

## Rare Blood Disorder Sureveillance Project

**Principal Investigator:** Dr. D.B.C. Ritchie, MD, FRCPC  
University of Alberta Hospital  
Assistant Professor  
Department of Medicine  
Division of Hematology

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### **1.0 Study Overview:**

Transfusion of blood has become a fundamental part of medical treatment since the second world-war. Separation of the cellular elements of blood products by centrifugation and fractionation of the remaining plasma using cold ethanol and other forms of precipitation, have made it possible to divide the blood up into components. These components can be used to replace the specific element(s) that are needed, making better use of this valuable resource. The efficiency of blood fractionation is significantly improved by pooling blood; pooled blood products, often made from pools of tens of thousands of donors, are now the mainstay of treatment of many disorders, but particularly a group of rare, and mostly genetic disorders, which I will refer to as the Rare Blood Disorders.

The Rare Blood Disorders include Hereditary AngioEdema, Primary Immunodeficiency, Congenital Neutropenia, Thallasemia, Sickle Cell Disease, Porphyria, Fabrys Disease, and some acquired auto-immune blood disorders including Aplastic Anemia, Pure Red Cell Aplasia, and Amegakaryocytic Thrombocytopenia. They share a number of common features: they are uncommon; their treatment requires the use of expensive blood products; they have genetic predisposition and are therefore likely candidates for gene therapy; and they have been complicated by adverse reactions from their treatment.

The most prominent adverse reaction to blood products has been the transmission of blood borne pathogens, including Hepatitis and AIDS. Justice Horace Krever reviewed errors in the handling of the testing and viral inactivation of blood product, and made recommendations including improved surveillance of blood products. We now know many of the pathogens that are transmitted by blood, and we know more about genetic changes that predispose people to bad outcomes from these infections, such as inheritance of the hemochromatosis gene which predisposes people infected with Hepatitis C virus to cirrhosis. The suppliers of blood products have developed new methods to screen for and remove pathogens from blood products, but patients, physicians, scientists, and government agencies remain concerned about the possibility of new and unknown agents entering the blood supply and causing problems in the future. The discovery of transmission of variant CJD by blood transfusion in the United Kingdom two years ago has raised concern. Gene therapy will likely allow people to avoid transfusion and thereby avoid these

problems, but gene therapy may well be complicated by other side effects; the use of viral gene therapy vectors has been complicated by liver failure and leukemia.

In addition, there has been intense interest over the last 15 years in the specific genetic mutations causing disease. This knowledge has become more important recently with the development of non-viral gene therapy, which targets specific mutations for correction within the chromosomes of living cells. Canadian researchers and the Public Health Agency of Canada are interested in identifying the genetic mutation that causes the Rare Blood Disorders as well as identifying other genetic factors that modify these disorders.

With funding from the Blood Safety Surveillance and Health Care Acquired Infections Division of the Centre for Infectious Disease Prevention and Control of the Public Health Agency of Canada, a surveillance lab has been established at the University of Alberta in Edmonton. This lab was developed under the supervision of the Association of Hemophilia Clinic Directors of Canada (AHCDC) as an archive of blood samples to look for known and emerging blood borne diseases, as well as known and emerging blood clotting gene changes. This project has been successfully audited by a multidisciplinary group including Health Canada, the AHCDC, and the Canadian Hemophilia Society in 2004, and more recently by the Centers for Disease Control in the US in 2005.

The recently developed Network of Rare Blood Disorders has asked the Public Health Agency of Canada to expand the current archive to include other patient groups who are exposed to blood products. The project described here will expand the current archive to establish a secure bank of samples to test for:

1. known and emerging adverse reactions from the treatment of Rare Blood Disorders with blood products and other treatments, focussing on blood borne pathogens at first
2. the genetic changes that predispose people to damage from these adverse reactions, such as the hemochromatosis mutations in people with hepatitis C, or iron overload from transfusion
3. known and emerging genetic changes causing or modifying the underlying rare blood disorder.

## **2.0 Study Design**

### **2.1 Consent**

Patients are usually sent a letter, approximately one month before their annual clinic appointment to remind them of their appointment date and time. At this time, we will attach a note to inform them of this project, along with the patient information sheet and the informed consent form. There will be clear instructions not to sign the informed consent sheet, until the participant is at the clinic for their annual visit, with either the nurse coordinator, or the physician investigator.

On the scheduled day of their annual clinic appointment, the research nurse and/or investigator will be present to obtain consent from patients who wish to participate in this study. The patient may be enrolled on any other visit to the clinic or associated hospital, as well.

The patient information sheet and consent form will be completely reviewed and then signed at this time.

The Physician Investigators and/or the Nurse Coordinator may obtain and receive telephone consent.

If the patient does not consent to participate in this study, at this time, we would like to ask them a few questions regarding their decision. They have the choice to either answer these questions or not, it is not mandatory.

### **2.1.1 Length of Study**

This is planned as a long-term project. In order to conduct serial testing of the blood samples for the purpose of surveillance, we will request re-consent every year for all participants, until such time as:

1. Patient no longer wants to participate in this project.
2. The project ends.
3. Patient dies.

### **2.1.2 Re-consent**

- i. We will ask all participants on a yearly basis, if they are willing to re-consent to continued yearly participation in this surveillance project.
- ii. If they re-consent, their participation would require additional blood and urine samples every year during their annual visit.
- iii. If they agree, they will be contacted by phone or mail when the designated time has expired, (no less than 8 months after the blood samples have been given) and an appointment will be booked for the participants to visit the clinic and give the samples.
- iv. When the participant arrives at the clinic, the nurse coordinator or participating physician will review the study and have them re-consent by way of signing the *Re-Consent form*. (See attached form)
- v. If the participant, changes his/her mind at this time and refuses continued participation, then the nurse/or physician will document this and ask if the study may keep the previous samples and any clinical information collected and proceed as they had initially consented for, one year prior.
- vi. If the person refuses to allow continued use of their samples, this will be documented. Then the samples and any clinical information collected will be destroyed. This will be documented on the Destruction of Samples Form.

All documentation will be maintained with the patient's case report forms.

## 2.2 Sample Collection

Blood and urine collected from people with rare blood disorders, who are at risk for transfusion transmitted diseases will be collected in vacutainer tubes labelled with a bar-coded registry number only and sent to the central surveillance laboratory in Edmonton.

**In addition, we are asking for permission to use any samples collected for and left over from other studies to be used as described above.**

No other identifying information will be on the tubes, so that the sample bank will have no information that can identify the patient. Blood from adults will be collected in two 6 ml EDTA tubes, and one PaxGene tube, while urine (10 ml) will be collected in a sterile tube. Blood from children will be collected in one 6 ml EDTA tube and urine (10 ml) will be collected in a sterile vacutainer tube. Samples will be placed in an approved shipping container provided by the surveillance lab, along with a phase-change pack that will prevent the samples from freezing, and will then be sealed. Shipping containers will be shipped by FedEx using an account number from the central surveillance lab.

In the lab, the blood will be processed to collect plasma (the liquid that the cells float in), DNA and RNA (the genetic material of the cells), and peripheral blood cells. These samples, as well as urine, will be given a new number, known as an inventory number, which will be used for further testing, (ie containing no information can link the sample to a patient) when samples are made available to any testing facility.

## 2.3 Data Collection

Demographic information for individual patients will not be used for this project. A database is under development with the Public Health Agency of Canada, that will create a unique patient ID number, which will be used for all data management related to an individual sample. Demographic data will be maintained only within the participating clinic or physicians office

## 2.4 Sample Testing

Samples will be tested only in labs specifically contracted to the surveillance lab. The contract will be supervised by the Network of Rare Blood Disorders (NRBD), and a research subcommittee made up from members of the Medical and Scientific Advisory Committee (MSAC) of each organization. Contracts will include the statement that:

No other testing than that described herein, will be done, and that test results will be reported back to the surveillance lab in a timely fashion. Samples will be labelled only with an inventory number, so that no identifying information is available to the testing facility. Data will therefore be doubly encoded, first when the registry number is generated, and subsequently when an inventory number is generated at the time aliquots are made. Reporting will be done in the form of a pre-formatted spreadsheet, that will facilitate transfer of data back to the clinic for that patient.

Notice will be given to all associated Research Ethics Boards of:

- i. Each new test. (This includes only tests covered by the consent provided here. Any test not covered in the consent must have a separate consent.)
- ii. Receipt of all results after the tests has concluded (not the results themselves).

#### **2.4.1 Sample Storage**

All blood samples will be stored at the Central Surveillance Laboratory located at the:

University of Alberta  
471 Medical Sciences Building,  
Edmonton, Alberta, T6G 2H7

under the direct supervision, and care of, of Dr. Bruce Ritchie. We plan to keep the samples indefinitely. We propose to apply for a long-term extension at 20 years or sooner if required.

#### **2.5 Handling of Results**

Test results will be sent in a timely fashion to the research subcommittee of the NRBD, to the NRBD which serves as an oversight board, and to the clinics that sent in the samples. The research committee and the oversight committee will only be given summarized data, while the clinics will be given test results linked to a registry number of the patient, so that the patient can be notified of their result. The Public Health Agency of Canada, has agreed to provide guidance for counselling patients who have been found to have evidence of exposure to a new blood borne pathogen.

As the project progresses, summary results may be reviewed and analyzed by other international committees, as designated by the Research Subcommittee of the NRBD. The data will be shared anonymously with international online databases of specific mutations.

### **3.0 Administrative Structure**

#### **3.1 The Research Subcommittee of the NRBD**

The Research Subcommittee of the NRBD is made up of NRBD members, specifically members of the Medical and Scientific Research Committees of the member organizations. They are responsible for overseeing research done on behalf of the NRBD

#### **3.2 The Surveillance Oversight Committee**

A Surveillance Oversight Committee will be made up of members of the Network of Rare Blood Disorders and an ethicist, currently Dr. John Dossetor, who was awarded

the order of Canada for his work on medical ethics. They will provide oversight and general recommendations about the handling of studies and results.

## **4.0 Scientific Rationale**

### **4.1 Surveillance Registry**

Details to follow

### **4.2 Patient Databases**

Public Health Agency of Canada is currently working with physicians and patient groups to develop national databases that will track the use of blood & recombinant products. These will be supported by the Canadian Blood Services and the Quebec Blood Secretariat. These databases are used for scientific purposes to track product use for recall, for post-marketing surveillance and so as to provide accountability of use, and data useful to plan future purchases.

### **4.3 Potential Transmission of blood-borne Disease in Products**

#### **4.3.1 Transfusion Transmitted Viruses**

Since the mid 1970s, treatment of many rare blood disorders has required replacement of missing factors with blood products has involved the use of pooled blood products. These pooled products are made from pools of thousands of donors, and in the past, they have transmitted hepatitis A, B, C, and G, human parvovirus, and HIV, the virus that causes AIDS. Now coagulation blood products are tested to remove any donors who have been exposed to known blood-borne infectious diseases and then the blood products are treated with techniques to inactivate any remaining undetected viruses. Effective methods of viral inactivation include the steps involved in the clotting factor purification process as well as pasteurization, solvent/detergent treatment, and nanofiltration. A combination of methods, usually three, is commonly used, since no method alone is effective against all blood-borne infectious agents. Since the introduction of modern effective methods of viral inactivation, used in combination, no cases of transmission of disease by these products have been discovered.

Despite the clear success of viral screening of donated blood and viral inactivation of pooled blood products, new infectious or viral agents continue to appear which may or may not cause disease upon transmission through blood products. More recently there have been problems with blood borne transmission of West Nile Virus, human parvovirus B19, variant CJD and others. Parvovirus B19 is of particular concern as it reaches very high levels in the blood of infected donors and is not cleared by solvent/detergent treatment, or pasteurization.

#### **4.3.2 Prion Diseases**

The prion agent that causes a disease known as scrapie in sheep can be transmitted in feed to cattle causing bovine spongiform encephalopathy (BSE) also known as Mad Cow disease. This in turn can be transmitted to humans who eat beef, causing

variant Creutzfeldt Jacob disease (variant CJD). Because of this concern Hema Quebec and the Canadian Blood Service have banned donors who have resided in the UK. In 1998, Health Canada placed a temporary “hold” on Recombinant FVIII, which was produced in tissue culture containing plasma protein from a man who later came down with CJD. The recombinant FVIII was cleared for use after studies showed that the donor in question had had classical CJD and not the variant form. Although there is concern about transmission of variant CJD, current evidence indicates that classical CJD is not transmitted by blood. In addition, Bayer Corp. has published the results of internal testing showing that prion agents are effectively cleared by their purification methods. Recently, assays for the agent causing CJD in biological fluids have been described. Within the past year, 2 patients have now been shown to have been infected with variant CJD by blood transfusion in the United Kingdom.

#### **4.3.3 Endogenous Retroviruses**

The normal DNA in all the cells of humans and other animals contains genetic sequences, which code for retroviruses, similar to HIV. These genetic sequences are known as endogenous retroviruses. They likely represent infection in distant ancestors, which integrated into the DNA of the infected animal and have been passed by inheritance and evolution from parent to offspring since. They then evolved separately in different animal species. These genetic sequences apparently do not cause disease, but concern has been raised, about the transmission of endogenous retroviral elements between species through transplantation of tissue. Pig cells in tissue culture can release porcine endogenous retrovirus (PERV) under appropriate conditions and these are able to infect human cell lines in tissue culture. Work by the Food and Drug Administration (FDA) in the US has found no evidence of transmission of PERV to humans, although there remain concerns by investigators and regulators. Use of porcine FVIII in patients with inhibitors is of concern in this context, although there has been no reported evidence of such transmission. The development of new tools, such as western blotting, will aid in screening for transmissible endogenous retroviral elements.

#### **4.4 Molecular Genetic characterization of Rare Blood Disorders.**

Modern molecular genetic techniques have revealed a wealth of information about the genetic defects leading to the Rare Blood Disorders. Matching of a genetic mutation with a biological change in the person affected provides a wealth of detail about the function and interaction of blood clotting factors. For instance, discovery of the interaction of the coagulation protein Factor V with the anticoagulant protein known as protein C, suggested that mutations in the Factor V gene (Factor V Leiden) which cause a mild hypercoagulable state, might modify the severity of bleeding in severe Hemophilia A.

Continued technical advances have greatly sped up the characterization of mutations causing disease. High speed DNA sequencing using robotic work stations, fluorescent nucleotides, and high speed capillary electrophoresis has allowed characterization of the entire human genome by both a commercial group (Celera Genetics) and an international consortium (HUMAN Genome Organization, HUGO), years earlier than expected. Silicon chip based sequencing has simplified

the characterization of known genes and silicon chip based hybridization can determine the level of activity of any known gene in different tissues. It is now possible to correlate the clinical/biological characteristics of individuals with disorders, with their genetic make-up. The collection of clinical data, DNA and RNA are key first steps in such work and are at the heart of this proposal.

Current gene therapy protocols for the most part involve viral vectors. Because of concerns about the interaction of these viral vectors with the genomes of their human hosts, these protocols require that DNA be banked prior to beginning treatment. A collection of DNA from all patients with rare blood disorders will greatly facilitate this work.

Additionally, it may be possible to correct point mutations in living animals using a technique known as chimeroplasty. Given recent problems with gene therapy using viral vectors, chimeroplasty may become the preferred method of gene therapy for the Rare Blood Disorders. Knowledge of the specific mutation in each patient will be critical prior to attempts to correct these mutations.

Genotyping is also important for predicting the progression of infectious diseases such as hepatitis C and HIV, which complicate the Rare Blood Disorders. In these situations, the genotype of the virus and its host can help predict the outcome of the disease.

We primarily propose to collect blood for banking of plasma, DNA and RNA for use in post marketing surveillance of blood products, in the first place for screening for known and emerging blood borne infectious diseases, and the genetic susceptibility of the affected individual. Our secondary purpose is to collect blood for banking of DNA, and RNA to identify the genetic mutations causing the Rare Blood Disorders and to characterize interacting genes.

## **5.0 Study Objectives**

### **5.1 Objective**

The objectives of this project are:

1. To collect a sample bank of plasma, DNA, RNA, and urine to screen for known and emerging blood borne diseases.
2. To study the genetic predisposition to:
  - a. Blood borne pathogens
  - b. Adverse events from the treatment of the patients' disorder
3. To identify the mutation leading to each consenting patient's rare blood disorder, and to characterize other known and yet to be discovered genes that affect the patients' disorder.
4. To collect encoded, non-nominal data into a central database from an electronic database set up with the assistance of Public Health Agency of Canada.

## **6.0 Study Population**

### **6.1 Patient Inclusion Criteria**

To participate in this surveillance project, the patient must have:

- A diagnosed rare blood disorder, requiring blood product treatment, including:
  - Hereditary AngioEdema,
  - Primary Immunodeficiency,
  - Thallasemia,
  - Sickle Cell Disease,
  - Porphyria
  - Fabry's Disease
  - Congenital Neutropenia
- Be between the ages of 0 – 101.
- Consent to be informed of all future testing results as they are discovered through this surveillance project.

A person must be willing to be informed of all the results which may be available as testing is done. Participation is not accepted if the participant refuses this. This is not an option. Therefore consent reads:

“I am aware that the results of all-future testing will be given to me.”

### **6.2 Patient Exclusion Criteria:**

The following is the list of the exclusion criteria:

- Persons who are unable to consent due to mental inability will not be able to participate in this study.
- Person that the investigator, and/or the co-investigator deem as unfit for this trial may not be enrolled in this study.
- Patients, who refuse to consent or participate, will not be enrolled in this study.
- Patients, who do not consent to be completely informed of all testing results as they are discovered, will not be eligible for this study.

### **6.3 Sample Size**

The sample size will be based on the total number of patients documented here in Canada with the help of the Public Health Agency of Canada . The incidence of HAE is estimated to be 1:10,000. Therefore, we estimate that 3000 Canadian suffer from HAE. Similarly, we expect X patients to be affected by Primary Immune Deficiency, X by Thalassemia, X by Sickle Cell Disease, X by Fabry's Disease, and so forth.

## **7.0 Study Design and Procedures**

### **7.1 Study Summary/Flow Chart**

The study begins at home with the brief letter to introduce the study, and the REB approved Patient Information sheet and Informed consent form.

Patients have specific instructions not to sign the Informed Consent Form until they are in the presence of the Physician Investigator and/or the Haemophilia Nurse Coordinator.

If the patient consents to participate in the surveillance study, the required blood and urine samples will be taken along with their routine tests. This will avoid having the patients accessed twice for samples.

If the patient does not consent, there will be no blood or urine samples taken.

After the lab assessment, the patients are then sent to the designated clinic office to continue with the rest of their appointments, for their Annual Comprehensive Clinic.

### **7.2 Procedure Description**

The study personnel will explain the procedure to the patient. Please refer to the Patient Information Sheet.

### **7.3 Informed Consent**

Informed consent can be obtained at this time, only if the patient information sheet has been reviewed, and all questions have been appropriately and completely answered. If the person understands the study and agrees to participate, the patient now signs the informed consent form. The original should remain on the chart and a copy given to the patient.

### **7.4 Evaluation of Inclusion/Exclusion Criteria**

After the participant agrees and signs the consent form, the study nurse will review the inclusion and exclusion criteria to ensure that the participant understands and accepts the responsibility of being informed of all the information that may be discovered with this project. If the patient meets all criteria, they will proceed with their participation in the study. If the patient does not meet all criteria, they will not continue their participation in the study.

We will also record the information regarding the reasons of non-participation in this study. We would like to ask why the person did not consent. Was it due to the project's objectives, being informed of all results, or other reasons? This information may help us analyze the needs and requirements of participants in the future. This information is not mandatory.

All the information will be recorded on the Enrolment Data sheets.

## **7.5 Laboratory Evaluation**

In addition to the routine blood work, qualified, and certified personnel will take an additional sample of blood and a urine sample.

For infants and children between the ages of 0 - 4 years of age, only 6 cc of blood will be taken, an equivalent of 1 teaspoon.

For all other participants age five or greater, 12-14.5 cc of blood will be taken, an equivalent of 2-3 teaspoons.

All patients will have 10ml of urine collected.

The samples will be obtained at this visit, and will be labelled and shipped (Per Protocol/ Lab Manual/ Standard of Practice) to the study centre:

Dr. Bruce Ritchie & Dr. Jonathan Hooton  
University of Alberta Hospital  
Department of Biochemistry  
Room 471, Medical Sciences Building  
Edmonton Alberta, Canada  
T6G 2H7

## **7.6 Data Management**

Clinical data will be collected through a separate project in which the Public Health Agency of Canada is developing patient registries. For this project, subject samples will be labelled only with a registry number, which will be used for all subsequent communication. No confidential data will be kept in the central lab. Communication with treating physicians will be done electronically, linking assay results to the clinical database wherever possible to assure accuracy.

Samples will be aliquotted into tubes with unique inventory numbers in the central lab. These inventory numbers will be used when samples are sent for testing, so that samples are doubly encoded. Assay results are requested back from testing labs on a pre-formatted spread-sheet to facilitate decoding of the inventory numbers, and transmission to participating clinics and physicians.

The central lab database is rigorously backed up. Data is backed up to a CD with every processing of blood. Additionally, the hard-drive is backed up to a second hard-drive nightly. CDs are kept in another building in case of disaster and exchanged on a weekly basis.

## **8.0 Study Completion/Withdrawal**

### **8.1 Study Completion**

There is no further requirement from the participant at this time.

## **8.2 Disposition of Blood Samples**

Once a blood sample is obtained, it will be labelled with the registry number and barcode, and shipped to the coordinating study site located at:

University of Alberta Hospital  
471 Medical Sciences Building  
Edmonton, Alberta. T6G 2H7

Under the direct supervision of Dr. Bruce Ritchie, the primary investigator identified in this project, the blood and urine samples will be processed, maintained and stored. Samples will be given an inventory number for storage, and the code linking the registry number and inventory number will not be released from the surveillance lab. (Please refer to the Laboratory Manual for guidelines of blood draws, certification documents of lab and personnel, and standards of practice.) Blood will be processed to purify DNA, RNA, and serum. These, as well as urine, will be split into aliquots and stored.

The samples that are taken for the purpose of this study will be stored at the coordinating study site; The University of Alberta Hospital, Medical Sciences Building 471, in Edmonton Alberta. These samples will be stored for the length of the study, so that they can be tested for emerging infectious agents, and emerging genes as they are identified.

### **8.3 Storage Failure:**

All the samples will be stored in a freezer, documented on the Clinical Lab Manual. In the case of electricity failure, this freezer has a continuous CO2 back up system, which immediately starts when the temperature increases from the set determined degree of -80C. There is also a subsequent alarm system which is linked with the security office, (University of Alberta Systems Monitoring) which enables them to immediately contact Dr. B. Ritchie, and/or Dr. J. Hooton, of this occurrence, so that measures can be taken to fix the problem and maintain the standard required for the storage of all the samples.

### **8.4 Testing of Samples**

Samples may be shared with other researchers working on emerging blood borne infectious agents or genetic changes affecting blood clotting only. Such work will be done under contract, which includes a statement that no other un-consented testing will be done and that the results will be reported back to the NRBD research subcommittee in a timely fashion. No other work or study may be conducted on these banked samples other than what is specifically outlined in this protocol, and covered in the contract between the NRBD and the testing facility. Studies of new or emerging blood borne infectious diseases or genetic defects, which are covered by the consent, but have not been previously described will be presented to the research committee of the NRBD for approval. Individual REB's will then be notified through the local Principle Investigator that such testing will be carried out, and given the opportunity to assure that such testing is covered by the consent.

Any other/new studies, not covered by current consent, will be considered by the research committee, but will not be carried out until appropriate consent is obtained. This must be presented as a peer reviewed protocol and approved informed consent form.

The genetic material and serum samples will not, in any way, be sold or used for any commercial purpose. No other work, other than what is outlined in this protocol can be done with the samples. Any additional research, not covered by this consent, must have a new informed consent and protocol.

The samples will be identified at the study centre only by the registry number, which has been predetermined, and labelled by the participating site. Samples sent to a testing facility will be labelled with an inventory number only. The code linking the registry and inventory number will be kept at the surveillance lab, and will not be shared outside the facility. The link of the registry number to the participant's name and other information will remain at the individual clinic site and will not be revealed to the surveillance lab or study centre. Thus, the identity of each participant will remain anonymous.

Each participant will be informed of all the results of consented genetic testing. Test results will be communicated to the clinic with the appropriate registry number, which will allow the clinics to identify the patient concerned. Participants will be notified of the results of such testing by the clinic physician at the first opportunity. The Public Health Agency of Canada will provide assistance in counselling patients about their results.

It is the responsibility of each site-specific co-investigator to inform each participant of the results of the genetic testing. However, it is the participants' responsibility to inform the site-specific co-investigator of any name change, move, change in contact number, etc. The site-specific co-investigator is then responsible for the maintenance and storage of all records for each participant for the purpose of this project.

All of the above information is specifically outlined in the patient information sheet and a copy will be given, and copies made readily available if the participant loses the original.

### **8.5 Study Withdrawal**

The patient is able to, at any time, to withdraw from the study. The patient is only required to contact his/her physician and inform them of their choice to withdraw. It is then, the site-specific co-investigators responsibility to contact the coordinating study centre and inform them of the patient's choice to withdraw. This will then enable the study centre to discard the samples, which prevents any further testing from that point.

### **8.5.1 Destroying of samples:**

If a patient refuses continued participation, at anytime after enrolment in to the study, they will need to inform the appropriate clinic investigator, who will then be responsible for informing the Central Lab.

As soon as verified notification is received, in the form of a signed and dated document, the central lab will take the remaining samples and destroy all of the remaining samples, when an independent observer is available to witness the destruction. This will be done according to the lab manual, under Standard of Practice.

### **8.5.2 If a patient expires**

If a patient expires, due to any circumstances, they have the decision at the time of enrolment to dictate what should happen to their samples.

The choices are:

- 1) All of their samples to be immediately destroyed upon receipt of the first notification of their death at the Central Lab.
- 2) Samples may remain in the study and can only be used in direct accordance of the specific protocol for which it is intended.

Ownership will remain in the responsibility of the governing committee directly involved with the study.

Therefore this will be included on the Informed Consents:

- I wish to have my samples destroyed upon my death.
- I wish to have my samples remain in the study, for further study, as outlined in my signed consent.

### **8.5.3 Consent for continued participation after death:**

At the time of death, where continued participation is consented by the deceased, notification to the family, significant other, and/or legal guardian will occur.

## **9.0 Statistical Consideration**

Statistical Evaluation will depend on the particular testing being done, which will determine the sample size, and statistical significance. Further statistical analysis will have to await decisions about specific testing.

## **10.0 Responsibilities of the Investigator**

### **10.1 Introduction**

This study will follow Good Clinical Practice guidelines, as established by Health Canada and the Food and Drug Administration (FDA).

The Public Health Agency of Canada acknowledges the GCP of the International Conference on Harmonization (ICH) but all research through Canadian Universities is also covered by the Canadian Tri-Council Policy Statement for Research involving Human Subjects (TCPS-1998).

The Therapeutics Products Program (TPP) of Health Canada is the national authority that regulates drugs, medical devices and other therapeutic products used in Canada. Clinical trials conducted in Canada are subject to Good Clinical Practice, the Declaration of Helsinki, and guidance regarding the conduct of clinical trials issued by TPP.

The purpose of these regulations and legal obligations is to define the standards and principles for the proper conduct of clinical trials that have been developed by the medical, scientific and regulatory communities. They are not to impede or restrict research.

The ethical standards defined with the GCP are intended to ensure that:

- Human subjects are provided with an adequate understanding of the possible risks of their participation in the study, and that they have a free choice to participate or not.
- The study is conducted with diligence and in conformation with the protocol in such a way as to insure the integrity of the finding;
- The potential benefits of the research justify the risks.

The primary investigator Dr. Bruce Ritchie responsibility for the following:

- To select qualified co-investigators
- Provide each co-investigator with the information that they need to properly conduct the study at their site/clinic.
- To assist each co-investigator with all/any aspects of this study, and to ensure that the trial is conducted professionally and ethically, abiding to all regulations and guidelines that have been developed for clinical trials.
- Ensuring proper monitoring of the investigators
- Ensuring that the study is conducted according to this Protocol.
- The primary investigator and the co-investigators are solely responsible for protecting the rights, safety, and welfare of all subjects entered into this project, and all information that may be obtained or discovered. All participants have the right to know, and must be informed of all information obtained and discovered as a result of their consent to use their blood for this project.

## **11.0 Legal and regulatory Consideration**

### **11.1 Compliance with Law, Audit, and Disbarment**

Each investigator, with current GCP standards and in conformity with the Canadian TCPS (1998) guidelines, is responsible to maintain complete and accurate study documentation in compliance with current GCP standards and all applicable federal and local laws, rules, and regulations related to the conduct of a clinical study.

Each investigator is required to make all study documentation properly available for inspection, review, or audit at each study site upon request by any appropriate regulatory agencies, the principal investigator, and its' representatives.

Any individuals ineligible from conducting or working on clinical studies, including those who are ineligible as a result of disbarment, will not be allowed to conduct or work on this project. The person is required to disclose immediately, in writing to the primary investigator if any of the above is, or may be applicable, as in the case of a pending, or threatened legal action.

## **12.0 Protection of Human Rights**

### **12.1 Subject Consent**

Each participating site will be provided with a sample patient information sheet and consent form. Although the use of these developed documents is recommended, these forms may be adapted to suit the needs of each institutional review board with the acknowledgement, acceptance, and approval of the primary investigator and the appointed study centre coordinators.

The patient information sheet must include the following:

- Purpose of the study, and the design
- Participation involvement
- Risks and benefits
- Withdrawal from the study at any time without prejudice to further treatment at any time
- Confidentiality/Maintenance of records

## **13.0 Research Ethics Boards (REB)**

Each co-investigator, with the continued support from the study centre, is responsible for obtaining approval of this project from their Research Ethics Board. The REB must also review any amendments, and approval must be in a form of written documentation. This approval document must be kept at each site, and a copy forwarded to the study centre

The study protocol and the appropriate final version of the patient information sheet and the consent form must have written approval from the REB before any patient enrolment into the study.

## **14.0 Protection of Subject Data**

The co-investigator, at each specified site is responsible for keeping and maintaining a record of all subjects participating in this project and link it to a registry number which has already been predetermined as a patient with a Rare Blood Disorder. However, no other persons will be able to link the designated patient number to the individual. This is the only identifier, which will be used to link the blood and urine samples taken and shipped to the study centre.

A confidentiality clause will be included in the patient information sheet to inform the patient that only authorized personnel, directly involved with this study, or regulatory authorities may have access to the records if deemed necessary, or for audits. All and any publications will never name a person, or provide any information by which any participant can be identified.

## **15.0 Modification of the Protocol**

In the case that an amendment is made to the protocol, each site-specific co-investigator will be notified in writing, along with the amended protocol. All amendments must be reviewed by each site specified REB and have written approval of such.

The research subcommittee of the NRBD, in consultation with the Surveillance Oversight Committee, will decide on whether or not significant changes in the protocol have been made. It is the responsibility of the Surveillance Oversight Committee to decide whether or not significant changes in the protocol have been made. If significant changes have been made, then consent for the changes must be obtained prior to doing the changed procedures. All necessary changes must be noted on associated documents. I.e. consent forms.

## **16.0 Study Records**

### **16.1 Documentation**

- All documentation is the responsibility of each site investigator. The responsibilities are listed below:
  - 1) Current and complete curriculum vitae for each site investigator. Signed and dated.
  - 2) REB membership list (kept on file).
  - 3) Approval of the protocol, patient information sheet and consent form from the REB. The study centre must be in approval of the patient information sheet and consent form if any changes were required, other than the original sent to the site.
  - 4) Certification of the laboratory that is used for the purpose of conducting this trial.
  - 5) Retain all approvals and correspondence with the IRB, and/ or study centre.  
All completed original informed consent forms with the required signatures.  
All shipment forms associated with this study

## **16.2 Subject Identification**

Each site must keep an enrolment log, for each patient that is screened for the study. (Patient name, age, date of visit, and whether or not the patient agrees/or not to participate in the trial. This form will be standardised to capture the same information at all participating clinics. See attached enrolment log).

## **16.3 Recording of Data**

The study centre will provide each site with a document for recording data, The Case Report Forms (CRF). This will help assist each site to a standard for data collection. (See appendix 3.)

## **16.4 Record Retention**

Each site is responsible for retaining the documents for a period no less than 15 years from the completion of the study. If the documents need to be relocated, the site must notify the study centre. Each site is responsible for providing the information to the study centre as to where the documents will be stored, and who may have access to them in case the need to re-access information in the future were to occur. The location must meet certain requirements; a locked, secured room, with a functional and working water sprinkler.

## **16.5 Laboratory Certification**

Each site must provide laboratory certification, which states that the lab chosen to participate in this study is accredited and all associated laboratory personnel to be certified as well, per guidelines and regulations. This document is to be kept on file.

## **16.6 Confidential Information**

All information related to this project is completely and entirely confidential. All confidential data remains the sole property of the NRBD. No information may be disclosed to others without the written permission from the study centre. At the discretion of the study centre, information from this study may be made available to Health Canada, other regulatory agencies (the FDA) and/or other physicians who are conducting similar studies.

## **16.7 Publication**

Authorship determination will be at the discretion of the research subcommittee of the NRBD, and the principle investigator Dr. Bruce Ritchie. Site-specific co-investigators are requested not to publish any results of this study without the written permission of the research subcommittee of the NRBD. Such permission will not be unreasonably withheld. A copy of any manuscript based on this data must be submitted to the research subcommittee of the NRBD at least 90 days before publication. The subcommittee will be allowed to comment on the manuscript and make comments and changes, as they deem necessary for the validity of the project. Additional publication can be submitted once the primary study analysis has been accepted for publication.