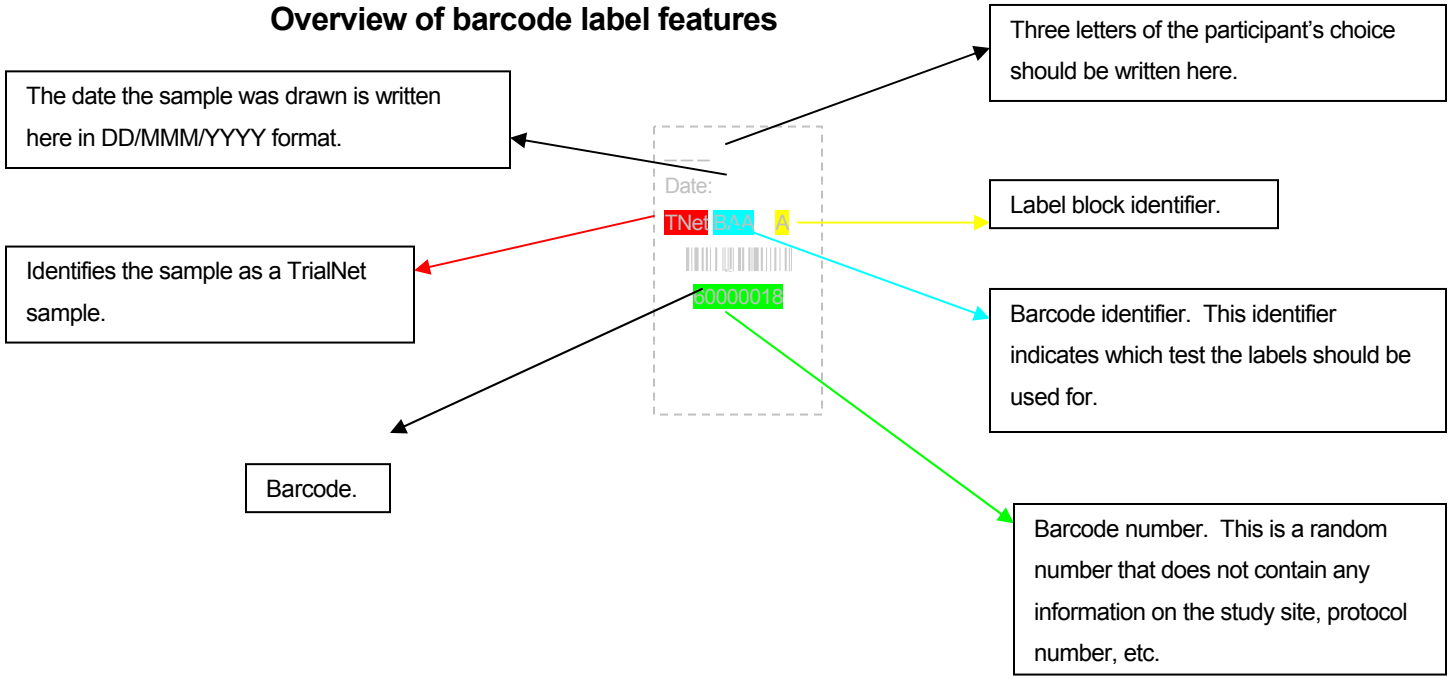
6. Barcode Label Sheets

Prior to the initiation of any study procedures all participating sites will receive batches of fan-folded barcode label sheets for every test that is run as part of the Anti-CD20 Trial. Each batch of label sheets will correspond to a single type of test, and each batch of labels on the sheet will be used for only one test. For example, each site will receive a batch of fan-folded barcode label sheets for the HbA1c test. Every time an HbA1c is run on a study participant, a new block of barcode labels will be used.

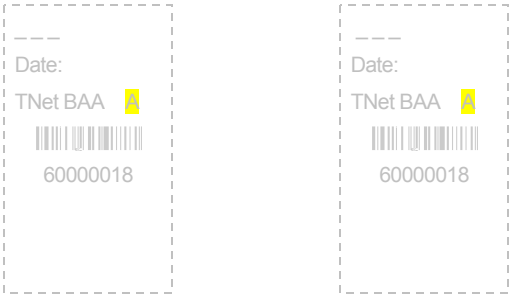
6.1. Format of Sheets and Labels

The barcode labels will be provided in fan-folded sheets. Each block of barcode labels is to be used for a single test. A letter in the upper right corner of the label distinguishes each block of labels. All labels in a single block will have the same letter in the upper right corner. One label from the block should be put on each of the three copies of the specimen transmittal form (total of three labels used). The remaining labels in the block should be placed on the specimen tube(s) or vial(s). When a test involves timed samples (such as the MMTT analysis), the labels will include the specimen type and the draw time. There may be a couple of extra barcode labels included in the block, in the event that any labels are damaged or lost. **It is absolutely crucial that every copy of the specimen transmittal form contains a barcode label from the SAME BLOCK as the labels placed on the specimen tubes or vials!** If this does not occur, there will be no way to link the laboratory samples to a study participant!

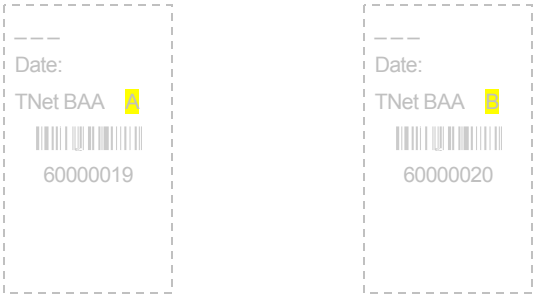
Overview of barcode label features



Examples of barcode labels from the SAME BLOCK:



Examples of barcode labels from DIFFERENT BLOCKS:



6.2. Barcode Label Identification

The following table summarizes the barcode labels that should be used for each test that is part of this study:

Test Name	**Barcode Identifier**		**Test Name**	**Barcode Identifier**
Autoantibodies	BAA		HbA1c	HBA1C
HLA/DNA	HLA		CBC with diff.	Analysis at local lab (no barcode label)
2-hour MMTT	MMT2		4-hour MMTT	MMT4
HIV/Hep B/Hep C Screening	SERO		EBV/CMV PCR	VIRVL
EBV/CMV Viral Serology	VIRST		Other Serology	SRLG
PK Analysis and HACA Levels	Barcode labels provided by Covance		ELISpot	ELSPT
Chemistries	CHEM		PhiX174 Serology	PHIX
Hepatitis A & Tetanus Serology	HEPT		T Cell Proliferation	PROL
Flow Cytometry	Barcode labels provided by ITN		Immunoblot	BLOT
Frozen PBMC/Plasma	Barcode labels provided by ITN		Tetramer	TETRA
RNA	Barcode labels provided by ITN			

The barcode identifier can be found above the barcode. This identifier makes it easier to differentiate between barcode labels for different tests.

6.3. Ordering Barcode Label Sheets

If more fan-folded barcode label sheets are needed, they should be requested from the TrialNet Coordinating Center.

Specimen Shipment Overview

Core β-Cell & Core Biochemistry Laboratory: Northwest Lipid Research Laboratories -- Seattle, WA								
Laboratory Test	**Frequency**	**Drawn In**	**Shipped As**	**Shipped In**	**Shipping Carton**	**Shipping Instructions**	**Shipping Frequency**	**TAT***
Chemistries	Screening, Months 3, 6, 12, 24	One 4ml red top tube	Serum	One 2 ml amber vial	cardboard box with Styrofoam insert	**Cold packs**	Daily / M-Th	5 days
HbA1c	Screening, Month 3, 6, 9, 12, 15, 18, 21, 24, 30 36, 42, 48	One 1.2 ml lavender top EDTA tube	Whole blood	Collection tube placed in a cardboard sleeve	cardboard box with Styrofoam insert	**Cold packs**	Daily / M-Th	5 days
HIV/Hep B/ Hep C	Screening	One 4ml red top tube	Serum	One 4 ml cryovial	cardboard box with Styrofoam insert	**Dry ice**	Daily / M-Th	8 days
2-hour MMTT	Months 3, 6, 18, 30,36, 42, 48	Glucose: Seven 1.2 ml gray top tubes	Plasma	Glucose: Seven 1.8 ml cryovials w/ gray insert	partitioned freezer storage box placed in a cardboard box with Styrofoam insert	**Dry Ice**	Daily / M-Th	7 days
		C-peptide: Seven 1.2 ml EDTA tubes		C-peptide: Seven 1.8 ml cryovials w/ lavener insert				NA
4-hour MMTT	Screening, Month 12, 24	Glucose: Eleven 1.2 ml gray top tubes	Plasma	Glucose: Eleven 1.8 ml cryovials w/ gray insert	partitioned freezer storage box placed in a cardboard box with Styrofoam insert	**Dry Ice**	Daily / M-Th	7 days
		C-peptide: Eleven 1.2 ml EDTA tubes		C-peptide: Eleven 1.8ml cryovials w/ lavender insert				NA

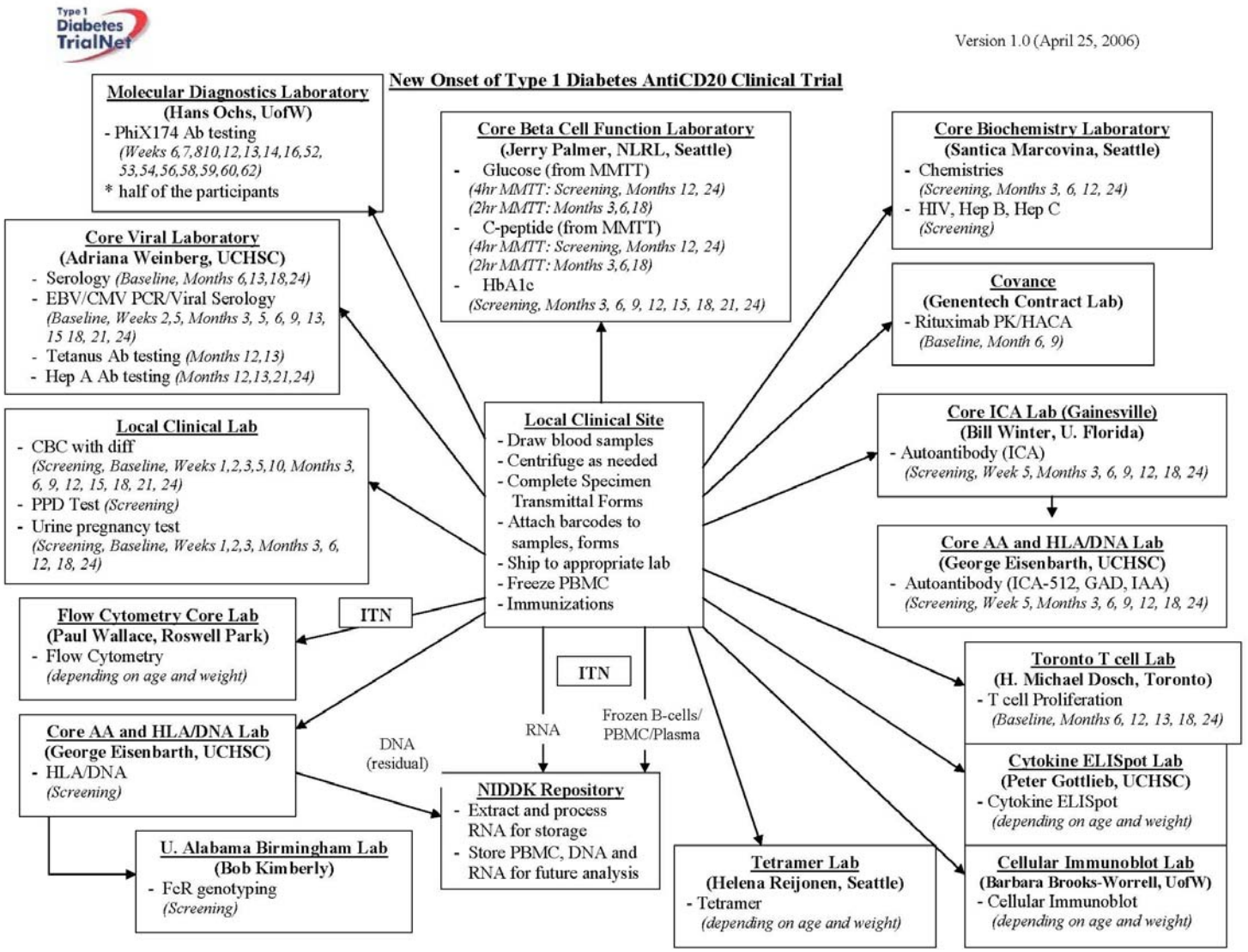
Cellular Immunoblot Laboratory: VA Medical Center -- Seattle, WA								
Laboratory Test	**Frequency**	**Drawn In**	**Shipped As**	**Shipped In**	**Shipping Carton**	**Shipping Instructions**	**Shipping Frequency**	**TAT***
Immunoblot	Refer to assigned Schedule of Assessments	For children 25-40 kg, two 10 ml green top tube and one 6 ml green top tube. For children over 40 kg and adults, three 10 ml green top tube.	Whole blood	6 ml or 10 ml green top tubes (Sodium heparin) placed in a bubble wrap sleeve placed in a Tyvek outer envelope	Ambient shipper	**Room temperature gel packs - 2**	Daily / M-W	NA
Tetramer Laboratory: Benaroya Research Institute -- Seattle, WA								
Laboratory Test	**Frequency**	**Drawn In**	**Specimen**	**Shipped In**	**Shipping Carton**	**Shipping Instructions**	**Shipping Instructions**	**TAT***
Tetramer	Refer to assigned Schedule of Assessments	For children 25-40 kg, two 10 ml green top tube and one 6 ml green top tube. For children 41-65 kg, three 10 ml green top tubes. For children over 65 kg and adults, two 10 ml green top collection tubes.	Whole blood	6 ml or 10 ml green top tubes (Sodium heparin) placed in a bubble wrap sleeve placed in a Tyvek outer envelope	Ambient shipper	**Room temperature gel packs - 2**	Daily / M-W	NA

Core ICA Laboratory: UFDRL Laboratory -- Gainesville, FL								
Laboratory Test	**Frequency**	**Drawn In**	**Shipped As**	**Shipped In**	**Shipping Carton**	**Shipping Instructions**	**Shipping Frequency**	**TAT***
Serum for autoantibodies	Screening, Week 5, Months 3, 6, 9, 12, 18, 24, 30, 36, 42, 48	One 2.6 ml SSG clotting activator tube	Serum	One 1.8 ml cryovial	partitioned freezer storage box placed in a cardboard box with Styrofoam insert	**Dry Ice - 5 lbs**	Daily / M-Th	10 days
Core Viral Laboratory: University of Colorado Hospital Clinical Laboratory -- Denver, CO								
Laboratory Test	**Frequency**	**Drawn In**	**Shipped As**	**Shipped In**	**Shipping Carton**	**Shipping Instructions**	**Shipping Frequency**	**TAT***
EBV/CMV PCR	Baseline, Weeks 2, 5 Months 3, 5, 6, 9, 13, 15, 18, 21, 24	One 2 ml lavender top EDTA tube	Whole blood	One 2 ml lavender top EDTA tube	cardboard box with Styrofoam insert	**Cold packs**	Daily / M-F	12 days/ reported as clinically needed
EBV/CMV Viral Serology	Baseline, Weeks 2, 5, Months 3, 5, 6, 9, 13, 15, 18, 21, 24	One 3 ml red top tube	Serum	One 1.8 ml cryovial placed in a Styrofoam shipment tube placed in a cardboard sleeve	cardboard box with Styrofoam insert	**Cold packs**	Daily / M-F	12 days/ reported as clinically needed
Other Serology	Baseline, Months 6, 13, 18, 24, 30, 36, 42, 48	One 3 ml red top	Serum	One 1.8 ml cryovial	partitioned freezer storage box placed in a cardboard box with Styrofoam insert	**Dry ice**	Daily / M-F	NA

Hepatitis A &Tetanus Serology	Hepatitis A: Months 12, 13, 21, 24 Tetanus: Months 12, 13	One 3 ml red top	Serum	One 1.8 ml cryovial	partitioned freezer storage box placed in a cardboard box with Styrofoam insert	**Dry Ice - 5 lbs**	Daily / M-W	NA
Covance Laboratories, Inc. -- Chantilly, VA								
Laboratory Test	**Frequency**	**Drawn In**	**Shipped As**	**Shipped In**	**Shipping Carton**	**Shipping Instructions**	**Shipping Frequency**	**TAT***
PK Analysis & HACA Levels	Baseline, Months 6, 9	One 4 ml red top tube	Serum	Two 1.8 ml cryovials		**Dry ice**	Batched Quarterly	NA
Cytokine ELISpot Laboratory: UCHSC, BDC -- Aurora, CO								
Laboratory Test	**Frequency**	**Drawn In**	**Shipped As**	**Shipped In**	**Shipping Carton**	**Shipping Instructions**	**Shipping Frequency**	**TAT***
Cytokine ELISpot	Refer to assigned Schedule of Assessments	For children 25-40 kg, two 10 ml green top tube and one 6 ml green top tube. For children over 40 kg and adults, three 10 ml green top tubes.	Whole blood	6 ml or 10 ml green top tubes (Sodium heparin) placed in a bubble wrap sleeve placed in a Tyvek outer envelope	Ambient shipper	**Room temperature gel packs - 2**	Daily / M-W	NA
Core HLA/DNA Extraction Laboratory: UCHSC at Fitzsimons -- Aurora, CO								
Laboratory Test	**Frequency**	**Drawn In**	**Shipped As**	**Shipped In**	**Shipping Carton**	**Shipping Instructions**	**Shipping Frequency**	**TAT***
HLA/DNA and FcR Genotyping	Screening	One 6 ml lavender top EDTA tube	Whole blood	One 6 ml lavender top EDTA tube	FedEX Clinical Pak	**Room temperature**	Daily / M-Th	NA

Immune Tolerance Network (ITN) -- Various Locations								
Laboratory Test	**Frequency**	**Drawn In**	**Shipped As**	**Shipped In**	**Shipping Carton**	**Shipping Instructions**	**Shipping Frequency**	**TAT***
Flow Cytometry	Refer to assigned Schedule of Assessments	Refer to ITN Manual of Laboratory Operations	Refer to ITN Manual of Laboratory Operations	Refer to ITN Manual of Laboratory Operations	Refer to ITN Manual of Laboratory Operations	Refer to ITN Manual of Laboratory Operations	Refer to ITN Manual of Laboratory Operations	NA
Frozen PBMC/Plasma	Refer to assigned Schedule of Assessments	Refer to ITN Manual of Laboratory Operations	Refer to ITN Manual of Laboratory Operations	Refer to ITN Manual of Laboratory Operations	Refer to ITN Manual of Laboratory Operations	Refer to ITN Manual of Laboratory Operations	Refer to ITN Manual of Laboratory Operations	NA
RNA (adults and children >65kg ONLY)	Baseline, Months 6, 12, 18, 24	Refer to ITN Manual of Laboratory Operations	Refer to ITN Manual of Laboratory Operations	Refer to ITN Manual of Laboratory Operations	Refer to ITN Manual of Laboratory Operations	Refer to ITN Manual of Laboratory Operations	Refer to ITN Manual of Laboratory Operations	NA
Local laboratory								
Laboratory Test	**Frequency**	**Drawn In**	**Shipped As**	**Shipped In**	**Shipping Carton**	**Shipping Instructions**	**Shipping Frequency**	**TAT***
CBC with Differential	Screening, Baseline, Weeks 1, 2, 3, 5, 10, Months 3, 6, 9, 12, 15, 18, 21, 24, 30, 36, 42, 48	One 2 ml lavender top EDTA tube	Whole blood	One 2 ml lavender top EDTA tube	Check with your lab	**Room temperature**	Daily	Check with your lab
Urine Pregnancy Test	Screening, Baseline, Weeks 1, 2, 3, Months 3, 6, 12, 18, 24 (qualifying female participants)	Urine collection cup	Urine	Test performed on Site	Not Applicable	Not Applicable	Not Applicable	NA

Molecular Diagnostics Laboratory: University of Washington School of Medicine -- Seattle, WA								
Laboratory Test	**Frequency**	**Drawn In**	**Shipped As**	**Shipped In**	**Shipping Carton**	**Shipping Instructions**	**Shipping Frequency**	**TAT***
PhiX174 Pre & Post Immunization Serology (if participant is enrolled)	Weeks 6, 12, 52, 58	Two 4 ml red top tubes	Serum	Two 1.8 ml cryovials	partitioned freezer storage box placed in a cardboard box with Styrofoam insert	**Dry Ice**	Daily or Batched Full Course / M-Th	NA
PhiX174 Serology (if participant is enrolled)	Weeks 6, 7, 8, 10, 12, 13, 14, 16, 52, 53, 54, 56, 58, 59, 60, 62	One 4 ml red top tubes	Serum	One 1.8 ml cryovials	partitioned freezer storage box placed in a cardboard box with Styrofoam insert	**Dry Ice**	Daily or Batched Full Course / M-Th	NA
Toronto T Cell Laboratory: Hospital for Sick Children -- Toronto, ON, Canada								
Laboratory Test	**Frequency**	**Drawn In**	**Shipped As**	**Shipped In**	**Shipping Carton**	**Shipping Instructions**	**Shipping Frequency**	**TAT***
T Cell Proliferation	Baseline, Months 6, 12, 13, 18, 24	For children 25-65 kg, one 6 ml green top tube. For children over 65 kg and adults, one 10 ml green top tube; draw an additional one 6ml green top tube @ months 6, 13, & 18 ONLY.	Whole blood	6 ml or 10 ml green top tube (Sodium heparin) placed in a Styrofoam shipment tube placed in a cardboard sleeve	FedEX Clinical Pak	**Room temperature**	Daily / M-W	NA


Type 1 Diabetes TrialNet
Version 1.0 (April 25, 2006)
New Onset of Type 1 Diabetes AntiCD20 Clinical Trial
Molecular Diagnostics Laboratory
(Hans Ochs, UofW)
- PhiX174 Ab testing
(Weeks 6,7,810,12,13,14,16,52, 53,54,56,58,59,60,62)
* half of the participants
Core Beta Cell Function Laboratory
(Jerry Palmer, NLRL, Seattle)
- Glucose (from MMTT)
(4hr MMTT: Screening, Months 12, 24)
(2hr MMTT: Months 3,6,18)
- C-peptide (from MMTT)
(4hr MMTT: Screening, Months 12, 24)
(2hr MMTT: Months 3,6,18)
- HbA1c
(Screening, Months 3, 6, 9, 12, 15, 18, 21, 24)
Core Biochemistry Laboratory
(Santica Marcovina, Seattle)
- Chemistries
(Screening, Months 3, 6, 12, 24)
- HIV, Hep B, Hep C
(Screening)
Core Viral Laboratory
(Adriana Weinberg, UCHSC)
- Serology (Baseline, Months 6,13,18,24)
- EBV/CMV PCR/Viral Serology
(Baseline, Weeks 2,5, Months 3, 5, 6, 9, 13, 15 18, 21, 24)
- Tetanus Ab testing (Months 12,13)
- Hep A Ab testing (Months 12,13,21,24)
Covance
(Genentech Contract Lab)
- Rituximab PK/HACA
(Baseline, Month 6, 9)
Local Clinical Lab
- CBC with diff
(Screening, Baseline, Weeks 1,2,3,5,10, Months 3, 6, 9, 12, 15, 18, 21, 24)
- PPD Test (Screening)
- Urine pregnancy test
(Screening, Baseline, Weeks 1,2,3, Months 3, 6, 12, 18, 24)
Local Clinical Site
- Draw blood samples
- Centrifuge as needed
- Complete Specimen Transmittal Forms
- Attach barcodes to samples, forms
- Ship to appropriate lab
- Freeze PBMC
- Immunizations
Core ICA Lab (Gainesville)
(Bill Winter, U. Florida)
- Autoantibody (ICA)
(Screening, Week 5, Months 3, 6, 9, 12, 18, 24)
Core AA and HLA/DNA Lab
(George Eisenbarth, UCHSC)
- Autoantibody (ICA-512, GAD, IAA)
(Screening, Week 5, Months 3, 6, 9, 12, 18, 24)
ITN
Flow Cytometry Core Lab
(Paul Wallace, Roswell Park)
- Flow Cytometry
(depending on age and weight)
ITN
Toronto T cell Lab
(H. Michael Dosch, Toronto)
- T cell Proliferation
(Baseline, Months 6, 12, 13, 18, 24)
Core AA and HLA/DNA Lab
(George Eisenbarth, UCHSC)
- HLA/DNA
(Screening)
DNA (residual)
RNA
Frozen B-cells/ PBMC/Plasma
Cytokine ELISpot Lab
(Peter Gottlieb, UCHSC)
- Cytokine ELISpot
(depending on age and weight)
NIDDK Repository
- Extract and process RNA for storage
- Store PBMC, DNA and RNA for future analysis
U. Alabama Birmingham Lab
(Bob Kimberly)
- FcR genotyping
(Screening)
Tetramer Lab
(Helena Reijonen, Seattle)
- Tetramer
(depending on age and weight)
Cellular Immunoblot Lab
(Barbara Brooks-Worrell, UofW)
- Cellular Immunoblot
(depending on age and weight)

Chapter 22. Procedures

This chapter contains instructions for temporarily and permanently transferring participants between sites.

1. Procedures for Temporary Transfer of Participants

The following are procedures for transferring participants from their primary site (originating site) to a secondary site (new site to where participant is being transferred). **Note:** Both sites should be aware of their institutions requirements before proceeding and the secondary site MUST have current IRB approval and full certification.

1) The Protocol RA should be notified ***prior*** to initiating any participant transfers.

2) The transferring participant must sign the secondary site's Informed Consent Form before this site may conduct any study-related procedures.

3) The primary site must complete a Participant Transfer Notification Form located in Appendix N and on the TrialNet Website.

4) The primary site must forward a copy of the participant's study file including copies of all available source documents and completed case report forms to the secondary site.

5) The secondary site must record the primary site's Site Number, and Screening ID Number and Letters assigned to the transferring participant by the primary site, on ALL case report forms and specimen transmittal forms completed for this participant. *Note: The primary site coordinator*

will receive all data form edits for this participant and will be responsible for coordinating the resolution of edits.

6) To ensure appropriate reimbursement, the secondary site must indicate on the RIT12 (Follow-up Visit Form) that the visit is occurring at a site other than the primary site (A.3) and record the secondary Site Number for reimbursement (A.3.a).

7) Both sites must maintain all original documents for visits that take place at their respective site.

8) Both site coordinators must note the transfer in the transferring participant's study file.

9) Copies of all available source documents, case report forms, and specimen transmittal forms completed at the secondary site must be sent to the primary site.

10) If the participant is scheduled to undergo an infusion visit at the secondary site, perform the following:

a) Both site coordinators must inform their pharmacists of the participant transfer.

b) The TNCC will coordinate communication between the primary and secondary site coordinators, and between the pharmacists, to review the transfer process.

c) The primary site pharmacist must fax a copy of the Randomization and Drug Dispensing Form for the transferring participant to the secondary site pharmacist. THIS FORM CONTAINS

CONFIDENTIAL INFORMATION AND MUST BE TRANSFERRED BETWEEN PHARMACISTS WITHOUT STUDY PERSONNEL INVOLVEMENT.

2. Procedures for Permanent Transfer of Participants

The following are procedures for transferring participants from their primary site (originating site) to a secondary site (new site to where participant is being transferred). **Note:** Both sites should be aware of their institutions requirements before proceeding and the secondary site MUST have current IRB approval and full certification.

1) The Protocol RA should be notified ***prior*** to initiating any participant transfers.

2) The transferring participant must sign the secondary site's Informed Consent Form before this site may conduct any study-related procedures.

3) The primary site must complete a Participant Site Transfer Form (RIT20) to ensure appropriate reimbursement and distribution of data form edits and lab results.

4) The primary site must forward a copy of the participant's study file including copies of all available source documents and completed case report forms to the secondary site.

5) Both sites must maintain all original documents for visits that take place at their respective site.

6) Both site coordinators must note the transfer in the transferring participant's study file.

7) If the participant is scheduled to undergo an infusion visit at the secondary site, perform the following:

   a) Both site coordinators must inform their pharmacists of the participant transfer.

   b) The TNCC will coordinate communication between the primary and secondary site coordinators, and between the pharmacists, to review the transfer process.

   c) The primary site pharmacist must fax a copy of the Randomization and Drug Dispensing Form for the transferring participant to the secondary site pharmacist. THIS FORM CONTAINS CONFIDENTIAL INFORMATION AND MUST BE TRANSFERRED BETWEEN PHARMACISTS WITHOUT STUDY PERSONNEL INVOLVEMENT.

3. Procedures for Requesting Testing of Viral Specimens

The Viral Lab will freeze and store ALL EBV/CMV PCR and Viral Serology specimens until study end unless clinically indicated. The following are procedures for requesting viral specimens to be tested.

1) Sites will notify the Viral Lab via email using the following template:

   Subject line: "TrialNet: A Late Add for Anti-CD20 Trial"
   TO: Lori.Brewster@uch.edu, Dennis.Long@uch.edu
   CC: anamaria.pacheco@uch.edu, Allan.Roem@uch.edu, Kathi.Wilcox@uch.edu, KOwens@biostat.bsc.gwu.edu,

ERaiden@biostat.bsc.gwu.edu, DPhoebus@biostat.bsc.gwu.edu, arvindm@biostat.bsc.gwu.edu

Email text:

Site #
Participant Screening ID Number and Letters
Study Visit(s)
Sample Number(s)
List of tests to be run (EBV PCR, EBV Viral Serology, CMV PCR, CMV Viral Serology) Note: If no list is provided all tests will be run

2) Sites will complete an Adverse Event Report Form (RIT13-Version 1.1, dated August 10, 2006) indicating in Question D.2.f. that testing of viral specimens was requested.

3) The Viral Lab will test all previously stored specimens for that participant. Analysis of future specimens collected for that participant will be determined on a case-by-case basis.

4) All positive viral loads will be reported as an alert value by the Viral Lab.

Chapter 23. Supplies

Case Report Forms (CRFs), Specimen Transmittal Forms (STFs), Participant Binders (pre-filled with all visit checklists, dividers, and CRFs and STFs through Month 24, except for phiX174 forms), barcode labels, and other study supplies are provided to all study sites by the TrialNet Coordinating Center (TNCC). Supplies obtained directly from The Coordinating Center are requested using the TrialNet Supply Order System and will be processed by TNCC staff. Sites will receive an initial quantity of screening supplies just prior to receiving approval to begin screening. Generally, sites should maintain a 3-month inventory or longer, sufficient for study needs. This is to avoid frequent reordering of supplies.

1. Ordering Clinical Supplies

A central supplies distributor will be responsible for processing orders of clinical supplies for blood collections and shipments. TNCC, through its subcontractor Fisher Bioservices, implemented a web based online order system for all supplies. "Authorized users", those individuals responsible for study supplies at the site, are provided with a login and password for the web order system. Webcast training on use of the web order system is provided by the TNCC.

1.1. Authorized Users

Only staff at your site that have been authorized to submit orders will be issued a user account (login and password). All Study and Trial Coordinators are authorized users when the site receives site certification from TrialNet. The Study or Trial Coordinator wishing to request additional user accounts for staff at their site should email a request to TNOrders@biostat.bsc.gwu.edu at the TNCC with "Request SOS User" in the subject line. The request should

identify the position or role (e.g., research staff) and provide the full name and email address of the new user.

1.2. TrialNet Supply Order System "SOS" (Web Application)

The website address for the TrialNet Supply Order System "SOS" is https://www.mckessonbio.com/bsdweb/trialnet/sitepage.asp. The website can also be accessed via a link on the TrialNet website at **TrialNet Studies Area - Order Clinical Supplies Online** https://www.mckessonbio.com/bsdweb/trialnet/sitepage.asp.

Technical support for problems on using the web order system is available. A User Manual may also be downloaded from the above website address.

Chapter 24. Reimbursement

This chapter describes reimbursement for protocol-related activities by the TrialNet Coordinating Center. Reimbursement is according to the NIDDK approved reimbursement schedule for the Anti-CD20 Trial. Reimbursement is provided for:

- Local laboratory services
- Local medical services
- Local pharmacy services
- Other services
- Study costs

Rates vary for Clinical Centers and Affiliate Sites with and without a GCRC, and International Sites. Refer to the TrialNet Fact Sheet of Reimbursement located on the TrialNet website for more information.

1. Reimbursement Schedule

The TrialNet Clinics will be paid for their screening, enrollment, and follow-up of study participants. NIDDK approved reimbursement schedules are posted on the TrialNet website. Payments issued to the Clinical Centers and Affiliate Sites are based on complete/accurate forms submitted to the GWU TNCC. All payments will be made in United States dollars. Payments should be generated and mailed within 30 days of the close of the payment period (see Fact Sheet for Reimbursement for details on payment periods). An Invoice Report and a detailed listing of services being reimbursed will be provided to the sites along with the payment.

Reimbursements for all components of a visit are made to the site that completed the visit. All applicable Case Report Forms have a place for recording the Site Number for reimbursement. This number is a unique number to identify the institution where the visit took place.

Chapter 25. Quality Control System of Samples

In order to determine the near-term repeatability of assays, reflecting all sources of potential errors, from the point of sample collection to statistical analysis, split duplicate analysis will be performed on specimens collected from study participants.

This program will allow an assessment of the overall measurement of external factors, as well as, internal factors in the assay laboratory that might affect the reproducibility of an assay. All Core Laboratories will participate in this program.

If feasible, specimens should be drawn from new onset studies to increase the likelihood of positive specimens. When possible, stored specimens will be held at primary testing centers, until it has been determined whether or not the sample is positive.

All TrialNet study participants are eligible, as long as blood volume is not a limiting factor, and the assay to be tested in the Split Duplicate program will be obtained during that particular visit. There are no additional visits specifically for Quality Control (QC) blood collections.

The anticipated goal is for each laboratory to assay 100 QC specimens every three months but this goal will be modified for some of the laboratories. A number of factors impact a laboratory from not reaching this objective. Due to variations in assay throughput, specimen processing, and testing (individual specimen verses batch), the number of specimens tested per laboratory will be greatly reduced from the expected goal in some laboratories.

Specimen numbers and goals will be specified for each assay. The guidelines will consider blood volumes, specimen integrity, number of

laboratories performing an assay, and a realistic number of specimens tested for a particular assay quarterly.

The type of collection tube needs to be considered in determining the handling of QC samples. Some specimens must be processed immediately and others can be batched. Whole bloods need to be shipped immediately. Once aliquoted and frozen, serum can be stored and shipped at a later time. Some assays will require duplicate specimens from multiple time points. The size of the collection tube will have to be increased for these timed collections. Pre- and post- infusion blood specimens will be collected for the QC program.

Specimens will be drawn from studies where yield is ample to provide specimens to the labs offering the same assay. The specimens must provide enough volume for the assay and sample storage in the event re-testing is necessary.

Specimens that have been stored by sites can be shipped for QC analysis at various times. If there is a large enough volume, samples can be shipped to the same testing labs at various intervals to measure assay drift over time.

A specially prepared Specimen Transmittal Form (STF) will be provided for this QC program. The specimens will be coded in a manner mimicking the participant specimens, blinding the laboratories as to the nature of these samples. The “Participant ID” Number will come from a separate QC list issued to the clinical sites. A barcode, containing the PID Number will be applied to the collection tubes, and they will be sent to the labs along with other labeled participant tubes that have been collected for the study. This number will appear the same as the PID numbers issued for the study participants but will allow TNCC to track the sample as a QC specimen.

In the event of discrepant results, the lab will rerun specimen samples to confirm results. If results remain discordant, new randomized aliquots will be sent to the laboratory for testing.

The QC data will be posted on the TrialNet web site and reviewed by the Mechanistic Outcomes Committee during its monthly meetings.

Appendix A. Participating Sites

California

Stanford University Medical Center
Stanford, CA

University of California, San Francisco
San Francisco, CA

Colorado

Barbara Davis Center for Childhood Diabetes
University of Colorado- Denver, CO

Florida

University of Florida
Gainesville, FL

University of Miami School of Medicine
Miami, FL

Indiana

Riley Hospital for Children
Indiana University - Indianapolis, IN

Maryland

University of Maryland Hospital
Baltimore, MD

Massachusetts

Joslin Diabetes Center
Children's Hospital Boston - Boston, MA

Minnesota
University of Minnesota
Minneapolis, MN

New York
Naomi Berrie Diabetes Center
Columbia University - New York, NY

Pennsylvania
Children's Hospital of Pittsburgh of UPMC
Pittsburgh, PA

Texas
University of Texas Southwestern Medical Center at Dallas
Dallas, TX

Washington
Benaroya Research Institute at Virginia Mason
Seattle, WA

Canada
The Hospital for Sick Children
Toronto, Ontario

Australia/New Zealand
Walter and Eliza Hall Institute of Medical Research
Parkville, Victoria

Italy
Vita-Salute San Raffaele University
Milan, Italy

Appendix B. Screening IDs

Clinic Name	Site Number	Screening Number Ranges
University of Florida	0001	5801 to 6100
University of Maryland	0210	4051 to 4300
Stanford University	0005	5201 to 5500
University of Miami	0006	4901 to 5200
Barbara Davis Center	0007	4601 to 4900
Joslin Diabetes Center	0008	4301 to 4600
University of Minnesota	0009	3401 to 3700
Benaroya Research Institute	0010	3701 to 4000
UCSF	0011	5501 to 5800
University of Texas	0012	3101 to 3400
Hospital for Sick Children – Toronto	0013	2801 to 3100
University of Pittsburgh	0014	2551 to 2800
Columbia University	0015	2201 to 2500
Indiana University	0016	1901 to 2200
San Raffaele Hospital	0017	1601 to 1900
Walter and Eliza Hall Institute	0019	1301 to 1600

Appendix C. Site Initiation Activities Checklist

05/01/2006

Site Initiation Activities for Anti-CD20 Trial

The following is an internal checklist for use by clinical sites that covers the basic steps for initiating the Anti-CD20 Trial. The checklist covers the following general areas:

- Providing TNCC with contact and other information
- Training and Certification
- IRB Approval and other necessary compliance documents
- Preparing for Implementation

Item	Completed	Date
Getting Started		
Confirm site interest in participation and timeline for implementation with TNCC.	☐	
Provide contact information for investigator(s) and coordinator(s) participating in the study.	☐	
Complete Form FDA 1572 and Financial Disclosure Form FDA 3455 (if applicable) for Principle Investigator and submit to TNCC along with updated CVs for all participating investigators.	☐	
Provide TNCC with name of coordinator(s) and email addresses where result alerts should be sent.	☐	
Provide TNCC with contact and shipping information for local pharmacy that will receive shipments of study medication (Note: The name of a contact at the pharmacy **must** be provided).	☐	
Provide TNCC with availability of –70 or –80 C degree freezer in pharmacy for storage of PhiX174 immunization.	☐	
Training and Certification		
Review current protocol, study procedures, and other study documents.	☐	
Complete protocol certification for all staff members.	☐	
Provide TNCC with pass-phrase for web randomization system and complete mock randomization as certification.	☐	
Complete other TrialNet-wide certifications, as needed (shipping, metabolic testing).	☐	
Complete DU7s for all staff members.	☐	
Provide name, location, and normal ranges to TNCC for local laboratory that will be performing the CBC with differential.	☐	

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05/01/2006

Item	Completed	Date
IRB Approval		
Prepare IRB submission and submit to local IRB.	☐	
Inform TNCC when protocol has been submitted to local IRB and expected date of review.	☐	
Respond to any issues raised by local IRB (TNCC can help with this).	☐	
Send approval letter and approved consents that reflect the appropriate version number and date of the Protocol to TNCC for review (Note: TNCC **must** review and approve consents before a site can start the study).	☐	
Preparing for Implementation		
Implementation can begin once protocol activation letter is received from the TNCC.	☐	
Complete site initiation call with Protocol Chair, PDT Chair, and TNCC (Contact Kim Owens, Protocol RA at TNCC, to arrange).	☐	
Receive laboratory supplies once approved by TNCC. (Use web online system for all re-orders.)	☐	
Receive screening study forms (if available) and barcode labels once approved by TNCC. If paper forms are unavailable, forms may be downloaded from the website for use. (Use order form available on website for all re-orders.) Note: Participant binders will be provided upon request for each participant enrolled.	☐	
Review shipping procedures for TrialNet laboratory samples and forms.	☐	
Review protocol and procedures with lab coordinator to ensure site is prepared for mechanistic sample collections.	☐	
Order study medication from TrialNet Central Pharmacy. Note: Initial supply of study medication will be on site prior to the first infusion (at baseline) once approved by TNCC.	☐	
Implementation		
TNCC recommends reviewing steps involved with first 1-2 visits and contact the TNCC with any questions.	☐	

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Appendix D. Volunteer Understanding Quiz Key

Type 1 Diabetes TrialNet

Anti-CD20 Study
VOLUNTEER UNDERSTANDING QUIZ (PARTICIPANT)

Form RITVQ
01 DECEMBER 2006
Version 1.2
Page 1 of 3

Screening ID: __ __ __ __ - __ Participant Letters: __ __ __

Volunteer's Understanding Quiz is based on the information that has been presented to you regarding this clinical research study. All of the questions are based on this information. The purpose of this quiz is to be sure you know the details of this clinical research study before you agree to be part of it. After you have finished the quiz, the research study team will go over the answers with you. The research study team will be sure to discuss any answers that were incorrect, because it is important to us that you understand the study.

Date that quiz was completed: / / (DAY / MONTH / YEAR)

Put an "X" or a check in the box next to the best answer(s) to each question.
You may take as much time as you want to answer these questions.

1. The reason I am being asked to be in this research study is:
   - ☒ 1 I have recently been diagnosed with Type 1 diabetes
   - ☐ 2 I am at high risk for developing Type 1 diabetes
   - ☐ 3 I have recently been diagnosed with Type 2 diabetes
   - ☐ 4 I do not know why I am being asked to be in this research study

2. The reason for doing this research study is to see:
   - ☐ 1 If giving experimental treatment *before* Type 1 diabetes starts will keep a person from getting Type 1 diabetes
   - ☒ 2 If giving experimental treatment within 12 weeks *after* Type 1 diabetes starts will help my insulin producing cells work longer by keeping them from being destroyed
   - ☐ 3 If testing my blood sugar more frequently will keep my Type 1 diabetes under control
   - ☐ 4 I do not know a reason for doing this study

3. If I decide to be in this research study, I will come to a study site for:
   - ☐ 1 One year
   - ☒ 2 At least two years and possibly up to four years
   - ☐ 3 Three years
   - ☐ 4 I do not know

4. If I decide to be in this research study, visits to the study site will be made *(check all that apply)*:
   - ☐ 1 Every week for the first month, then every six months for the next four years
   - ☒ 2 Every week for the first month and two weeks after the last treatment visit, then every three months for the next two years (visits at 10 weeks, 5 months, and 13 months can be done locally or at the study site)
   - ☐ 3 Every week for the first three months then every month for the rest of the research study
   - ☒ 4 If I continue to make insulin at the end of two years, I will have additional visits every 6 months for two more years
   - ☐ 5 I do not know

5. If I agree to be in this research study, I will have to *(check all that apply)*:
   - ☒ 1 Let my study team know about any health problems that occur whether or not I think they are important because I am in the study
   - ☒ 2 Stay in the treatment group I am assigned to until the research study ends
   - ☒ 3 Keep all appointments at the clinic
   - ☐ 4 I do not know

Type 1 Diabetes TrialNet	**Anti-CD20 Study** **VOLUNTEER UNDERSTANDING QUIZ (PARTICIPANT)**	**Form RITVQ** 01 DECEMBER 2006 Version 1.2 Page 2 of 3
	Screening ID: __ __ __ __ - __	Participant Letters: __ __ __

6. My assignment to a treatment group will be random. This means:
   - ☒ 1 I will have a 2 out of 3 chance of getting the medicine and a 1 out of 3 chance of getting the placebo (A placebo is a "pretend" medicine that looks like the real medicine, but is not active)
   - ☐ 2 I can choose which treatment group I want to be in
   - ☐ 3 The doctor decides which treatment group I will be in
   - ☐ 4 I will be put in the treatment group that is best for me to be in
   - ☐ 5 I am not sure of how I will be assigned to a treatment group

7. My participation in this research study is voluntary. This means:
   - ☐ 1 I must stay in this research study until the entire research study ends
   - ☐ 2 I can choose to not be in this research study or to stop being in this research study at any time, but I will not get as good of diabetes care if I do
   - ☒ 3 I can choose to not be in this research study or to stop being in this research study at any time and no one will be mad at me, I will still receive the same diabetes care
   - ☐ 4 I do not know what voluntary means

8. The risks of being in this research study may include *(check all that apply)*:
   - ☐ 1 There are no risks to being in this research study
   - ☒ 2 Higher risk of getting certain infections
   - ☐ 3 Higher risk of weight gain
   - ☐ 4 Getting an allergic reaction as the drug is being given
   - ☒ 5 Less protection from vaccines

9. The guaranteed benefits of being in this research study include *(check all that apply)*:
   - ☐ 1 I will not need to take insulin anymore
   - ☒ 2 There are no guaranteed benefits to being in this research study
   - ☐ 3 I will not need to check my blood sugar as frequently
   - ☐ 4 I will no longer have diabetes

10. If I decide to be in this research study, I will have to pay for *(check all that apply)*:
    - ☐ 1 The procedures and tests that will be required
    - ☐ 2 The study medicines I will be taking
    - ☒ 3 My diabetes treatment supplies (e.g., insulin, needles, insulin pump supplies)
    - ☐ 4 I do not know what I will have to pay for

11. If I decide to be in this research study, I will get an IV infusion of the study medicine rituximab, or a placebo:
    - ☐ 1 One Time
    - ☐ 2 Two Times
    - ☒ 3 Four Times
    - ☐ 4 Daily
    - ☐ 5 I do not know how many IV infusions of study medicine I will get

Type 1 Diabetes TrialNet	**Anti-CD20 Study** **VOLUNTEER UNDERSTANDING QUIZ (PARTICIPANT)**	**Form RITVQ** 01 DECEMBER 2006 Version 1.2 Page 3 of 3
	Screening ID: _ _ _ _ _ - _ _	Participant Letters: _ _ _

12. If I decide to be in this research study, my diabetes management plan will require that I *(check all that apply):*

- ☐ 1 Take insulin injections only every other day and test my blood sugars when I don't feel well
- ☒ 2 Report my insulin use (i.e., number of injections, type of insulin, use of an insulin pump) and blood sugar results to the study site every two weeks
- ☐ 3 Use an insulin pump and test my blood sugars before bedtime only
- ☒ 4 Check and record my blood sugars at least four times a day
- ☐ 5 I do not know how often I will need to take insulin or test my blood sugars

13. As part of this study, depending on my previous immunization status, I will be given immunizations for:

- ☐ 1 Flu
- ☐ 2 Pneumonia
- ☒ 3 Tetanus/Diphtheria and Hepatitis A
- ☐ 4 I do not know what immunizations I will be given

14. If I choose to do so, I will also be given another immunization of PhiX174. This will be given as a:

- ☐ 1 An injection beneath the skin
- ☐ 2 An injection into a muscle in my arm or leg
- ☒ 3 Two intravenous immunizations spaced 6 weeks apart given in the first and second year of the study
- ☐ 4 I do not know how this is given

Appendix E. Eligibility and Deviation Review Form

Type 1 Diabetes TrialNet

ELIGIBILITY AND DEVIATION REVIEW FORM **Form ELIG**
10JUN2005
Page 1 of 1

Study: PIN: __ __-__ __-__ __ __ __-__ Issue Number: ___ ___ ___

A. GENERAL INFORMATION

1. Date of review request: / / MM DD YYYY
2. Date response needed by: / / MM DD YYYY

B. GENERAL SUBJECT INFORMATION

1. Age (years): ___ ___ ___
2. Sex: ☐ Male ☐ Female
3. Date of diagnosis with type 1 diabetes *(if applicable)*: / / MM DD YYYY
4. Date of screening visit *(if applicable)*: __ __/__ __/__ __ __ __ MM DD YYYY

C. ELIGIBILITY ISSUE DETAILS

1. Provide a brief description of the eligibility issue/deviation that requires review:

2. Provide a brief justification for the subject's enrollment into the study:

D. RELEVANT INFORMATION FROM STUDY DOCUMENTS

TNCC USE ONLY

1. Eligibility reviewed? Y N
   IF YES,
   a. Date of review: __ __/__ __/__ __ __ __ MM DD YYYY
   b. Reviewer ☐1 TNCC ☐2 Committee Chair ☐3 Full Committee
   c. Eligibility decision: ☐1 Eligible ☐2 Not Eligible
   If NO,
   a. Reason not reviewed:
2. Comments:

Appendix F. PPD Test

Updated: November 3, 2005

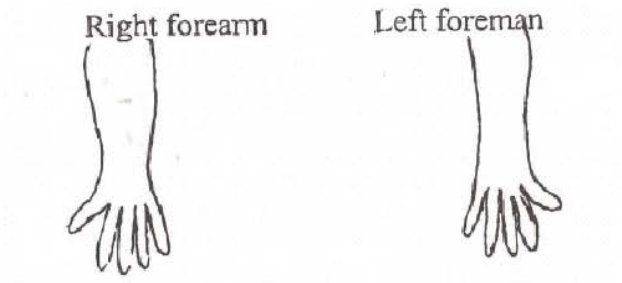
PPD Test Reading Guidelines

Reading the Test: Please measure the TB test site at 48-72 hours post injection.

Given date/time: _____
Read date/time: _____

There will be one site to measure and report. Using the scale provided on the bottom of the page, measure in millimeters and write the results below. If there is no reaction, write that as well. **Note:** The results of the test are based on the size of the induration **only**, not the size or extent of redness. The size or extent of redness **DOES NOT** need to be measured and recorded.

Measure the largest width of induration. You must feel the induration.



Skin Test Type	Induration Width
1.	mm

Subject's name: _____
Name of person reading test: _____
Title: _____ Facility: _____ Date: _____ Time: _____

Please fax to: (_ _ _)-_ _ _-_ _ _ _
Thank you!

Dear Study Participant,

The results of the tuberculosis (PPD) test need to be read within 48 to 72 hours of administering the test. The results of the test must be read by a trained healthcare professional that has experience in reading these tests.

Please make an appointment to have your result read on one of the following dates:

_____ or _____
(48 hours) *(72 hours)*

Have your local healthcare professional complete the section below.

Fax the completed form to <Name> at <phone>

Dear Healthcare Professional,

_____ received a tuberculosis (PPD) test at our site on the date below. Please measure in millimeters the largest width of redness and the largest width of any lump. Record the results in the box below. If there is no reaction, indicate that as well.

Date Given	Indicate Site Right / Left Forearm	Date Read	Lump Width	Redness Width
			mm	mm

Name and Title of person reading test: _____

Facility: _____ Phone Number: _____

Questions or Concerns Contact us at <phone number>

Appendix G. Mixed Meal Tolerance Test (MMTT) Procedure

Background

The MMTT assesses the participant's insulin production capacity. The Anti-CD20 Trial includes both 2-hour and 4-hour MMTTs. The 2-hour MMTT will be administered at Months 3, 6, and 18, and the 4-hour MMTT will be administered at Screening and Months 12 and 24.

Clinic Preparation

The following are the steps that need to be taken to adequately prepare the clinic for the upcoming participant visit:

- Retrieve the participant's study materials.
- Make sure you have all appropriate supplies for tests scheduled
- Make sure you have all appropriate specimen transmittal forms pertinent to the scheduled visit. If this is the Screening, Month 12, or Month 24 visit, the appropriate specimen transmittal form is RIT99M4. If this is the Month 3, 6, or 18 visit than the appropriate specimen transmittal form is RIT99M2.
- Affix all barcode labels to the appropriate cryovials.
- Prepare for the shipment of laboratory specimens making sure you have all necessary supplies including adequate dry ice.

Supplies Needed

Item	Number Needed
Specimen Tubes	
1.2 ml gray top tube (glucose)	7* or 11†
1.2 ml lavender top EDTA monovette tube (C-peptide)	7* or 11†
Cryovials	
1.8 ml Nunc cryovials (glucose, C-peptide)	14* or 22†
Color Code Caps	
gray (glucose)	7* or 11†
lavender (C-peptide)	7* or 11†
Syringes and Needles	
18-22 gauge intra-catheter or butterfly needle	1
10 ml syringe	2
Solutions	
0.9% Sodium Chloride Inj. U.S.P.	x
BOOST High Protein	x
Paperwork	
Specimen Transmittal Form	x
RIT99M2 or RIT99M4†*	x
Federal Express Pre-Printed Labels:	
Northwest Lipid Research Laboratories	x
Bar Code Labels	x
Indelible marking pen	x
Equipment	
polyfoam shipping box	x
packing tape	x
ziplock bag	x
ice/refrigerator	x

Supplies Needed Continued

Item	Number Needed
dry ice	x
dry ice label (*FedEx*)	x
Biohazard label	x
IV tubing	1 line
3-way stop-cock	2
T-connector extension tubing (optional)	x
Multi-Adapter Luer Lock, IST	2
clock/timer	x
rack (for tubes)	x
sterile strip bandage	x
ice bath (C-peptide)	x
IV poles	1
tourniquet	x
Band-Aids	x
First-aid tape	x
latex gloves	x
heating pads (optional)	2
alcohol wipes	x
2 x 2 sterile gauze pads	x
Medications	
EMLA cream (optional, recommended for children)	x
Snack	x

* Supplies needed for a 2-hour MMTT

† Supplies needed for a 4-hour MMTT

Participant Preparation

The participant should be reminded at the prior visit to the clinic about the upcoming MMTT and should be given guidelines to be followed prior to the test. Within one week of the scheduled visit, a letter should be sent to the

participant reminding him/her of the upcoming appointment and repeating the guidelines that need to be followed.

The participant should be reminded:

a. To fast for at least 8 hours (but not more than 16 hours) prior to the test. The participant should start fasting the night before the test, and should continue fasting up until the start of the test. The participant should not eat or drink anything except for water. This means no coffee, tea, sodas, cigarettes, alcohol, or chewing gum during the fasting period.
b. To refrain from vigorous exercise during the fasting period.
c. To refrain from working during the night preceding the morning of the test.
d. To discontinue taking any prescription medications that must be taken on a daily basis.
e. To eat a high carbohydrate diet (see below for sample) for 3 days prior to testing.
f. In children, application of EMLA cream is encouraged.
g. Water consumption is encouraged, especially in small children

The **eligibility checklist** should be reviewed prior to starting the test to be sure that the participant has followed the above guidelines.

Sample High Carbohydrate Diet

The number of grams of carbohydrates is listed in parentheses for each serving of food. You may substitute any items from the lists below and may add as many servings of meats or vegetables as desired. The menu below includes more than 150 grams of carbohydrate. Remember, you must eat at least 150 grams, but you can eat more if you want to. The menu below is only an example, so it can be modified to suit your tastes.

Breakfast	4 oz. orange juice (15)
	4 oz. milk (whole, low fat, 1%, or non-fat) (5)
	3/4 cup dry cereal (20)
	2 tsp. sugar (10)
	1 slice toast (15)
	1 tbsp. jam or jelly (15)
Lunch	12 oz. can of regular soda (not diet) (30)
	2 slices of bread for a sandwich (30)
	1 brownie or 1 banana (15)
Dinner	1 medium size potato (20)
	1 serving of vegetables (5)
	1 cup ice cream or 1/2 cup sherbet (30)
Snack	2 cups popcorn (10)

10 grams	**20 grams**	**30 grams**	**50 grams**
1/2 cup cooked cereal	1 medium apple	1 jelly donut	1 piece pie
1/2 grapefruit	1 piece plain sponge cake	2 toaster waffles	1 pastry
6 vanilla wafers	1 cup plain yogurt	1 cup rice	1 piece frosted cake
12 grapes	1 cake donut	1 cup pasta	1 cup fruit salad
1 cup strawberries	1 in. square fudge	1 slice pizza	1 ice cream soda
8 oz. tomato juice	1 serving canned fruit	4 Oreos	1 milk shake
1 plum	4 oz. cranberry juice	6 date or prunes	8 oz. prune juice
1 Fig Newton	1 hamburger bun	1 plain croissant	1 Milky Way bar
5 Ritz Crackers	1 medium corn on the cob	1 bagel	4 oz. raisins
4 saltines		8 oz orange juice	

Directions For Use Of Local Anesthetic Cream

For participants who may wish to use a local anesthetic cream, the anesthetic cream and instruction sheet should be sent to the participant or parent in advance so that it can be applied prior to the appointment.

Procedure for Mixed Meal Tolerance Test (MMTT)

The MMTT will be performed at Screening, Months 3, 6, 12, 18, and 24. The tests done at Screening, Month 12, and Month 24 will be 4 hours in length, whereas the tests done at other times will only be 2 hours in length. The test uses a standard oral mixed formula meal (Boost) composed of liquid sucrose, soy protein, casein, and soy oil.

Do not proceed with performing the MMTT if the participant manifests unequivocal elevation of fasting plasma glucose (> 200 mg/dl on the participant's home blood glucose meter). The participant should ideally have a fasting glucose level in the range defined by **70 to 200 mg/dl** the morning of the test. Any participants with values outside of this range should have the test rescheduled.

Preparation for MMTT

Because a large number of factors may affect the MMTT, care must be taken to properly prepare participants for the test.

Dietary guidelines:

- High (>150 g) CHO diet for at least 3 days prior to the test
- 10 hr fasting (maximum 16 hr)
- 10 hr abstinence from coffee, tea, caffeine containing drinks, cigarettes, alcohol, vigorous exercise

- Water consumption is encouraged, especially for young children

If the participant has not consumed sufficient dietary carbohydrate before the test, the insulin secretory response to the mixed meal stimulus may not be as great as it should be and the test results may be unreliable. Therefore, the participant must consume a high carbohydrate diet, with ≥ 150 grams carbohydrate per day, for a minimum of three full days prior to testing.

- The test should be **rescheduled** if the participant has a blood glucose (measured on his/her home meter) **less than 70 mg/dl or greater than 200 mg/dl**.
- The test must begin between the hours of **7 and 10AM**.
- The participant should remain seated during the performance of the test.
- The test is started in the morning after a night's sleep. Participants may not work during the night preceding the morning of the test.
- The test should be postponed for at least one week after any intercurrent infectious illness or other stress.

Insulin Guidelines

- Long-acting insulin can be administered the day before the test is scheduled.
- Corrective insulin (Humalog (H) and NovoLog) can be administered up to two hours before the test.
- Regular (R) insulin can be administered up to six hours before the test.
- Participants on insulin pumps (continuous insulin infusions (CSII)) should continue with the normal basal rate, but a Humalog (H) or NovoLog bolus may be added up to 2 hours prior to the test, and Regular (R) bolus up to 6 hours prior to the test.

Mixed Meal Dose

The test meal (Boost) is given at a dose of 6 ml per kilogram body weight. Maximum dose is 360 ml. Boost is supplied in 8 fluid ounce cans.

Test Procedures

1. The MMTT must begin between 7:00 - 10:00 a.m. for proper interpretation.
2. Obtain the weight of the participant and calculate Boost meal size = 6 ml/kg, up to 360 ml, 1lb = 0.45 kg

   The MMTT test uses a standard oral mixed meal formula (Boost®, Mead Johnson Nutritional Division, Evansville, Indiana) composed of liquid sucrose, soy protein, casein, and soy oil. **The test meal is given at a dose of 6 kcal/kg body weight, at 1 kcal/ml to a maximum of 360 kcal.**
3. The participant should remain sitting or resting in bed quietly throughout the test. The participant can engage in quiet, non-strenuous activities such as reading, playing cards, watching TV and may walk to the bathroom between blood draws if necessary (but should otherwise remain in resting position until the test is completed). It is recommended that participants not be asked to answer questions for the purpose of completing case report forms during the MMTT.
4. Place an I.V. line into an antecubital vein, using an intracatheter/butterfly needle (usually 20 or 22 gauge depending upon the size of the participant). The **intracatheter** may be kept patent between samples with a slow saline drip or heparinized saline solution (as per the guidelines of your institution) in a 20 ml syringe, injecting about 2-3 ml after each blood draw.
5. Before the procedure, fill several 3 ml syringes with luer-lock tips with 1 ml normal saline solution to flush the **adapter** after each blood draw.

This is only necessary if the blood sampling is more than 3 minutes apart.

6. Obtain baseline samples:
   - The first sample should be taken at least 10 minutes after establishing the line(s) and when participant is calm and relaxed (if possible, depending on age) - this is the "-10 minute" sample
   - The second sample should be taken just prior to drinking the Boost - this is the "0 minute" sample
7. Meal consumption - Start the clock at the beginning of the drink. The dose of Boost must be completely consumed within **five (5) minutes**.
8. Obtain post-meal blood samples.
9. Samples are taken at 15, 30, 60, 90, and 120 min after time 0' (if this is a 4-hour test, samples should also be taken at 150, 180, 210 and 240 minutes)
   - A timer should be turned on at 0 min
   - The actual start time for each blood draw should be recorded on the MMTT specimen transmittal form

Sampling Protocol

Time (min)	**Glucose Sample Taken** **1.2 ml gray top tube**	**C-peptide Sample Taken** **1.2 ml lavender top EDTA tube**
-10	X	X
0	X	X
Drink Boost		
15	X	X
30	X	X
60	X	X
90	X	X
120	X	X
150†	X	X
180†	X	X
210†	X	X
240†	X	X

† Samples only taken at these times during a 4-hour MMTT

10. If a clogged line, missed sample, or other deviations from the protocol occur, these must be noted on the "Comments" section of the MMTT specimen transmittal form.
11. Termination of MMTT
    - Test is terminated after the blood sample at 120 minutes for a 2-hour MMTT, or 240 minutes for a 4-hour MMTT. At that time, the indwelling cannula(e) will be withdrawn, pressure applied and a sterile strip bandage applied.
    - Upon completion of the test, the participant should have a snack, for example peanut butter or cheese crackers, coffee, milk or ginger ale.

Procedures for blood preparation, storage and shipping

A. **Glucose**: For each time point, 1.2 ml of blood is drawn in a 1.2 ml gray top tube (-10, 0, 15, 30, 60, 90, 120, *150, 180, 210 and 240* min):
   - Label each 1.8 ml cryovial with the appropriate bar-coded label from an unused MMTT barcode label sheet. Apply the labels vertically and snap on appropriate color codes (**Glucose = Gray**).
   - Immediately invert the tube gently 6 times, avoid jarring or shaking.
   - Place the tube upright in an ice bath or in a refrigerator.
   - Centrifuge within 1 hour of drawing for 10-15 minutes.
   - Transfer serum to an appropriately labeled (check both sample type and time point) 1.8 ml cryovial. Screw top on tightly.
   - Freeze samples at -20° C or ship on dry ice the same day.
   - **Ship samples only on Monday, Tuesday, Wednesday** or **Thursday.**
   - Affix both Biohazard and dry ice label on box indicating shipment of blood specimens on dry ice, according to instructions provided on the Specimen Transmittal Form. Record the FedEx waybill number on the

exterior of the box in case the waybill becomes separated from the box.

B. **C-peptide**: For each time point, 1.2 ml of blood is drawn in a 1.2 ml lavender top tube (-10, 0, 15, 30, 60, 90, 120, *150, 180, 210 and 240* min):

- Label each 1.8 ml cryovial with the appropriate bar-coded label from an unused MMTT barcode label sheet. Apply the labels vertically and snap on appropriate color codes (**C-peptide = Lavender**).
- Immediately invert the tube gently 6 times, avoid jarring or shaking.
- Place the tube upright in an ice bath or in a refrigerator.
- Centrifuge within 1 hour of drawing for 10-15 minutes.
- Transfer serum to an appropriately labeled (check both sample type and time point) 1.8 ml cryovial. Screw top on tightly.
- Freeze samples at -20° C or ship on dry ice the same day.
- **Ship samples only on Monday, Tuesday, Wednesday** or **Thursday.**
- Affix both Biohazard and dry ice label on box indicating shipment of blood specimens on dry ice, according to instructions provided on the Specimen Transmittal Form.

Note: Each Core lab will return shipping boxes via Federal Express; please affix a return address label on the inside flap of the box.

Documentation

The appropriate specimen transmittal form must accompany all samples sent to the laboratory. Specimen transmittal form **RIT99M2** is the appropriate form for a 2-hour MMTT. Specimen transmittal form **RIT99M4** is the appropriate form for a 4-hour MMTT. Please do **not** mark in sections labeled for **Laboratory Use Only**.

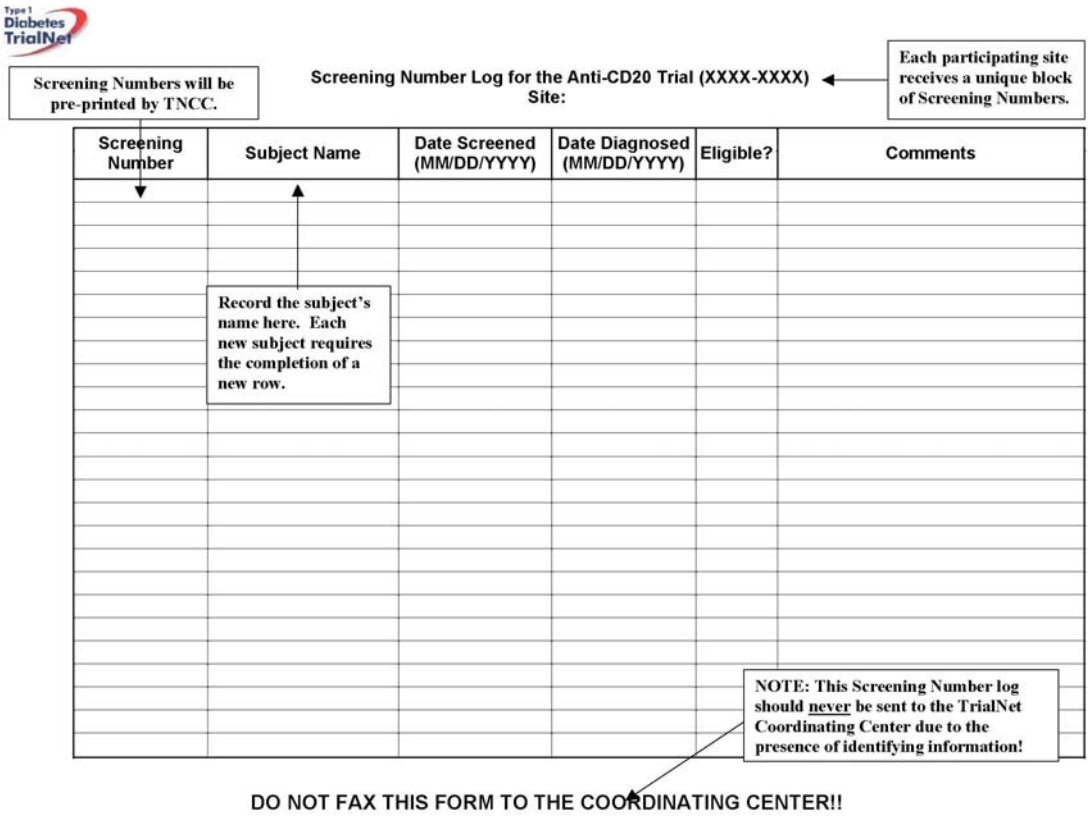
- The **white copy** of this form should be mailed to the TrialNet Coordinating Center
- The **yellow copy** should be sent to the Core Beta Cell Function Laboratory, along with the specimens
- The **pink copy** should be kept at the clinic.

Shipping Address for MMTT Samples

Address: Specimen Processing
Northwest Lipid Research Laboratories
401 Queen Anne Avenue North
Seattle, WA 98109-4517

Phone: (206) 685-3327

Appendix H. Blank Screening Log



Appendix I. EMINENT and Local Pharmacy Forms

Type 1 Diabetes TrialNet - Anti-CD20 Trial (Protocol TN-05)

AGENT REQUEST FORM - RITUXIMAB

Instructions: Type or print clearly all information except signature. Complete all sections except for box labeled **For EMINENT Use Only**. Sign the form in the space provided. All requests received by 2:00 PM EST weekdays will be shipped to arrive 2nd business day by 4:30 pm. Requests pertaining to refrigerated drug products will be shipped by Overnight Service to arrive by next business day 10:00 am. If drug is needed overnight, check **"Yes"** in the Overnight field. If an overnight shipment is needed for Saturday and clinic personnel will be available to receive it, check **"Yes"** in the Saturday field.

FAX or Express Mail to:

EMINENT Services Corporation
7495 New Technology Way
Frederick, MD 21703

Phone: (240) 629-1972
FAX: (240) 629-3298

Clinical Investigator's Name:

Ordered by:

Telephone number: Site No.:

Date ordered: Date needed by:

Overnight: Yes Saturday: Yes

For EMINENT Use Only

Order # _____

Protocol No.	Current Inventory	Agent Name	Unit	Quantity Required	Comments
TN05		Rituxan 10 mg/mL 10 mL Vial	Vial		
TN05		Rituxan Placebo 0 mg/mL 10 mL Vial	Vial		
TN05		Rituxan 10 mg/mL 50 mL Vial	Vial		
TN05		Rituxan Placebo 0 mg/mL 50 mL Vial	Vial		

TYPE Pharmacy shipping address below:
(No P.O. Box Numbers Please)

Initial Request

Subsequent Request

Authorized Signature (Clinical Site) Date

Authorized Signature (Client) Date

0052_Req_TN05 Rev.042706

PHARMACY DRUG REQUEST FORM
Anti-CD20 Trial (Protocol TN05)

Site #: _____

Participant Name: _____

Screening ID: _____ **Participant Letters:** _____

Randomization #: _____ **Randomization Date:** _____

Check One:

Initial Request ☐ **Subsequent Request** ☐

Height: _____ **cm** **Weight:** _____ **kg**

Participant BSA: _____ **m^2**

BSA = square root{[height(cm) X weight(kg)] ÷3600}
If additional space is needed to perform calculation, use back of form.

Rituximab/Placebo Dose Requested: _____ **mg**

Dose = BSA X 375

Requested by: _____ **Date:** _____

Version 1.0 (April 14, 2006)

Type 1 Diabetes TrialNet - Anti-CD20 Trial (Protocol TN-05)

AGENT RETURN FORM

RETURN ONLY AGENTS SUPPLIED BY EMINENT SERVICES CORPORATION

The Agents listed below were returned by: _____

Address: _____

_____

_____

Site: _____

INSTRUCTIONS FOR INVESTIGATOR

1. Type or print clearly all information-one item per line. Follow all sections.
2. DO NOT mark in the shaded areas.
3. Sign and date list.
4. Pack the agents/vials to minimize breakage and spillage.
5. Enclose the completed list with the agents and return to:

Diabetes TrialNet- Anti-CD20 Trial (0052)
c/o EMINENT Services Corporation
7495 New Technology Way

Protocol No.	Agent Name	Strength & Dosage Form	Quantity	Lot #	For EMINENT Use Only	
					Rec. Code	Checked by:
TN05	Bacteriophage PhiX174 0.8 mL Vial	Vial				
TN05	Bacteriophage PhiX174 1.8 mL Vial	Vial				
TN05	Bacteriophage PhiX174 2.8 mL Vial	Vial				
TN05	Hepatitis A (Havrix®) 0.5 mL	Prefilled Syringe				
TN05	Hepatitis A (Havrix®) 1.0 mL	Prefilled Syringe				Verified by:
TN05	Diptheria Tetanus Adsorbed 0.5 mL	Prefilled Syringe				
TN05	Rituximab / Placebo Injection 500 MG 50 ML VIAL	Vial				Date:
TN05	Rituximab / Placebo Injection 100 MG 10 ML VIAL	Vial				

To be completed by site
Individual preparing this list:
(if other than the investigator)

_____ Name _____ Title

_____ Signature _____ Telephone No.

Comments:_____

0052_Ref_TN05 Rev.040606

Type 1 Diabetes TrialNet - Anti-CD20 Trial (Protocol TN-05)

AGENT REQUEST FORM

Instructions: Type or print clearly all information except signature. Complete all sections except for box labeled **For EMINENT Use Only**. Sign the form in the space provided. All requests received by 2:00 PM EST weekdays will be shipped to arrive 2nd business day by 4:30 pm. Requests pertaining to refrigerated drug products will be shipped by Overnight Service to arrive by next business day 10.00 am. If drug is needed overnight, check **"Yes"** in the Overnight field. If an overnight shipment is needed for Saturday and clinic personnel will be available to receive it, check **"Yes"** in the Saturday field.

FAX or Express Mail to:

EMINENT Services Corporation
7495 New Technology Way
Frederick, MD 21703

Phone: (240) 629-1972
FAX: (240) 629-3298

Clinical Investigator's Name:

Ordered by:

Telephone number: Site No.:

Date ordered: Date needed by:

Overnight: ( ) Yes Saturday: ( ) Yes

For EMINENT Use Only

Order #_____

Protocol No.	Current Inventory	Agent Name	Unit	Quantity Required	Comments
TN05		Bacteriophage PhiX174, 0.8 mL	Vial		Weighing less that 38 kg.
TN05		Bacteriophage PhiX174, 1.8 mL	Vial		Weighing 39 kg - 84 kg.
TN05		Bacteriophage PhiX174, 2.8 mL	Vial		Weighing 85 kg or more.
TN05		Hepatitis A (Havrix®), 0.5 mL, 5 Syr Pack	Pack		
TN05		Hepatitis A (Havrix®), 1.0 mL, 5 Syr Pack	Pack		
TN05		Diphtheria Tetanus Adsorbed(Decavac™), 0.5 mL, 10 Syr Pack	Pack		

TYPE Pharmacy shipping address below:
(No P.O. Box Numbers Please)

( ) Initial Request

( ) Subsequent Request

_____ _____
Authorized Signature (Clinical Site) Date

0052_Req_TN05 Rev.071607

PHARMACY VACCINE NOTIFICATION FORM
Anti-CD20 Trial (Protocol TN05)

Site #: _____

Participant Name: _____

Screening ID: _____ **Participant Letters:** _____

Randomization #: _____ **Randomization Date:** _____

Weight: _____ **kg**

Target Date for 1st Bacteriophage phiX174 Vaccination: _____
Enter "NA" if participant chooses not to receive phiX174.

Target Date for Diphtheria Tetanus Vaccination: _____

Target Date for 1st Hepatitis A Vaccination: _____

Submitted by: _____ **Date:** _____

Version 1.0 (April 14, 2006)

PHARMACY VACCINE REQUEST FORM
Bacteriophage phiX174
Anti-CD20 Trial (Protocol TN05)

Site #: _____

Participant Name: _____

Screening ID: _____ **Participant Letters:** _____

Randomization #: _____ **Randomization Date:** _____

Weight: _____ **kg**

Check One:

Initial Request ☐ **Subsequent Request** ☐

Bacteriophage phiX174 **Dose Requested:** _____ **ml**

Dose = 0.022ml X weight (kg) of participant
If additional space is needed to perform calculation, use back of form.

Requested by: _____ **Date:** _____

Version 1.0 (April 14, 2006)

PHARMACY VACCINE REQUEST FORM
Diphtheria Tetanus Toxoids Adsorbed (DECAVAC™)
Anti-CD20 Trial (Protocol TN05)

Site #: _____

Participant Name: _____

Screening ID: _____ **Participant Letters:** _____

Randomization #: _____ **Randomization Date:** _____

Diphtheria Tetanus **Dose Requested: 0.5 ml pre-filled syringe**

Requested by: _____ **Date:** _____

Version 1.0 (April 14, 2006)

PHARMACY VACCINE REQUEST FORM
Hepatitis A (Havrix®)
Anti-CD20 Trial (Protocol TN05)

Site #: _____

Participant Name: _____

Screening ID: _____ **Participant Letters:** _____

Randomization #: _____ **Randomization Date:** _____

Check One:

Initial Request ☐ **Subsequent Request** ☐

Hepatitis A Vaccine Dose Requested:

0.5 ml pre-filled syringe ☐
Child

1.0 ml pre-filled syringe ☐
Adult **(≥ 18 years old)**

Requested by: _____ **Date:** _____

Version 1.0 (April 14, 2006)

Type 1 Diabetes TrialNet - Anti-CD20 Trial

Investigational Drug Accountability Record

Page No._____

Name of Institution	Protocol No. **TN05**

Drug Name, Dose Form and Strength: **Diptheria Tetanus Adsorbed (Decavac™) 0.5 mL Prefilled Syringe**

Protocol Title: Effects of Rituximab on the Progression of Type 1 Diabetes in New Onset Subjects

Investigator

Date	Patient's Name	Patient's Number	Dose	Quantity Dispensed/ Received	Balance Forward / Balance	Manufacturer and Lot No.	Recorder's Initials

0052_ACC_TN05 Rev.051106

Type 1 Diabetes TrialNet - Anti-CD20 Trial

Investigational Drug Accountability Record

Page No._____

Name of Institution	Protocol No. **TN05**

Drug Name, Dose Form and Strength: **Hepatitis A (Havrix®) 0.5 mL Prefilled Syringe**

Protocol Title: Effects of Rituximab on the Progression of Type 1 Diabetes in New Onset Subjects

Investigator

Date	Patient's Name	Patient's Number	Dose	Quantity Dispensed/ Received	Balance Forward / Balance	Manufacturer and Lot No.	Recorder's Initials

0052_ACC_TN05 Rev.040306

Type 1 Diabetes TrialNet - Anti-CD20 Trial

Investigational Drug Accountability Record

Page No._____

Name of Institution	Protocol No. **TN05**

Drug Name, Dose Form and Strength: **Hepatitis A (Havrix®) 1.0 mL Prefilled Syringe**

Protocol Title: Effects of Rituximab on the Progression of Type 1 Diabetes in New Onset Subjects

Investigator

Date	Patient's Name	Patient's Number	Dose	Quantity Dispensed/ Received	Balance Forward / Balance	Manufacturer and Lot No.	Recorder's Initials

0052_ACC_TN05 Rev.040606

Type 1 Diabetes TrialNet - Anti-CD20 Trial

Investigational Drug Accountability Record

Page No._____

Name of Institution	Protocol No. **TN05**

Drug Name, Dose Form and Strength: **Bacteriophage PhiX174 0.3 mL Vial**

Protocol Title: Effects of Rituximab on the Progression of Type 1 Diabetes in New Onset Subjects

Investigator

Date	Patient's Name	Patient's Number	Dose	Quantity Dispensed/ Received	Balance Forward / Balance	Manufacturer and Lot No.	Recorder's Initials

0052_ACC_TN05 Rev:040606

Type 1 Diabetes TrialNet - Anti-CD20 Trial

Investigational Drug Accountability Record

Page No._____

Name of Institution	Protocol No. **TN05**

Drug Name, Dose Form and Strength: **Bacteriophage PhiX174 1.3 mL Vial**

Protocol Title: Effects of Rituximab on the Progression of Type 1 Diabetes in New Onset Subjects

Investigator

Date	Patient's Name	Patient's Number	Dose	Quantity Dispensed/ Received	Balance Forward / Balance	Manufacturer and Lot No.	Recorder's Initials

0052_ACC_TN05 Rev:040606

Type 1 Diabetes TrialNet - Anti-CD20 Trial

Investigational Drug Accountability Record

Page No._____

Name of Institution	Protocol No. **TN05**

Drug Name, Dose Form and Strength: **Bacteriophage PhiX174 2.8 mL Vial**

Protocol Title: Effects of Rituximab on the Progression of Type 1 Diabetes in New Onset Subjects

Investigator

Date	Patient's Name	Patient's Number	Dose	Quantity Dispensed/ Received	Balance Forward / Balance	Manufacturer and Lot No.	Recorder's Initials

0052_ACC_TN05 Rev:040306

Appendix J. Administration Guidelines

Rituximab is a chimeric murine/human monoclonal antibody approved for the treatment of B cell non-Hodgkin's lymphoma. The antibody binds to the CD20 antigen on the surface of the B cells and mediates B cell depletion. It has been shown to be effective in autoimmune diseases such as Rheumatoid Arthritis. Since there is a high frequency of infusion related reactions – follow administration guidelines carefully.

Rituximab/Placebo Administration

Participants will receive 4 doses of rituximab/placebo at 375 mg/m² IV/dose, each a week apart.

- Calculate dose. 375 mg/m² BSA
  - BSA = square root [[height (cm) * weight (kg)]/3600] or use nomogram
  - BSA*375 = DOSE _____mg
  - Have two staff members check dose calculation
  - Receive dose from pharmacy rituximab/placebo at concentration of 4 mg/ml
- Pre-medicate subject
  - Acetaminophen (dose _____) and Diphenhydramine (dose _____)
- Infuse rituximab/placebo in peripheral vein. Hang as IVPB with either NS or D5W

FIRST INFUSION –

- begin at 50 mg/hr and escalate rate in 50 mg/hr increments every 30 minutes to a maximum of 400 mg/hr
- Vital signs at minimum- every 15 min for first hour, then every 30 minutes with each rate increase until maximum dose. At maximum dose (400mg/hr), vital signs every hour.

FIRST INFUSION		
Time (min)	Infusion dose (mg/hr)	Infusion rate (cc/hr)
0-30	50	12.5
30-60	100	25
61-90	150	37.5
91-120	200	50
121-150	250	62.5
151-180	300	75
181-210	350	87.5
211-240 +	400 (max rate)	100

SUBSEQUENT INFUSIONS –

- If no problems during previous infusion, begin at 100 mg/hr, then increase by 100 mg/hr every 30 minutes until 400 mg/hr
- Vital signs at minimum, every 15 min for first hour, then every 30 minutes with each rate increase until maximum dose. At maximum dose (400mg/ml), vital signs every hour.

SUBSEQUENT INFUSION		
Time (min)	Infusion dose (mg/hr)	Infusion rate (cc/hr)
0-30	100	25
31-60	200	50
61-90	300	75
91-120	400	100
121-150	400	100
151-180 +	400 (max rate)	100

Possible Infusion-Related Events

- Fever, chills, rigors, nausea
- Hypotension
- Anaphylaxis
- Cardiac symptoms (MI, Ventricular fibrilation, Cardiogenic shock), Acute respiratory distress syndrome

Mild Reaction

- Temporarily interrupt infusion and notify physician
- Medicate as appropriate
  - Consider redose with acetaminophen and diphenhydramine (up to 2 doses)
  - Bronchospasm or mild hypotension: consider bronchodilators and/or saline infusions
- Upon improvement of symptoms, the infusion can be resumed at one-half the previous rate. If the reduced rate is tolerated for 30 minutes, then the infusion rate may be increased to the next closest rate on the infusion schedule.

Moderate to Severe Reaction

- Stop infusion and notify physician
- Medicate as appropriate
  - Pressor support for hypotension
  - Epinephrine for bronchospasm
- DO NOT RESTART INFUSION OR ADMINISTER ANY SUBSEQUENT DOSING

Appendix K. Key Points Quiz: Teaching Tool for Personnel Administering Drug

Rituximab/Placebo Administration
REVIEW "quiz" OF KEY POINTS

I. DRUG CAUTIONS

QUESTIONS	ANSWERS
1. MILD Infusion reactions to rituximab are common. (true/false)	True
2. The first Rituximab infusion should be rescheduled if the subject took an antihypertensive med that day. (true/false)	True
3. Common mild reactions include (choose all that apply) a. Fever b. Chills c. Joint pain	a, b
4. Severe reactions include (choose all that apply) a. Shortness of breath b. Bleeding c. Hypotension	a, c

II. SUBJECT A

You have a subject for their 2nd infusion who had no problems with their first
Height = 210 cm
Weight = 100 kg

QUESTIONS	ANSWERS
1. What is the dose of rituximab for this infusion?	BSA = Square root [(210 * 100)/3600] = 2.42 m^2 Dose = 2.42 * 375 = 905.7 mg
2. What is the infusion rate to start	100 mg/hr
3. If there are no problems with the infusion, approximately how long will infusion last?	About 3 hours
4. Will subject need pre-med of acetaminophen and diphenhydramine?	Yes

III. SUBJECT B

You have a new subject
Height = 160 cm
Weight = 64 kg
Age = 19

QUESTIONS	ANSWERS
1. What is the dose of rituximab for first administration? a. Explain process and calculation	Two staff members need to calculate and confirm dose BSA= Square root [(160 * 64)/3600] = 1.69 m^2 Dose = 1.69 * 375 = 632.45 mg
2. What is the initial infusion rate?	50 mg/hr
3. If no problems occur with the infusion, approximately how long will infusion last?	About 3.5 hours
4. How frequently will you be assessing VS?	Every 15 minutes for the first hour then every 30 minutes
5. What dose of acetaminophen will you use before infusion?	Adult = 650 mg po
6. What dose of diphenhydramine will you use before infusion?	Adult = 50 mg po
7. How many total doses of acetaminophen and diphenhydramine can you use?	Total of three
8. If subject has chills what do you do?:	a. stop infusion, notify physician b. give acetaminophen and diphenhydramine c. restart rituximab infusion at ½ previous rate for 30 minutes, increase as tolerated to scheduled dose
9. If subject has severe hypotension, what do you do?	a. stop infusion, notify physician b. saline and pressors c. do NOT give any further drug now or later
10. What is the maximum infusion rate of drug?	400 mg/hr
11. What is the starting infusion rate for subsequent doses? a. If subject had chills during first infusion b. If subject had hypotension requiring pressor support during earlier infusion c. If subject had no problems during first infusion	a. 50 mg/hr b. No further drug c. 100 mg/hr

Appendix L. Infusion Orders

Rituximab/Placebo Infusion Orders

Protocol TN-05 – Effects of Rituximab on Progression of Type 1 Diabetes in New Onset Subjects

Patient Name: _____ MRN: _____

Study ID number: _____ DOB: _____

- ❑ Admit to CRC
- ❑ Assure subject signed informed consent and copy is on file in CRC
- ❑ Before FIRST DOSE: if subject takes anti-hypertensives, assure they were D/C'd ≥12 hrs ago
- ❑ Weight (kg) _____ & height (cm) _____
- ❑ Calculate BSA (m^2) = square root of [(ht in cm x wt in kg)/3600]: _____
- ❑ Calculate dose = $375/m^2$: _____
- ❑ Request Rituximab from pharmacy
- ❑ Urine sample for pregnancy testing, if applicable (must be negative to proceed)
- ❑ Insert peripheral IV line
- ❑ Pre-infusion blood samples
- ❑ Pre-medicate subject 30 min before start of infusion
  - Acetaminophen [adults: 650 mg po, children: 40-650 mg po – based on wt]
  - Diphenhydramine [adults: 50 mg po, children: 12.5-50 mg po – based on wt]
- ❑ Verify total dose: _____ mg (concentration should be 4 mg/ml)
- ❑ Vital signs (BP, HR, RR, Temp) prior to start of infusion, q15 min for the 1st hour of the infusion, then q30 min with each rate increase until maximum dose. At maximum dose (400mg/ml), VS every hour until discharge.
- ❑ **PRECAUTION: Anaphylaxis is possible. Crash cart must be available.**
- ❑ Administer infusion via IV pump – DO NOT administer IV push or bolus.
- ❑ **First Infusion (Week 0):**
  - Start infusion at 50 mg/hr (12.5cc/hr)
    - ➢ If no hypersensitivity or infusion-related reactions occur, increase rate in 50 mg/hr increments every 30 minutes, to a maximum of 400 mg/hr.

Mild hypersensitivity or an infusion-related event:

INTERRUPT INFUSION temporarily

- May re-medicate with acetaminophen & diphenhydramine q 3-4 hours prn
- May treat mild hypotension with saline infusion
- May treat bronchospasm with bronchodilators
- Notify physician
- If improvement of symptoms, infusion can be resumed at one-half the previous rate and increased by 50 mg/hr q30 mins as tolerated.

Moderate or Severe hypersensitivity or infusion reactions (e.g. those requiring pressor support for hypotension or treatment with epinephrine for bronchospasm):

STOP INFUSION permanently

- Notify physician
- Pressor support for hypotension
- Epinephrine for bronchospasm
- DO NOT restart therapy or provide subsequent doses

- **Subsequent Infusions (Weeks 1/2/3):**
  - If the subject tolerated the first infusion, start infusion at 100 mg/hr (25cc/hr).
    - If no hypersensitivity or infusion-related reactions occur, increase rate in 100 mg/hr increments every 30 minutes, to a maximum of 400 mg/hr.
  - If the subject experienced infusion-related reactions during a prior infusion, start infusion at 50 mg/hr (12.5 cc/hr) and follow orders for first infusion.

  Mild hypersensitivity or an infusion-related event:

  INTERRUPT INFUSION temporarily
  - May re-medicate with acetaminophen & diphenhydramine q 3-4 hours prn
  - May treat mild hypotension with saline infusion
  - May treat bronchospasm with bronchodilators
  - Notify physician
  - If improvement of symptoms, infusion can be resumed at one-half the previous rate and increased by 50 mg/hr q30 mins as tolerated.

  Moderate or Severe hypersensitivity or infusion reactions (e.g. those requiring pressor support for hypotension or treatment with epinephrine for bronchospasm):

  STOP INFUSION permanently
  - Notify physician
  - Pressor support for hypotension
  - Epinephrine for bronchospasm
  - DO NOT restart therapy or provide subsequent doses

Post-Infusion:
- Once study drug administration is complete, flush tubing with normal saline.
- Keep IV line patent for approximately 60 minutes.
- **Call physician** if:
  a. BP: systolic >180 or <80, diastolic >100 or <50 **and/or if ≥20% change from baseline**
  b. Temp >100°F
  c. Rash or serious AE
- Remove IV catheter.

Adverse Events:
- Record all AEs, including start and stop times, on visit worksheets.

Discharge:
- If VS out of range or if AE has occurred, do not discharge subject from CRC until study coordinator or physician has checked off on subject's discharge.

Week 0: Orders noted: _____ **(RN signature) Date:** _____

Week 1: Orders noted: _____ **(RN signature) Date:** _____

Week 2: Orders noted: _____ **(RN signature) Date:** _____

Week 3: Orders noted: _____ **(RN signature) Date:** _____

2

Appendix M. Assessment Schedule

TrialNet

Anti-CD20 Trial Schedule of Assessments

Version 1.2 (01/19/07)

	Screening[2]		Baseline	Week 1	Week 2	Week 3	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Week 12 / Month 3	Week 13	Week 14	Week 16 / Month 4	Week 19 / Month 5	Week 26 / Month 6	Week 39 / Month 9	Week 52 / Month 12	Week 53	Week 54	Week 56 / Month 13	Week 58	Week 59	Week 60	Week 62	Week 65 / Month 15	Week 78 / Month 18	Week 91 / Month 21	Week 104 / Month 24
Visit Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
Assessments For ALL Participants																													
History and Physical Exam[4]		X[2]	X	X	X	X					X					X[2]	X	X[2]								X	X[2]	X	X[2]
Adverse Events Assessments			X	X	X	X					X				X	X	X	X								X	X	X	X
CBC with Differential	X	X	X	X	X	X					X	X				X	X	X								X	X	X	X
Chemistries	X											X																	
PPD Test	X															X		X											X
HIV, Hepatitis B and C	X																												
Urine Pregnancy Test	X	X	X	X	X						X					X		X								X		X	
Serum for Autoantibodies	X					X					X					X	X	X								X		X	
Rituximab/Placebo Infusion		X	X	X	X																								
PK Analysis and HACA Levels		X														X	X												
HbA1c	X										X					X	X	X							X	X	X	X
4-hour MMTT	X																	X											X
2-hour MMTT											X					X										X			
HLA/DNA, FcR Genotyping	X																												
EBV/CMV PCR		X		X		X					X				X	X	X				X				X	X	X	X	X
EBV/CMV Viral Serology		X		X		X					X				X	X	X				X				X	X	X	X	X
Other Serology[1]		X														X					X					X		X	
Tetanus Pre-Immunization Serology																		X											
Tetanus Intramuscular Immunization																		X											
Tetanus Serology																					X								
Hepatitis A Pre-Immunization Serology																		X									X		
Hepatitis A Intramuscular Immunization																		X									X		
Hepatitis A Serology																					X								X
PhiX174 Pre-Immunization Serology							X				X							X				X							
PhiX174 Intravenous Immunization							X				X							X				X							
PhiX174 15-Minute Post Immunization Specimen							X				X							X				X							
PhiX174 Serology								X	X	X		X	X	X						X	X	X		X	X				

Serology[1]: Antibody titers to other childhood immunizations and illnesses will be measured on these samples.

Physical Exam[2]: Prior to administration of drug.

Screening Visit[2]: May take place several weeks prior to Baseline Visit. Screening MMTT must be within one month (37 days) of randomization.

Physical Exam[4]: including a neurologic assessment at visits designated by *.

TrialNet

Anti-CD20 Trial Schedule of Assessments

Version 1.2 (01/19/07)

	Screening		Baseline	Week 1	Week 2	Week 3	Week 5	Week 6	Week 7	Week 8 / Month 2	Week 10	Week 12 / Month 3	Week 13	Week 14	Week 16 / Month 4	Week 19 / Month 5	Week 26 / Month 6	Week 39 / Month 9	Week 52 / Month 12	Week 53	Week 54	Week 56 / Month 13	Week 58	Week 59	Week 60	Week 62	Week 65 / Month 15	Week 78 / Month 18	Week 91 / Month 21	Week 104 / Month 24
Visit Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
For Children 25-40kg																													
Flow Cytometry		X				X					X					X	X	X			X						X		X
Frozen PBMC/Plasma		X				X					X					X		X									X		X
T cell Proliferation		X														X		X			X						X		X
ELISpot or Immunoblot or Tetramer (alte matrig)[5]		X															X				X							X	
For Children 41-45kg																													
Flow Cytometry		X				X					X					X		X			X						X		X
Frozen PBMC/Plasma		X				X					X					X		X									X		X
T cell Proliferation		X														X		X			X						X		X
ELISpot or Immunoblot or Tetramer (alte matrig)[5]		X														X		X			X						X		X
For Adults and Children >65kg																													
Flow Cytometry		X				X					X					X		X			X						X		X
Frozen PBMC/Plasma		X				X					X					X		X									X		X
T cell Proliferation		X														X		X			X						X		X
Immunoblot[5]		X														X		X			X						X		X
ELISpot[5]		X														X		X			X						X		X
Tetramer[5]		X														X		X			X						X		X
RNA		X														X		X									X		X

ELISpot, Immunoblot, Tetramer[5]**:** Dependent on HLA results.

Appendix N. Temporary Transfer Notification Form

July 18, 2006

Participant Transfer Notification Form for the Anti-CD20 Trial

The Primary Site must complete this form ***prior*** to the Secondary Site initiating any study related procedures for the transferring participant.

Fax the completed form to the Protocol RA (Kim Owens) at 301-881-0179 AND the Site Coordinator at the Secondary Site. The TNCC will acknowledge receipt by signing the form below and faxing it back to the Site Coordinator at the Primary Site. Both sites must keep a copy of the completed form in the participant's study file.

Screening ID Number: ____ ____ ____ ____ - ____

Randomization Number: ____ ____ ____ ____

Primary Site Number: _____

Secondary Site Number: _____

Check visit(s) being attended at the Secondary Site:

☐ 2	Baseline	☐ 10	Week 10	☐ 18	Month 12	☐ 29	Month 24
☐ 3	Week 1	☐ 11	Month 3	☐ 21	Month 13	☐ 99	Other
☐ 4	Week 2	☐ 15	Month 5	☐ 26	Month 15		
☐ 5	Week 3	☐ 16	Month 6	☐ 27	Month 18		
☐ 6	Week 5	☐ 17	Month 9	☐ 28	Month 21		

If OTHER, Specify: _____

Reason for transfer:

_____
_____
_____
_____

Primary Site PI's Signature: _____

Primary Site Coordinator's Signature: _____

Date: _____

TNCC Notification of Receipt: _____

Date: _____

Appendix O. Eligibility Report and Lab Results

Anti-CD20 Subject Eligibility Report

15AUG06

Site Number:
Screening Number:
Letters:

		Eligible
Diagnosis Information		
Date of diagnosis:	*07FEB2006*	
Date subject must be randomized by,if eligible (w/in 100 days):	*18MAY2006*	
Date 4-hour MMTT was performed:	*03MAY2006*	
Days from diagnosis MMTT was performed (at least 21 days):	*85*	
Date 4-hour MMTT expires (37 days):	*09JUN2006*	
Participant Information		
Age: *	*17*	*Y*
Laboratory Results		
Stim.C-peptide: >= 0.2pmol/ml (0.6ng/ml)?	*N/A*	*Y*
Detectable autoantibodies ?		*Y*
--Anti-GAD65 AA:	*Positive*	
--Anti-ICA512:	*Negative*	
--MIAA: *	*Positive*	
--Islet Cell Antigen:	*Positive*	
Hepatitis B screening:	*Negative*	*Y*
Hepatitis C screening:	*Negative*	*Y*
HIV screening:	*Negative*	*Y*
HLA received by TNCC:	*Yes*	

Lab results by visits for Anti-CD20 Study 15AUG06

Site Number:
Screening Number:
Letters: Sex:
Date of Birth:
VisitNo: 1-Screening Visit Date:

ResultName	Description of Result Name	Sample No	Col Date	Results	Normal range of result
ALB	Albumin(g/dL)	79511022	03MAY06	4.2	3.4-4.8
ALP	Alkaline phosphatase(U/L)	79511022	03MAY06	70.0	12-17:(M)<390,(F)<187;Adults:(M)40-129,(F)35-104
ALT	Alanine aminotransferase(U/L)	79511022	03MAY06	13.0	M:10-40,F:7-35
AST	Aspartate aminotransferase(U/L)	79511022	03MAY06	22.0	M:15-40,F:13-35
BIC	Bicarbonate(mmol/L)	79511022	03MAY06	22	22-29
CAL	Calcium(mg/dL)	79511022	03MAY06	9.1	8.4-10.2
CHL	Chloride(mmol/L)	79511022	03MAY06	103.1	96-108
CHOL	Cholesterol(mg/dL)	79511022	03MAY06	127	Normal:<200,Borderline:200-239,High:>=240
CREA	Creatinine(mg/dL)	79511022	03MAY06	0.78	M:0.5-1.2,F:0.4-1.1
GGT	Gamma glutamyl transferase(U/L)	79511022	03MAY06	6	M:8-31,F:5-36

Appendix P. Tanner Staging System

Stage	Pubic Hair	Breast	Penis	Testes
I	Preadolescent	Preadolescent	Preadolescent	Preadolescent
II	Sparse, long, lightly pigmented, downy straight hair	Breast bud; breast and papilla elevated, with increased areolar diameter	Slight enlargement	Enlarged scrotum, pink, texture roughened
III	Increased pigmentation, more curly	Enlarged breast and areola with no contour separation	Increased length	Increased length
IV	Adult type, but less	Areola and papilla form secondary mound	Glans enlarged, increased breadth	Enlarged, darker in color
V	Adult distribution with spread to medial thighs	Nipple elevated, areola contour continuous with breast	Adult size	Adult size

Adapted from Tanner, JM. Growth at Adolescence, 2nd ed. Oxford, England, Blackwell Scientific Publications, 1962.