

REDS-III

Brazil Sickle Cell Study

Aim A

Manual of Operations

December 2017

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Chapter 1 – Introduction

1.1 Overview of REDS-III

The Recipient Epidemiology and Donor Evaluation Study–III (REDS-III): Brazil International Component is a research program sponsored by the US National Institutes of Health, National Heart, Lung, and Blood Institute (NHLBI) to study the safety and availability of the blood supply in Brazil. The program includes several studies in the United States, as well as international collaborations with blood banks and hospitals in Brazil, South Africa and China. General objectives of the REDS-III program are to ensure the safety and efficacy of blood banks and transfusion medicine practices. This study is being conducted to better understand the clinical and genetic factors that impact patients with Sickle Cell Disease in Brazil and get a better understanding of the impact of transfusion on their care and treatment. The study will be conducted by the six REDS-III blood centers; the central data coordinating center; and the central laboratory. Below is a list of the participants.

- Blood Centers
 - Fundação Hemope (Recife, Pernambuco)
 - Fundação Pro Sangue/Hemocentro (São Paulo, São Paulo)
 - Fundação Hemominas (Belo Horizonte, Minas Gerais)
 - Fundação Hemominas (Juiz de Fora, Minas Gerais)
 - Fundação Hemominas (Montes Claros, Minas Gerais)
 - Fundação Hemorio (Rio de Janeiro, Rio de Janeiro)

- Data Coordinating Center
 - RTI International (Rockville, Maryland)

- Central Laboratory
 - Blood Systems Research Institute (San Francisco, California)

1.2 Study Objectives

This large program focuses on transfusion practices and predictors of health outcomes in patients with Sickle Cell Disease (SCD). The four primary aims of this study are: 1) Aim A - Establish a cohort of SCD patients with a comprehensive centralized, electronic database of detailed clinical, laboratory and transfusion information, as well as establish a repository of blood samples to support biological studies relevant to SCD pathogenesis and transfusion complications, such as transfusion-transmissible infections and alloimmunization; 2) Aim B - Characterize changes in markers of inflammation in response to transfusion by analyzing chemokine/cytokine panels and microparticles in serial pre- and post-transfusion specimens; 3) Aim C - Identify single nucleotide polymorphisms (SNPs) that contribute to the risk of red blood cell alloimmunization in SCD by performing a genome-wide association (GWA) study in transfused SCD patients; and, 4) Aim D – Characterize risk of HIV and HIV outcomes in the

Brazilian SCD population and compare SCD outcomes among HIV sero-positive and sero-negative SCD patients. Patients are enrolled from six clinical care sites affiliated with the participating four REDS-III Brazil hemocenters. The main cohort aims to enroll 3000 SCD patients.

This manual describes the procedures for implementing Aim A.

1.3 Tasks for Participating Hemocenters

The six hemocenters participating in this study are responsible for:

- Selecting collaborators and research assistants from the center who will be responsible for the collection and reliability of the data;
- Sending information from the hemocenter in the development of the REDS-III Sickle Cell Disease Study protocol;
- Managing and supervising recruitment, sample collection and the collection of clinical and socio-demographic data from all patients according to the rules established in the procedures manual;
- Responding to all questions from participating patients and ensuring he/she is informed of all the implications of their consent to participate in the study;
- Ensuring that participant consent was obtained and documented prior to enrollment;
- Ensuring that the assigned, unique REDS-III participant identification code (ID) is used to label all survey materials (samples, questionnaires and reports);
- Collecting and processing the samples according to the instructions described in Chapter 4 of this manual;
- Entering data in the study management system (SMS) and database (DIDB) according to the user guide (see documents: "DIDB User's Guide" and "SMS User's Guide"); and,
- Contributing to the development of analyzes and scientific articles to disseminate the results obtained in the research.

Chapter 2: Enrolling Patients

The Brazil SCD Study will target enrollment of 3000 active sickle cell patients from the 6 hemocenters throughout Brazil. A description of the patients to enroll in this study is described in this chapter.

2.1 Patient Eligibility

The number of patients with sickle cell disease treated at each center is listed in **Table 1**. Only active patients (with at least one clinical visit in the last 3 years) will be eligible for the study.

Table 1. Hemocenters and Patient Population

Hemocenter/Clinic	City	Number of Patients	Active Patients
Hemope	Recife	1365	1185
Hemominas	Belo Horizonte	2934	2348
	Juiz de Fora	483	413
	Montes Claros	903	789
Hemorio	Rio de Janeiro	3830	3027
ITACI	São Paulo	157	137
Total		9670	7945

A random sample of patients was selected from all active patients with SS, SC, Sβ+, or Sβ0 enrolled at participating centers. Only these patients will be eligible for the study and will be registered in the SMS with a unique study identifier. The goal of enrollment in the study will be 3,000 patients divided among the hemocenters, as shown in **Table 2**. An oversample of eligible patients was chosen to account for a refusal rate of up to 30%.

Table 2. Recruitment Goals by Hemocenters

Hemocenter/Clinic	City	Active Patients	Eligible Patients	Patient Recruitment Goal
Hemope	Recife	1185	787	550
Hemominas	Belo Horizonte	2348	1213	850
	Juiz de Fora	413	358	250
	Montes Claros	789	502	350
Hemorio	Rio de Janeiro	3027	1287	900
ITACI	São Paulo	137	137	100
Total		7945		3000

2.2 Recruitment Documents

Recruitment into this study is targeted to a specific set of patients at the participating hemocenters. To ensure recruitment occurs effectively, the study will use an electronic, web-based study management system (SMS) to manage the many tasks involved with participation.

The following documents are required for successful recruitment into the study. They must be pre-printed prior to enrollment and maintained for the duration of the study:

- Informed consent document/s approved by the local Research Ethics Committee (2 copies for each enrollment – **Appendix 1**)
 - Depending on age of the participant the following forms should be completed:
 - 18 or older: Informed consent signed by participant
 - Less than 18: Informed consent signed by parent/legal guardian
 - Less than 18 but older than 7: Child assent signed by child (accompanied by parental/legal guardian consent)
- Reimbursement receipts (2 copies for each eligible patient – **Appendix 2**)
- Recruitment form & Specimen Collection form (1 copy of each - PDF file extracted from the SMS and saved in the study computer – **Appendix 3**)

2.3 Identification Numbers and Labels

To manage the many participation activities, each patient will have 3 types of unique ID numbers assigned to him/her:

1. Medical Record/Hospital ID: Patient registration number in the Hemocenter. This number will be printed on the recruitment and data/specimen collection materials for each eligible patient. This ID is defined by the Hemocenter.
2. Participant ID: Patient identification number in the study and is assigned by the study staff at the time of enrollment. This number could be affixed to materials or even pre-printed when generated from the SMS. For enrollment, it is necessary to affix the participant ID labels on the following documents:
 - a. Informed Consent form
 - b. Specimen Collection form
3. Specimen ID: Identification number for the specimens that each participant provides to the study (over the course of their participation). Specimen ID labels should be affixed to the following documents/materials:
 - a. Informed Consent form
 - b. Recruitment form
 - c. Specimen Collection form
 - d. Whole blood tubes
 - e. Aliquots tubes

2.4 Recruitment Visits

Patients should be recruited to participate in the study prior to a routine blood collection for a routine clinical visit. When approaching a participant for enrollment, the research assistant should explain the study and what is expected of the patient, in addition to giving the patient the opportunity to ask any questions they may have. Research assistants must follow the recruitment script (**Appendix 1**) developed for this study.

Each week, the research staff will print out a report and recruitment documents for eligible patients who have a scheduled blood collection in the upcoming week. This information will be provided from the scheduling system at each hemocenter.

For patients who miss a scheduled blood draw, study staff will call the patient to describe the study and ask them to participate at their next scheduled medical visit or plan a visit specific to enrollment into the study. Study personnel can approach participants selected for the study at any point that they are at the hemocenter or clinic for routine clinical care.

2.5 Materials for Recruitment

Research assistants should have the following materials prepared for each recruitment:

- One set of participant and sample ID labels;
- 2 copies of the informed consent;
 - one for the participant
 - one for the patient study folder
- 2 copies of transportation reimbursement receipt;
 - One for the participant
 - One for the patient study folder
- SMS generated patient recruitment forms (with participant ID affixed)
- Specimen Collection form (with both labels affixed)
- 2 x 6 mL EDTA tubes (with the sample ID affixed) in a zip lock bag

Each site coordinator will work with the assistants to have a few packets prepared and available in the study space.

2.6 Reviewing and Signing the Consent Form

Each participant must review and understand all the requirements for participation prior to study enrollment. The participant must sign the one copy of the informed consent documents (**Appendix 3a-c**) before they can complete any study activities. For patients under the age of 18, the consent form must be signed by the parents or legal guardian. And for the patients aged 7 to 18 years, in addition to the consent of the parent or legal guardian, the patient must also sign the assent form.

The Consent Form should be presented to the patient for reading. If the patient has difficulties or is unable to read, the research assistant should assist him/her. Before signing the consent, the research assistant must also confirm that the patient understands what is asked of him/her and is willing to participate in the study. The informed consent requires the following information: participants name, signature, date of signature, and the name of the study staff enrolling the patient into the study.

2.7 Study Enrollment Tasks

After signing the appropriate consent/assent documents, the research assistant must perform the following tasks:

1. Update and confirm the patient's personal data on the recruitment form (mother's name, address, telephone).
2. Paste the sample ID tags: into the tubes, both copies of the consent form, and the recruitment and specimen collect forms.
3. Put all materials needed for collection in a clear plastic zip lock bag: tubes, specimen collection form, sample ID labels for aliquots.
4. Deliver the plastic bag to the phlebotomist. Before collection, he/she must check the patient's data contained in the collection form and confirm the draw occurs with the person enrolled in the study.
5. After collection, save the sample until the interview is completed.
6. Update the SMS (see **Appendix 5**) with the patient's current personal contact information, update the demographic data, agreement to participate, confirmation of signed informed consent, attendance at the clinic visit. Only after this is completed will the patient interview become available to complete.
7. Conduct the patient interview using the DIDB system (see **Appendix 6** for more details).
8. Obtain the participant's signature on the reimbursement receipt (**Appendix 2**) and provide the money.
9. Provide 1 copy of the consent form to the patient and place one copy in the participant's study folder.

10. Update the SMS with the collection of the whole blood tubes (only after this is done can the laboratory lab technician update the SMS with details of the processing, creation, and storage of the aliquots).
11. After the interview, submit the samples and the Specimen Collection form to the study laboratory technician for processing and storage.
12. At the end of each week, collect all the signed specimen collection forms from the laboratory technicians to confirm processing of specimens occurred.
13. Assign a physician at the local hemocenter to be responsible for completing the medical record review/abstraction and update the SMS. The site coordinator should aid the research assistant in making assignments to the study physicians.
14. After completing the medical record, ask responsible physician to review the data and revised, if necessary, prior to entering/saving the data in DIDB.
15. Follow up with the responsible physicians at each hemocenter on the status of the medical record reviews and do so until all have been entered and confirmed.

The assigned physician is responsible to enter data into the DIDB on a daily/regular basis, details of which are provided in **Appendix 6**.

NOTE: All recruitment data must be updated in SMS EVERY day. It is through this system that the enrollment goal will be monitored by those responsible for the study.

2.8 Organization of Enrollment Documents

To ensure success enrolling as many patients as possible, it is important to set up and maintain an organization at each hemocenter. Below are three types of folders that should be utilized during the entirety of this study.

- Folder 1 - Refusal documents:
 - This will include the recruitment forms for patients who refused to participate in the study.
- Folder 2 – Patient enrollment documents:
 - Recruitment form
 - Signed informed consent/s form
 - Receipt of reimbursement form
 - Completed sample collection form
 - Printed and completed questionnaires, if used

- Folder 3 - Non-attendance / non-attendance documents:
 - Completed recruitment forms for patients who were not approached because they did not attend the blood center or because the approach was not possible (lack of time, goal reached, etc.).

For the three follow up visits, similar folders should be set up, except that documents related to the additional tasks should be added to the original participant folder (folder 2).

2.9 Schedule for Enrollment Activities

Recruitment of eligible patients will occur over a period of 15 months. Between October and December 2013, the study will undergo a pilot period, where fewer patients will be enrolled so that the procedures and processes (recruitment, specimen collection, specimen processing/management, patient interview and medical form collections). Full enrollment will begin in January 2014, with the goal of registering a total of 3,000 patients by December 2014. To achieve this goal, the number of patients to enroll from each hemocenter each week is shown in *Table 3*.

Table 3: Weekly Enrollment Goal (assuming 4 weeks per month) by Hemocenter

Hemocenter/Clinic	City	Patient Recruitment Goal	Weekly Patient Recruitment Goal
Hemope	Recife	550	10-11
Hemominas	Belo Horizonte	850	15-16
	Juiz de Fora	250	4-5
	Montes Claros	350	6-7
Hemorio	Rio de Janeiro	900	16-17
ITACI	São Paulo	100	1-2
Total		3000	

2.10 Materials for Follow Up Visits

Additional genetic studies were added to the Brazil SCD protocol in 2014, so a new informed consent document was created. Efforts to approach the enrolled participants in the study began during Follow Up Visit 1.

To ensure the availability of these uniquely valuable human specimens for longer term study, a biobank was established at the University of Sao Paulo. A consent form was developed and enrolled participants have been approached to request permission to store all remaining specimens from the collections they provide to the study.

All relevant US and Brazilian administrative and ethical committee approvals were obtained and are maintained for the duration of this study.

For the follow up visits, the research assistants should have the following materials prepared:

- One set of participant and specimen ID labels;
- At first follow up visit appropriate:
 - 2 copies of the genome wide association studies informed consent;
 - One for the participant
 - One for the patient study folder
 - 2 copies of biobank informed consent:
 - One for the participant
 - One for the patient study folder
- 2 copies of transportation reimbursement receipt:
 - One for the participant
 - One for the patient study folder
- SMS generated patient recruitment forms (with participant ID affixed or pre-printed)
- Specimen Collection form (with specimen ID label affixed or pre-printed)
- Specimen collection tubes:
 - Follow up visit 2: 2 x 4 mL EDTA tubes + 1 x 3 mL Tempus tube (with the sample ID affixed to all tubes) in a zip lock bag
 - Follow up visit 3: 1 x 4 mL EDTA tube + 1 x 4 mL Serum Separator tube (SST) + 1 x 3 mL Tempus tube (with the sample ID affixed to all tubes) in a zip lock bag

Chapter 3: Data Collection

At enrollment and each subsequent visit, many types of data are collected. This chapter describes the procedures for collecting the socio-demographic, patient interview/risk factor and medical history data. The data collection forms are provided in **Appendix 4**.

3.1 Socio-demographic data

The patients eligible for this study were randomly selected from all of the active patients at each participating hemocenter. Data for these patients (chart number, date of birth, sex, etc.) were extracted from the hemocenter medical systems and loaded into the study management system (SMS) by staff at the University of São Paulo (USP). Any changes to these data should be noted on the recruitment form and updated into the SMS. More detailed socio-demographic data will be collected by the researcher at the time of the interview with the patient.

3.2 Modules

The patient questionnaires are available directly through the DIDB web system (see instructions in the **Appendix 6**), which is only accessible via the SMS. If for any reason you are not able to enter the data directly into the DIDB, it is acceptable to complete a hardcopy version of the questionnaire. Data from the hardcopy questionnaire should be entered into the DIDB only by approved study staff. Please use clear and legible handwriting to complete hardcopy documents to minimize errors when these data are entered into the DIDB. Instructions on entering hardcopy data can be found in the same **Appendix 6**. Consent must be signed prior to performing the interview or collecting medical record data, and the SMS must be updated for the link to the DIDB to be active for a participant.

3.3 SCD Interview Questionnaire

Modules 1 and 2 of the "SCD Interview Questionnaire" include socio-demographic questions that should be asked directly to participants. In no case should the interviewees' responses be induced or suggested. The interview should be held in a private location, the research assistant should always remind the patient that the information collected is CONFIDENTIAL. For the interview to be well conducted, the patient should feel free to answer the questions. The interview has questions that can cause discomfort to the patient, so it is very important to maintain a professional posture, ask, listen and record all the answers naturally and above all with great respect.

3.3.1 SCD Medical Record Questionnaire

Modules 3 to 15 of the "SCD Medical Record Questionnaires" should be completed directly from the participant's medical records, as described in the Filling Manual (see "Manual Filling Interview Questionnaires and Medical Record"). Modules 11 and 14, in some blood centers, will be extracted directly from the electronic records. After the SMS is updated with the patient enrollment and date of informed consent, the physician or other study staff responsible for collecting data from the medical record will have 15 days to finalize this collection. Reminders will be generated weekly for any medical record abstractions that are beyond 15 days.

3.3.2 Medical Record Questionnaire/Abstract Modules

There are up to 13 modules that should be collected for each enrolled participant; some of the modules may not be relevant to a patient and will not need to be completed. They are described briefly here. Refer to **Appendix 4** for more details.

3. Hospital admissions
4. History of surgeries
5. Bone Marrow Transplants
6. Medications
7. Diagnostic tests
8. Serological Results
9. Laboratory Tests
10. Iron Status
11. Immuno-hematological data
12. Transfusion history
13. Transfusions history in the last year
14. Transfusion data in the last year
15. Clinical History

If specific medical conditions are identified, supplemental data will be triggered and collected. These supplemental data include:

1. Anaplastic episodes
2. Acute splenic sequestration
3. Ischemic Stroke
4. Silent Cerebral Infarct
5. Hemorrhagic stroke
6. Seizures
7. Hepatic Sequestration
8. Pulmonary Embolism
9. Acute Chest Syndrome
10. Pulmonary Hypertension Catherization

11. Pulmonary Hypertension Echocardiogram
12. Avascular necrosis
13. Priapism
14. Proteinuria
15. Chronic Renal Failure
16. Acute Renal Failure
17. Bacteremia/Sepsis/Meningitis

3.3.3 Follow Up 1, 2, and 3 Data Collection Issues

For Follow Up visits 1, 2 and 3, the same physician will be assigned to complete the medical record abstraction, as often as possible.

The research assistants should interview the participant at a medical visit during the period of each follow up. If a participant is not able or willing to come back to the hemocenter just to complete the interview, the research assistants should complete the by telephone.

The months included in each of the follow up periods for this study are:

Follow Up 1 – October 2014 – June 2016

Follow Up 2 – June 2016 – January 2018

Follow Up 3 – January 2018 – September 2018

The SMS will define the start and end dates for each follow up abstraction range for each participant and provide that information to the physician assigned to completing the Medical Record Questionnaire.

Chapter 4: Specimen Collection and Processing

Along with establishing the cohort and all the related medical and clinical data for enrolled patients, the REDS-III Brazil International Program will establish a comprehensive and robust biospecimen repository. The quality of this repository will have an impact long term and offer REDS-III investigators and other researchers with a valuable source of biological data to answer important questions among sickle cell patients in Brazil.

4.1 Overview

Peripheral whole blood will be collected from each consenting subject at study enrollment and during the 2-year and 3-year follow-up visits. These collections will involve EDTA, Tempus and Serum tubes, depending on the study visit (see **Table 4**).

Each whole blood tube will be identified by a unique sample ID label (see **Figures 4, 5, and 6**). Each sample label will also include a tube id (e.g. T1, T2) that can be used to identify which whole blood tube links to a specific set of plasma or packed cell (PRWBC) aliquot labels if more than one EDTA whole blood tube is used for collection. The specimen volume from each tube will be aliquoted, inventoried and placed in a -20° or -80°C freezer for storage. A summary of the specimens projected to be collected and aliquoted from each donor is described below in *Table 4.1*.

Visit	Max Volume	EDTA		SST	Tempus
		Plasma	Packed Cells	Serum	
Enrollment*	12 mL	6 x 1 mL	6 x 1 mL	N/A	N/A
Follow Up 2	11 mL	4 x 1 mL	4 x 1 mL	N/A	3 mL
Follow Up 3	11 mL	2 x 1 mL	2 x 1 mL	3 x 1 mL	3 mL

** 6 mL EDTA tubes were collected originally, but decreased to 4 mL aliquots when the Tempus tube was added to the collection.*

4.2 Laboratory Supplies

Materials and supplies for collecting, processing, storing, recording and shipping of the specimens will be provided by the São Paulo blood center, REDS-III Central Laboratory (BSRI) and RTI.

4.2.1 Brazil Blood Centers

Each of the blood centers in Brazil will provide the following materials for the collection and processing of the patient blood samples:

- All blood collection supplies (e.g. Vacutainer tubes, tourniquets, alcohol pads etc.)
- Blood processing equipment and supplies (e.g. centrifuge, pipettors, pipette tips, transfer tubes, tube racks, gloves, lab coats, safety glasses etc.)
- Specimen storage equipment (e.g. -80°C mechanical freezers)

These materials should be covered under the capitated per-patient enrollment payments.

4.2.2 Central Laboratory – Blood Systems Research Institute (BSRI)

Blood Systems Research Institute will:

- Provide technical support for specimen collection and processing
- Receive, store, and then forward specimens selected for testing in the US
- Coordinate with RTI regarding updates to the BSI-II system

4.2.3 Central Coordinating Center/Brazil Central Laboratory – University of Sao Paulo (USP), School of Tropical Medicine & School of Computer Science

This group will provide:

- Freezer storage boxes
- Freezer box grid inserts
- Shippers and dry ice (or related costs) for shipment of materials to USP on a quarterly basis
- Shippers and dry ice (or related costs) for shipment of materials from USP to BSRI, as requested
- Study and specimen management systems
- Other materials, as needed

4.2.4 Data Coordinating Center, RTI

RTI will:

- Design, print and ship the labels needed for the study to Brazil (Hemominas for distribution to all study sites), specifically the Aliquot IDs, freezer box IDs, and the participant IDs.
- Collaborate with the study team to develop the processes for collection and management of biospecimens.
- Maintain a web-based system (BSI-II) for specimen inventory and tracking in the United States.

4.3 Collection of Specimens

The most important task for this study is to standardize the collection and processing of blood samples across all blood centers. A blood sample that is not collected, labeled, and processed correctly will most likely have a negative impact on the current study and on future downstream applications and assay results. Therefore, it is important that blood samples be collected and processed according to established procedures to ensure that assay results reflect biological change and not collection or process variability.

Blood should be collected from participants at enrollment, follow up 2, and follow up 3. The specific processing of the specimen tubes collected at each visit are included in **Figures 3, 4 and 5**.

4.3.1 Specimen Integrity

Ideally, all whole blood tubes collected for this study should be centrifuged and processed within 6 hours of collection. However, this may prove to be logistically impossible at some sites. For any EDTA whole blood tubes that a site is unable to process within six hours of collection should be refrigerated (+2° to +8°C) as soon as possible to maintain specimen and analyte stability. Sites should store these whole blood tubes in a refrigerated condition only up to 48 hours. If samples are collected on a Friday afternoon after processing has occurred for the day, lab techs can keep the samples in the refrigerator until the following Monday for processing (up to 72 hours post collection). **Every effort should be made to collect and process whole blood samples on the same day.** Following specimen processing, all specimen aliquots should be transferred to -80°C storage as soon as possible. The Tempus tube requires storage at -20°C for a few days prior to longer term storage at -80°C.

4.4 Aliquot ID Labels

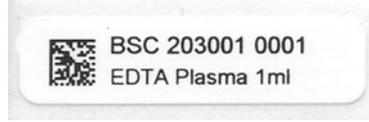
RTI will print and send the specimen ID labels for use by each blood center to Hemominas in Belo Horizonte (BH). The site coordinator at Hemominas BH will distribute the aliquot ID labels sets to each site. Every month on the in-country study team call, each site coordinator will report on the status of the materials at each site and review the blood collections completed to date. The Sickle Cell Disease Study Management System (SCD SMS) will track all aspects of the blood collection and every aliquot made. The sites should **ONLY** use labels that are printed and provided by the DCC.

4.4.1 Label Design

The specimen ID labels provided by the DCC are designed and printed with the following information; a 2D matrix barcode, barcode translation text, specimen description and other identifiers (see **Figure 1**). The labels and printed text are designed to remain affixed to tubes and vials, survive exposure to a number of common laboratory environments, a range of

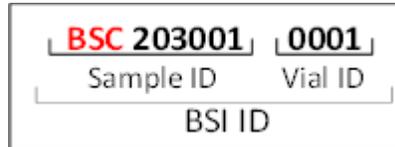
freezer storage temperatures (-20 °C to -199 °C), direct contact with dry ice and some chemical solvents and disinfectants.

Figure 1. Example Specimen Label Format



The specimen ID format is a triple alpha/six-digit identifier followed by a four-digit vial ID (see **Figure 2**). The combination of identifiers is known as the BSI ID. When used with the DCC biospecimen management system (BSI-II) the ID has the capability to identify and track each individual whole blood tubes collected and all resulting aliquots that are created.

Figure 2. BSI ID Format



4.4.2 Label Assignment and Usage

The specimen ID labels consist of 20 labels per subject and are specific to the collection at each scheduled study visit (with except Follow Up Visit 1). The number of tubes collected depends on the visit type. As shown in **Figures 3, 4 and 5**, each collection has its own processing schema:

Figure 3 – Enrollment Visit Processing Scheme

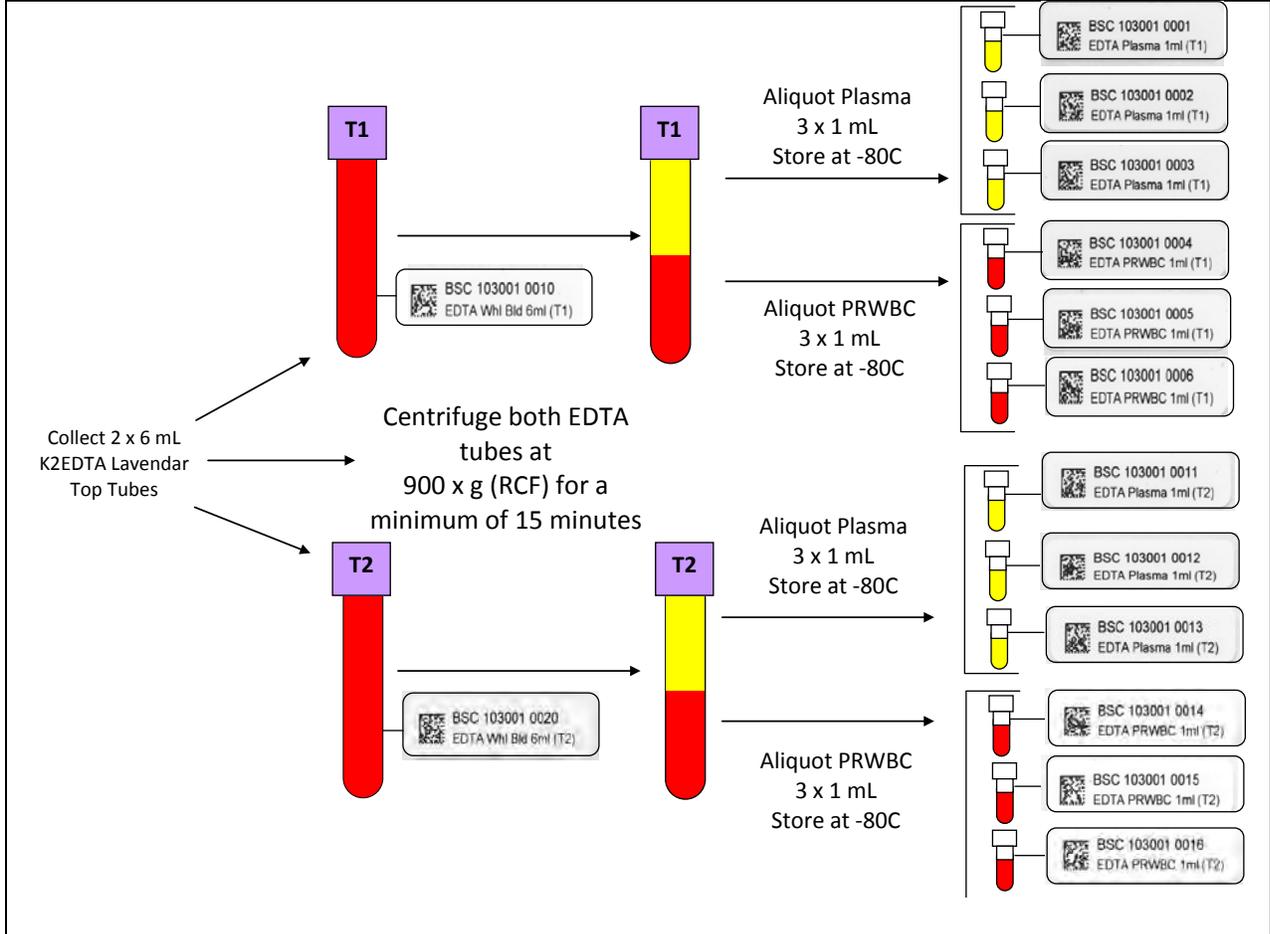
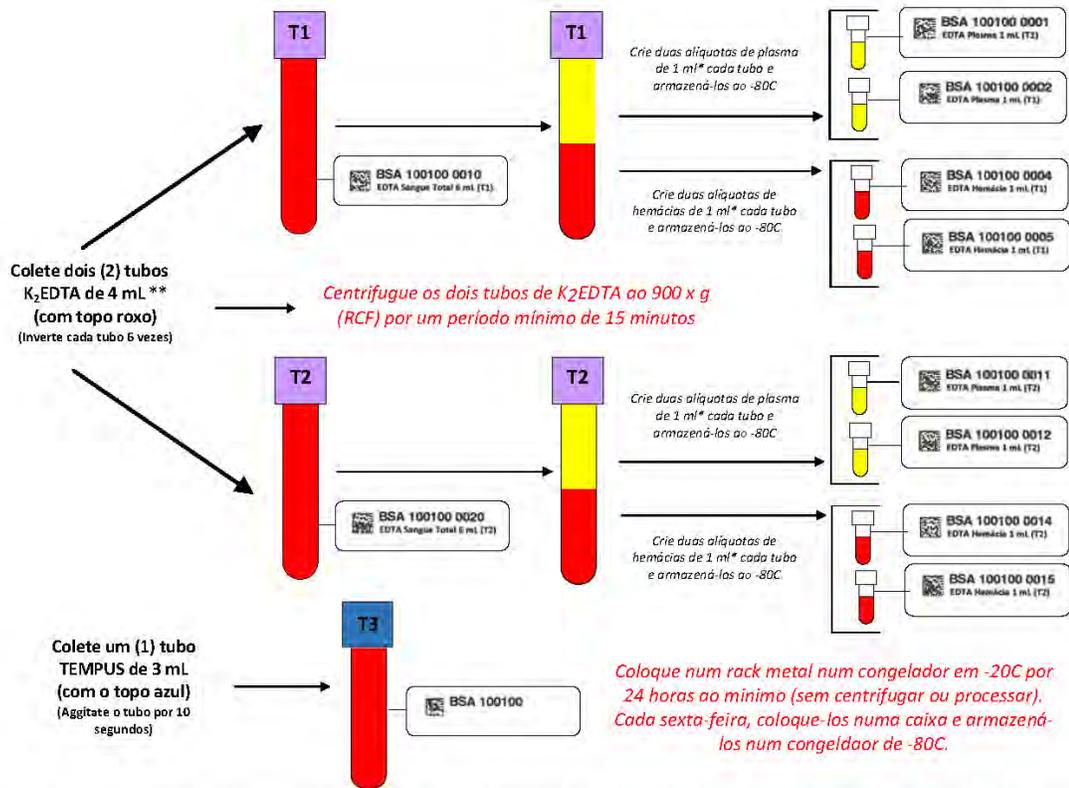
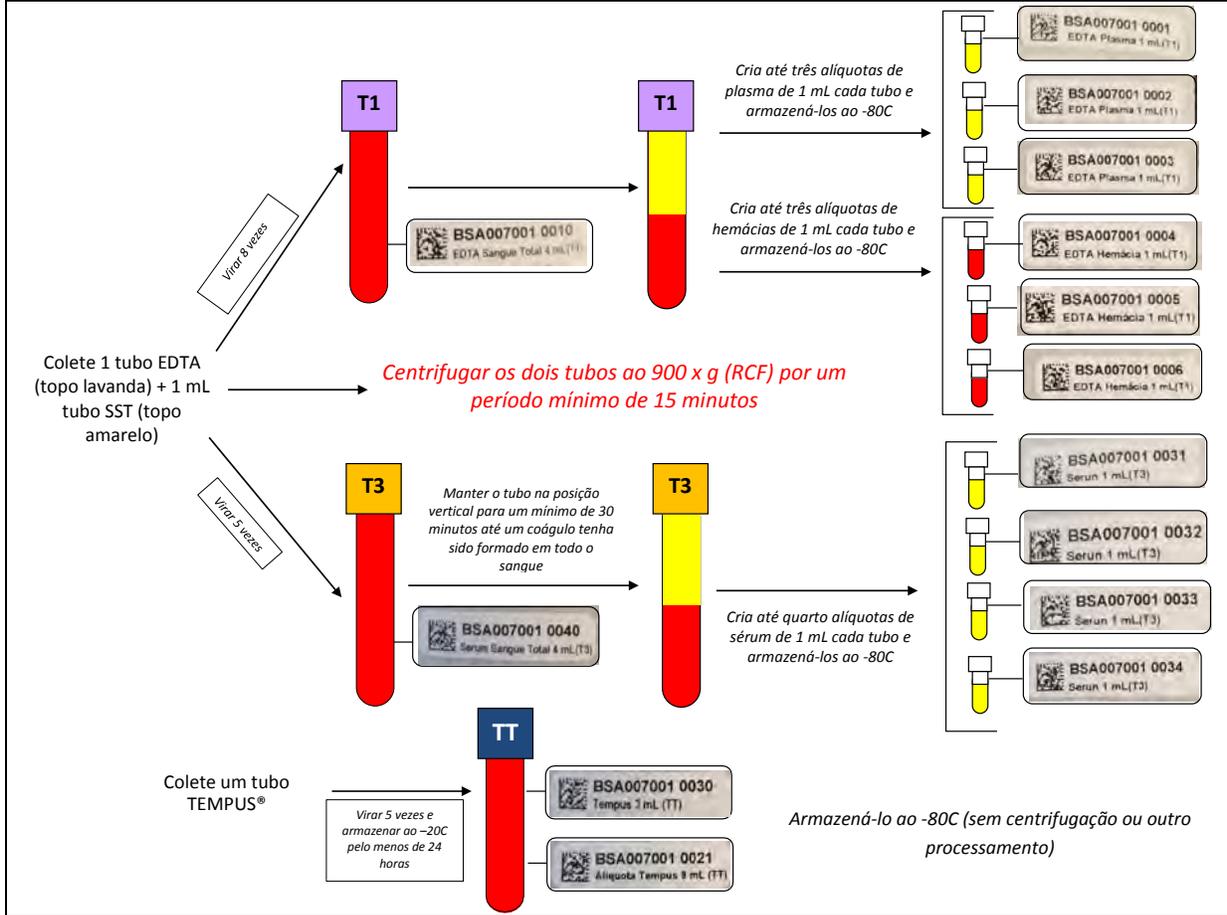


Figure 4 – Follow Up Visit 2 Processing Scheme



* A segunda aliquota pode ter volume maior ou menor que 1 ml, pois deve ser o que restou depois que a primeira aliquota foi criada. O esquema para aliquotagem deve ser o esquema aprovado para Anemia Falciforme. ** As etiquetas são imprimidas com 6ml, mas os tubos EDTA tem limite de volume de 4 mL.

Figure 5 – Follow Up Visit 3 Processing Scheme



4.5 Label Whole Blood Collection Tubes

It is critical that the whole blood tubes are labelled correctly and that the specimen ID is linked with each participant ID. There are labels on each specimen label set that are intended to go on the patient folder, the informed consent (at enrollment), and the specimen collection form. As described earlier, each collection varies slightly in the tubes collected and aliquots to be created. It important the research assistants pay close attention to the visit type and apply the correct sample labels on the whole blood tubes prior to delivering them to the phlebotomy service at the beginning of each day. In **Figures 6, 7 and 8**, the sample IDs that should be applied to the whole blood tube labels are identified by color by visit and tube type: **red/purple** = EDTA tube; **blue** = Tempus tube; **gold** = SST (serum separator tube).

Figure 6 – Enrollment Specimen/Aliquot Label Set

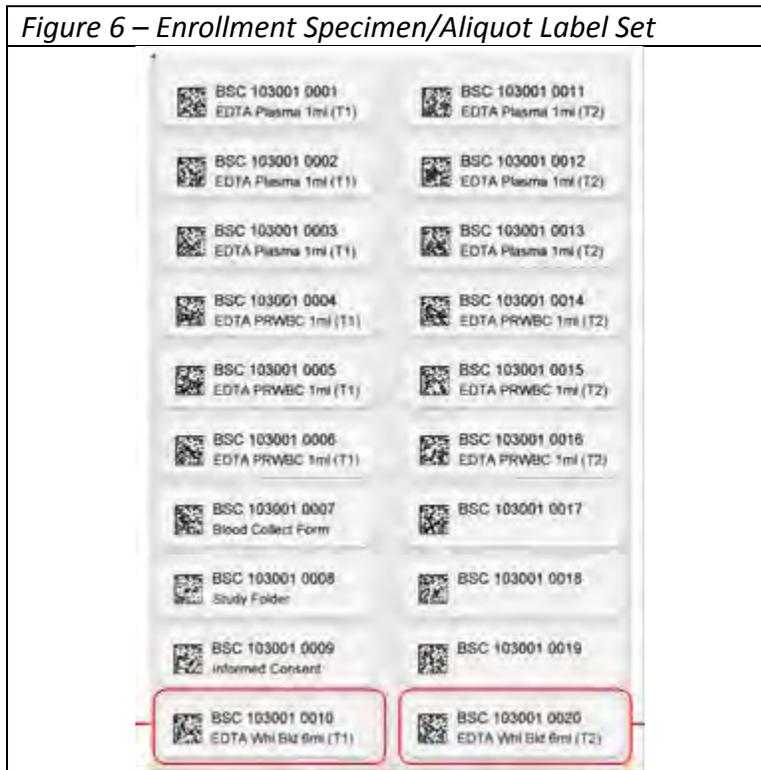


Figure 7 – Follow Up 2 Specimen/Aliquot Label Set



Figure 8 – Follow Up 3 Specimen/Aliquot Label Set



4.6 Collect Whole Blood Tubes

Handle and process all whole blood specimens according to your institution's biospecimen safety guidelines and policies.

Collection of the whole blood will occur at the phlebotomy department at each hemocenter.

- Research assistants will prepare a blood collection packet for each participant, including the following steps:
- Prior to blood collection, label the EDTA, SST (serum) and/or Tempus tubes with the correct *Specimen ID* labels (see **Figures 6, 7, and 8**).

This remainder of this section applies to tasks performed by the phlebotomists.

- Perform phlebotomy procedure using your institution's standard procedures and universal safety precautions.
- Immediately after each tube is filled with whole blood:
 - EDTA tubes – slowly invert the tube 8-10 times to mix the blood and EDTA additives.
 - SST tubes – slowly invert 5 times; allow this tube to sit for at least 30 minutes until a clot has formed.
 - Tempus Tube – vigorously shake for 10 seconds.
- Maintain the collected tubes at room temperature in an upright position.

Order of draw for the collections with multiple tube types is:

1. Serum Separator tube (SST)
2. EDTA tube
3. Tempus tube

If there are any issues with collecting all the planned whole blood tubes at a visit, the following samples are considered highest priority for collection:

- Enrollment – EDTA
- Follow Up 1 – Tempus tube (then the EDTA tube)
- Follow up 2 – SST tube (then EDTA tube and finally the Tempus tube)

Research assistants should contact the in-country study coordinator at Hemominas if there are any questions or exceptions to the collection scheme during a visit.

4.7 Process Specimens

Once the whole blood specimens are collected, the research assistant will collect the packet with the blood collection form and the tubes and update the SMS to document the collection, and then take the zip lock to the processing laboratory.

These following sections apply to tasks performed by the laboratory technicians.

- Upon receipt in the processing laboratory, verify the sample ID on the tubes to ensure all tubes are properly labeled and accounted for from each study subject.
- Collected whole blood tubes should be **processed within six hours of collection**. If this is not possible, please store the whole blood tubes upright in a +4°C refrigerator until processing can occur.
- Each collected tube should be maintained at room temperature in an upright position.
- Document any problems with the tubes on the blood collection form, in the observations section.

4.7.1 Centrifuge EDTA Whole Blood Tubes

In order separate the whole blood into its the components, laboratory technicians will need to centrifuge the EDTA and SST tubes:

- Centrifuge each EDTA and SST whole blood tube at room temperature (18-25°C) for a minimum of 15 minutes at 900 x g (relative centrifugal force, rcf) with no brake, or otherwise specified by your equipment or institutional guidelines.
- During centrifugation label the cryovials for the appropriate amount of whole blood tubes.
- Following centrifugation, carefully handle the separated whole blood tubes to prevent the plasma from remixing with the packed cells. Maintain the tubes in an upright position at room temperature.

4.7.2 Label Cryovials

Accurate labeling of the aliquots is critical to linking assay data to the correct study participant. To maximize time:

- Affix the corresponding specimen ID labels to 2 mL cryovials for the storage of plasma, packed cells, and serum while the whole blood tubes are in the centrifuge (see **Appendices 7, 8 and 9**).
- For the Tempus tube at Follow Up visit 3, also apply the aliquot specimen ID label to the Tempus tube as indicated in **Appendix 9**.

Because the barcode is a 2D matrix, each aliquot should be wrapped around the cryovial tube, starting with the right end of the label as to not cover the 2D barcode. Labels should not be applied vertically.

4.8 Create Specimen Aliquots

Handle and process all blood specimens according to your institution's specific biospecimen safety guidelines and policies.

The procedures for creating the aliquots from the centrifuged whole blood tubes is described below for each collection type:

4.8.1 Enrollment collection

- After centrifugation, the whole blood in each:
 - EDTA tube will separate into two layers
 - 1) an upper layer that contains the plasma, and
 - 2) a lower layer that contains the packed red and white blood cells
- EDTA tubes:
 - Carefully handle all EDTA centrifuged tubes to prevent the plasma from remixing with the packed cells.
 - Place each EDTA tube in a rack with the appropriate labeled cryovials. Match each EDTA tube to its specific set of labeled cryovials. **Prior to aliquotting, verify that the sample ID (e.g., BSC 123456) number is the same on all tubes.**
 - Gently remove the stopper from the EDTA whole blood tubes. Using a pipettor carefully collect and dispense 1.0 mL of plasma into the appropriate cryovials labeled plasma. If there is more or less than 1.0 mL of plasma remaining in the tube, please aliquot all remaining volume into the third aliquot (0003 or 0013).
 - After all the plasma is aliquoted, use a vortex mixer at slow speed or tap the tube against your finger, to re-suspend the packed cells and ensure a homogenous cell concentration. Dispense 1.0 mL of packed cells into each cryovial that is labeled PRWBC. If there is more or less than 1.0 mL of PRWBC remaining in the EDTA tube, please aliquot all remaining volume into the third aliquot (0006 or 0016) and change the volume (defaulted to 1.0 mL) in the SMS to reflect the actual volume stored.
 - Repeat the above steps for all EDTA tubes.
- Assign the aliquots into their respective freezer boxes using the SMS (see **Appendix 5** for details).
- All aliquot cryovials should be stored in freezer specimen boxes at -80°C mechanical freezers.

4.8.2 Follow Up 2 Collection

- After centrifugation, the whole blood in each:
 - EDTA tube will separate into two layers
 - 1) an upper layer that contains the plasma, and
 - 2) a lower layer that contains the packed red and white blood cells
- EDTA tubes:
 - Carefully handle all EDTA centrifuged tubes to prevent the plasma from remixing with the packed cells.
 - Place each EDTA tube in a rack with the appropriate labeled cryovials. Match each EDTA tube to its specific set of labeled cryovials. **Prior to aliquotting, verify that the sample ID (e.g., BSC 123456) number is the same on all tubes.**
 - Gently remove the stopper from the EDTA whole blood tubes. Using a pipettor carefully collect and dispense 1.0 mL of plasma into the appropriate cryovials labeled plasma. If there is more or less than 1.0 mL of plasma remaining in the tube, please aliquot all remaining volume into the second aliquot (0002).
 - After all of the plasma is aliquoted, use a vortex mixer at slow speed or tap the tube against your finger, to re-suspend the packed cells and ensure a homogenous cell concentration. Dispense 1.0 mL of packed cells into each cryovial that is labeled PRWBC. If there is more or less than 1.0 mL of PRWBC remaining in the EDTA tube, please aliquot all remaining volume into the second aliquot (0004) and change the volume (defaulted to 1.0 mL) in the SMS to reflect the actual volume stored.
- Tempus tube:
 - Place the Tempus Tube into a metal rack and place the rack into a -20°C mechanical freezer. Keep the tube in the freezer for at least 24 hours.
 - Every Friday, remove the Tempus tubes that have been stored for at least 24 hours, and assign the tube to its respective freezer box using the SMS (see **Appendix 5a** for details).
- Assign the aliquots into their respective freezer boxes using the SMS (see **Appendix 5a** for details).
- All aliquot cryovials should be stored in freezer specimen boxes at -80°C mechanical freezers.

4.8.3 Follow Up 3 Collection

- After centrifugation, the whole blood in each:
 - EDTA tube will separate into two layers
 - 1) an upper layer that contains the plasma, and
 - 2) a lower layer that contains the packed red and white blood cells
 - SST (serum) tube will separate into two layers
 - 1) an upper layer that contains the serum, and
 - 2) a lower layer that contains the clot and other components

- EDTA tube
 - Carefully handle all EDTA centrifuged tubes to prevent the plasma from remixing with the packed cells.
 - Place each EDTA tube in a rack with the appropriate labeled cryovials. Match each EDTA tube to its specific set of labeled cryovials. **Prior to aliquotting, verify that the sample ID (e.g., BSC 123456) number is the same on all tubes.**
 - Gently remove the stopper from the EDTA whole blood tubes. Using a pipettor carefully collect and dispense 1.0 mL of plasma into the appropriate cryovials labeled plasma (0001). If there is more or less than 1.0 mL of plasma remaining in the tube, please aliquot all remaining volume into the second aliquot (0002).
 - After all of the plasma is aliquoted, use a vortex mixer at slow speed or tap the tube against your finger, to re-suspend the packed cells and ensure a homogenous cell concentration. Dispense 1.0 mL of packed cells into each cryovial that is labeled PRWBC (0003). If there is more or less than 1.0 mL of PRWBC remaining in the EDTA tube, please aliquot all remaining volume into the second aliquot (0004) and change the volume (defaulted to 1.0 mL) in the SMS to reflect the actual volume stored.
- Serum tube:
 - Gently remove the stopper from the SST (serum) whole blood tube. Using a pipettor carefully collect and dispense 1.0 mL of serum into the appropriate cryovials labeled serum (0031, 0032). If there is more than 1.0 mL but less than 1.8 mL of serum remaining in the tube, please aliquot all remaining volume into the third aliquot (0033). **DO NOT STORE THE CLOT/REMAINING MATERIALS IN THE SST TUBE.**
- Tempus tube:
 - Place the Tempus Tube into a metal rack and place the rack into a -20°C mechanical freezer. Keep the tube in the freezer for at least 24 hours.
 - Every Friday, remove the Tempus tubes that have been stored for at least 24 hours, and assign the tube to its respective freezer box using the SMS (see **Appendix 5a** for details).
- Assign the aliquots into their respective freezer boxes using the SMS (see **Appendix 5a** for details).
- All aliquot cryovials should be stored in freezer specimen boxes at -80°C mechanical freezers.

4.9 Freezer Box Labels

Aliquots will be stored in freezer boxes once they are created. To manage the many boxes that are filled, each box will have a unique ID assigned to it. The labels are printed in a duplicate label set (see **Figure 9**) that will include a 2D barcode and readable text that identifies the box for storage. One label (top) should be affixed to the lid of the box and the second label (bottom) to the base of the box (see **Figure 10**).



All 2 mL cryovials should be stored in standard 2” specimen boxes with a 9 x 9 insert (See **Figure 11**). The 9 mL Tempus tubes should be stored in 3” specimen boxes using a 7 x 7 insert.

4.10 Specimen Data Management

The REDS-III SCD study performed will use the web-based SMS to track and record all aliquots for each EDTA blood tube. Details on this system are found in **Appendix 5**.