1. Title: REDS-III South Africa Transfusion in Pregnancy (TIP) Study

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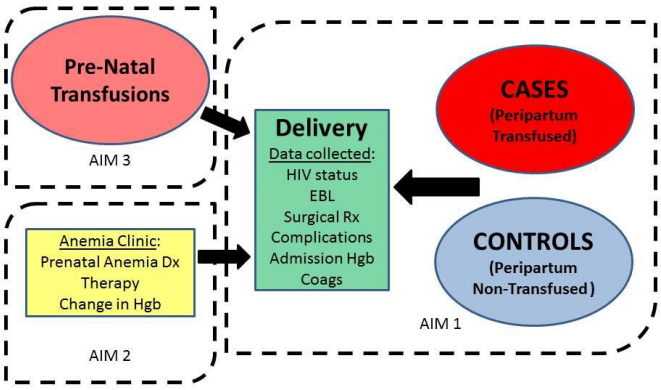
2. Concept Synopsis and Study Schema

Both globally and in South Africa, obstetric hemorrhage (OH) adversely impacts maternal health, and is still the foremost contributor to obstetric mortality[1-3]. Both lack of early recognition of associated risk factors for recurrent OH as well as failure to provide effective peripartum care contribute to maternal morbidity and mortality[2]. Consequently, both OH and high rates of blood transfusion prescribed for OH are primarily encountered in resource poor countries; this is the case evident in South Africa[4,5]. While data exists for OH and maternal mortality, there is little detailing of blood use in this population and little research on transfusion practice, whether blood is administered appropriately or whether associated with untoward effect.

Preliminary data from our recently completed REDS-III obstetric hemorrhage (OH) pilot study have indicated that although the risk of OH is not significantly increased in HIV positive versus HIV-negative women (OR = 1.12, 95% CI 0.88-1.43 p=0.3460), there is a significantly increased risk of transfusion in HIV positive women as compared to their HIV negative counterparts controlling for age, parity, mode of delivery, and hospital (for transfusion, OR = 1.54, 95% CI 1.24-1.91 p<0.0001). This unexpected finding has public health significance due to the high prevalence (25%-30%) of HIV in South African pregnant women. While the rates of OH (~2.5%) approximated those reported in high resource settings, the frequency of blood transfusion (2.8%) appears significantly higher in South Africa.



In order to improve our understanding of the relationship between HIV, pregnancy, anemia, OH and bleeding and their contribution to risk of receiving a transfusion, we plan to conduct a 3-tiered study (see schematic below) ***(Aim 1) to determine the risk factors for allogeneic blood transfusion among a sample of peripartum South African women, (Aim 2) to characterize antenatal anemia in both HIV positive and HIV negative patients, and (Aim 3) to evaluate patients who receive antenatal transfusion (>48hrs prior to delivery).*** Please see appendix I for detailed flowchart.

Aim 1: In order to test our hypothesis that HIV positive patients are indeed more likely to be transfused, even after controlling for parity and advanced maternal age, we propose to conduct a case control study of 1200 cases (women who are transfused in the peripartum period) and 2400 controls (non-transfused peripartum women) using medical chart review, administration of a clinical questionnaire and laboratory investigation to determine the predictors of transfusion risk. We will also capture data on management, outcomes and comorbid disease. Our postulated hypotheses for increased risk of transfusion among HIV positive patients include (i) a higher

incidence of antenatal anemia, (ii) HIV related coagulopathy and/or (iii) variability in institutional and physician transfusion practice for HIV positive patients.

Aim 2: We hypothesize that HIV infection is associated with a higher prevalence of anemia of chronic disease and a blunted response to oral iron therapy for iron deficiency anemia, which could explain the higher incidence of transfusion among HIV positive women. Therefore we will evaluate - using a cross-sectional study design (with minimal prospective follow-up of patients with iron deficiency anemia) - all patients who are newly referred to the specialist antenatal clinics (AAC) at Chris-Hani Baragwanath Hospital (CHB) using medical chart review, administration of a clinical questionnaire and laboratory investigation. Specifically, we will perform a standard panel of tests on all anemic patients to complete evaluation of their underlying anemia. From discussions with our collaborators at CHB, we anticipate that an enrollment of 500 patients is feasible.

Aim 3: Finally, we recognize that a subset of patients is transfused in the antenatal period (i.e. > 48 hours prior to delivery) and was not captured by our REDS-III OH pilot study or previous studies. We plan to evaluate all patients who fall into this category i.e. are transfused prior to the peripartum period, again using a cross-sectional study design (medical record review).

We expect to attain target enrollment within 2 years for all 3 study aims (21 months for Aim 2 and 24 months for Aims 1 and 3). Aims 1 and 3 will be conducted at all 4 clinical sites used in the OH pilot study (CHB, King Edward VIII Hospital, Mowbray Maternity Hospital and Groote Schuur Hospital), while aim 2 will be confined to CHB only.

There are several compelling reasons for conducting this study. (1) First, our proposed Phase 2 study would both expand as well as validate the salient findings from the pilot OH study that HIV is indeed associated with increased risk of transfusion. (2) A more complete understanding of the mechanisms underpinning risk of transfusion is imperative: if proven to be a function of antecedent anemia, this would motivate for more intensive measures to identify and treat patients in the antenatal period. (3) The management of antenatal anemia, especially in HIV positive patients in a resource-constrained setting lacks for comprehensive laboratory investigation. Our study may help to provide contemporary insight into the spectrum of diagnoses being encountered, thereby informing clinical management and guidelines. (4) Our aim to evaluate antenatal transfusion practice is novel, and complements our other study aims. Through identification of patients who escape detection through extant referral methods, our study could help to define risk factors for antenatal transfusion, again allowing for early identification and management of high-risk patients. (5) Finally, we plan to collect limited data on babies of HIV positive mothers. Although underpowered, this would begin to examine the relationship between anemia, OH, and perinatal maternal to child transmission of HIV. If enhanced by anemia and OH, this would lend further support to early management of high-risk patients.

3. Table of Contents

4. Protocol

4.1 Background and Significance: All 3 Aims

4.1.1 Background:

Both globally and within South Africa, OH adversely impacts maternal health, and is still the foremost contributor to obstetric mortality[1-3]. Both lack of early recognition of associated risk factors for recurrent OH, e.g., history of postpartum hemorrhage or retained placenta, high parity, polyhydramnios, etc.,[3] as well as failure to provide effective peripartum care, e.g., active management of the third stage of labor, contribute to maternal morbidity and mortality[2]. Consequently, both OH and high rates of blood transfusion prescribed for OH are primarily encountered in resource poor countries; this is the case evident in South Africa[4,5].

While data exists for OH and maternal mortality, there is little detailing of blood use in this population. In a recent review of the contribution of lack of transfusion to maternal mortality[1], only two articles from South Africa[6,7] were cited, neither of which was within the past decade nor -specifically- related to blood utilization. Although both the International Confederation of Midwives (ICM) and the International Federation of Gynecology and Obstetrics (FIGO) recommend that there be "blood transfusion facilities in all centers that provide comprehensive health care (secondary and tertiary levels of care)"[2], there has not been consideration toward transfusion practice, whether blood transfusion is administered appropriately or whether associated with untoward effect. This has been echoed through communication with clinical collaborators in South Africa.

Preliminary data from our recently completed REDS-III obstetric hemorrhage (OH) pilot study have indicated that although the risk of OH is not significantly increased in HIV positive versus HIV-negative women (OR = 1.12, 95% CI 0.88-1.43), there is a significantly increased risk of transfusion in HIV positive women as compared to their HIV negative counterparts controlling for age, parity, mode of delivery, and hospital (for transfusion, OR = 1.54, 95% CI 1.24-1.91). This unexpected finding has public health significance given the high prevalence (25%-30%) of HIV in the obstetric population in South Africa. The OH pilot study surveyed 15,744 peripartum women who were admitted to 4 major obstetric units in South Africa over a four-month period. We found that 2.5% of all patients surveyed sustained OH and 2.8% received transfusion. These two patient subsets overlap substantially, but women may have OH without transfusion or vice versa.

While rates of OH (reported as postpartum hemorrhage [PPH]) in high resource settings are similar to that encountered in South Africa the frequency of blood transfusion is significantly higher in South Africa. For example, PPH increased in the US from 2.3-2.9%[8] from 1994-2006 and from 4.1-5% in Canada[9] ; these figures are similar to the 2.5% of OH observed in our pilot study. Despite concern over increased use of blood transfusion in selected high resource settings (notably the US)[10], the data suggest that blood transfusion is still a rare event in these settings[11]. From 1998 to 2005 blood transfusion increased from 0.24% to 0.46% in the US[12]; this is still five- to ten-fold lower as compared to the incidence of 2.8% observed in our study in South

Africa. One hypothesis is that this stems from high rates of antenatal anemia in HIV positive patients, leading to lower admission hemoglobin and an increased probability of transfusion, per our pilot data (see **Figures 1 and 2**). We also postulate that HIV related coagulopathy and variability in transfusion practice may contribute to the high rates of transfusion. Liberal transfusion practice invites two problems: first, up to 30% of pregnant women are HIV positive in South Africa[13,14] where there is little appreciation for the clinical or biological ramifications of blood transfusion in this vulnerable population. Second, blood is a limited resource. This is highlighted across Africa where only 40% of transfusion demand is currently being met.[15]

Figure 2. Distribution of admission hemoglobin values by HIV status—Pr (>F) <0.001

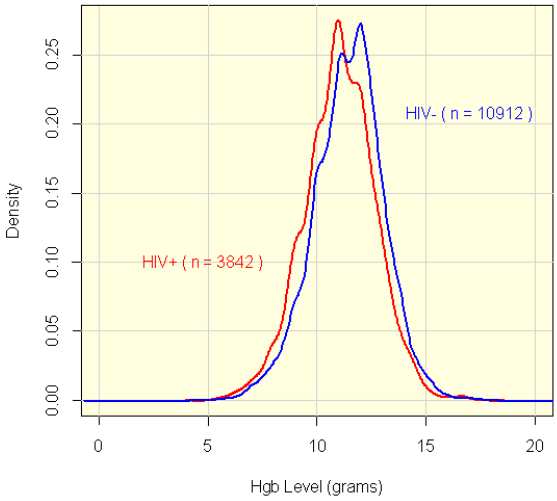
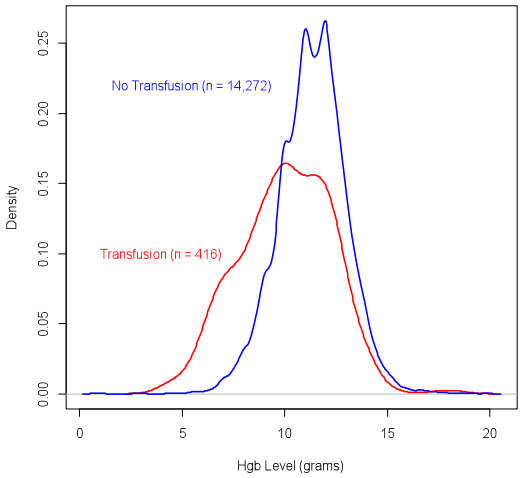


Figure 1. Distribution of admission hemoglobin values by transfusion status—Pr (>F) <0.001



The relationship between OH, transfusion, anemia and pregnancy, is complex and ever more challenging in the setting of HIV. Anemia, specifically, has a multitude of causes in the setting of HIV.[16,17] HIV has a direct, adverse effect on bone marrow stromal cells; allied with aberrant cytokine production (e.g. tumor necrosis factor and interleukin-6) and immune dysregulation. Renal deficiency, which is common in HIV, also affects erythropoietin production, and potentiates bleeding risk through uremic platelet dysfunction, thereby contributing to anemia. Furthermore, although not definitively proven, it is postulated that HIV may affect erythropoiesis directly through infection of hematologic progenitor stem cells. Indeed, the mechanism of hematologic failure in HIV remains an ongoing and unresolved research interest. The adverse effect of HIV on hematopoiesis is not confined to red cell production and other cytopenias –particularly thrombocytopenia- are well described in HIV.

Secondary causes of anemia in the HIV positive patient are extensive and include nutritional deficiencies (e.g. iron, folate and vitamin B12), infections (both opportunistic e.g. TB and mycobacteria other than TB, as well as conventional pathogens e.g. parvovirus B19), malignancies (e.g. Hodgkins and non-Hodgkins lymphomas), and drugs (including selected antiretroviral agents). [16,17] The causes of anemia are not mutually exclusive thereby introducing compound risk, accounting for the high prevalence of anemia in HIV.

Independent of HIV status, anemia in pregnancy is common and includes both physiological effect as well as pathological mechanisms. Physiological anemia occurs as a result of the relative increase in the plasma volume (50%) vs. red cell mass (25%) in pregnancy.[18,19] This hemodilutional effect is maximal during the second trimester, accounting for the need to interpret hemoglobin values, cautiously, in conjunction with gestational age. By 26-28 weeks gestation the hemoglobin may decrease up to 2g/dL as compared to pre-pregnancy levels.[18]

Nutritional anemia –specifically iron deficiency- is also frequently encountered in pregnancy[20], particularly in resource constrained settings. The demand for iron increases from 0.8 mg/day in early- to 7.5 mg/day in late pregnancy. While gastrointestinal absorption increases with gestational age, dietary intake is insufficient in 20% of pregnancies to prevent iron deficiency anemia in the absence of supplementation.[19] Evaluation of iron deficiency in pregnancy may be challenging. Both hemoglobin and ferritin (a marker of hepatic iron stores) can prove unreliable where used alone given the hemodilutional effects of pregnancy. Ferritin is also an acute phase reactant that increases non-specifically in inflammation and/or infection, further limiting it's utility in pregnancy. The use of Transferrin receptors (TfR) may address some of the limitations of other markers. TfR are expressed on the surface of erythrocytes and increase during iron deficiency, while soluble transferrin receptors (sTfR) are TfR that are shed into the circulation. sTfR are proportionate to TfR on the erythrocytes and are influenced less by gestational changes and inflammation, rendering them a more sensitive and reliable index of iron deficiency than ferritin, hemoglobin or MCV alone[21].

In order to improve our understanding of the tenuous relationship between HIV, pregnancy, anemia, OH, and bleeding risk, all of which are expected to contribute to risk of transfusion, we plan to conduct a 3-tiered study that includes the antenatal anemia clinic, the antenatal transfusion setting and, the peripartum period. These will afford an opportunity for a global overview of transfusion in pregnancy. Specifically, our Phase 2 study is seeking ***(Aim 1) to determine the risk factors for peripartum allogeneic blood transfusion among a sample of South African women with high HIV prevalence; (Aim 2) to characterize antenatal anemia in both HIV positive and negative patients; and (Aim 3) to evaluate patients who receive antenatal transfusion.*** Although the study is primarily focused on blood transfusion in pregnancy, we will also collect data on OH under Aims 1 and 3 given the strong association between OH and risk of transfusion (OH Pilot Study OR=94.38, 95%CI 71.16-125.19).

4.1.2 Significance:

There are several compelling reasons for conducting this study. First, despite the high morbidity and mortality from OH, there is a paucity of data pertaining to blood use in this setting. Our preceding OH pilot study began to characterize transfusion practice in the obstetric population through a focus on the peripartum period where the majority of OH is noted to occur. Our proposed Phase 2 study would both expand as well as validate the salient findings from that study. Specifically, it would test our hypothesis that HIV does indeed confer an independent risk of transfusion. The postulated reasons that might

contribute to this risk include antenatal anemia, coagulopathy, and/or variability in institutional/ physician practice with respect to management of HIV positive patients.

A complete understanding of the mechanisms underpinning an increased risk of transfusion is imperative: if proven to be a function of antecedent anemia, this would motivate for more intensive measures to identify and treat patients in the antenatal period. Similarly, an improved understanding of coagulopathy in this setting would inform transfusion guidelines in OH. While recognized as a more challenging undertaking, the determination that there is a lower threshold for transfusion among physicians when confronted with HIV positive patients would support a broad educational initiative regarding evidence based transfusion practice. Blood transfusion is not without risk to patients and - particularly in Africa - is a limited resource.

Second, improved management of antenatal anemia in a resource-constrained, yet high HIV prevalence, setting of South Africa could reduce the downstream need for red blood cell transfusion. The preliminary findings from our pilot study support the hypothesis that the observed high rates of blood transfusion in peripartum obstetric patients stem from reasons independent of OH alone and antenatal anemia is the most likely contributing factor.

Our study may help to provide contemporary insight into the spectrum of diagnoses being encountered, as well as offering information on laboratory tests that demonstrate the greatest diagnostic yield given limited available resources. This study therefore impacts clinical management and guidelines, directly. Furthermore, we postulate that the high prevalence of anemia in both HIV positive and negative patients in the obstetric population impact on the downstream risk of OH and transfusion. Anemia, through altered rheological effects adversely affects hemostasis. Therefore, should patients later develop OH, they are more likely to need transfusion. The lower starting hemoglobin renders the patient less likely to tolerate blood loss and reduced blood viscosity with adverse impact on rheology confers a propensity toward bleeding. We anticipate that anemia is prevalent in this population despite the high penetrance of antiretroviral drugs (ARVs) (~80%). We recognize that the mechanism of anemia and hematological failure in HIV, despite use of ARVs, is a major research focus that could exceed the scope of this study. However, through characterization of antenatal anemia in HIV positive patients (with capture of the ARV treatment regimens), we will begin to identify some of the contributing factors. We will also investigate – specifically- the response to iron therapy in HIV positive patients.

Third, our aim to evaluate antenatal (>48 hours pre-delivery) transfusion practice is novel and complements our other study aims. Specifically, it would serve to identify patients who escape detection through extant referral methods. While a proportion of these patients will require transfusion from anatomical bleeding (e.g. placenta previa, trauma etc.), a subset is expected to have unaddressed antenatal anemia. Our study could help to define risk factors for antenatal transfusion, again allowing for early identification and management of high-risk patients.

Finally, we plan to collect limited data on babies of HIV positive mothers. Although the study is not powered to test this hypothesis, it will begin to examine the relationship between anemia, peripartum transfusion and vertical transmission of HIV. If transmission is shown to be enhanced by anemia and/or transfusion, this would lend further support to early management of high-risk patients.

4.2. Aim 1 Protocol

This section will address protocol activities for Aim 1.

4.2.1 Objectives

4.2.1.1 Primary

Specific Aim 1: To determine the risk factors for peripartum allogeneic blood transfusion among a sample of South African women.

Hypothesis: Transfused patients in the peripartum period are more likely to be HIV positive. The association between transfusion risk and HIV status remains even after controlling for parity, mode of delivery, and advanced maternal age.

4.2.1.2 Secondary

Secondary analyses will determine risk factors for peripartum transfusion other than HIV. We expect reasons for increased risk of transfusion include OH, antenatal anemia, coagulopathy, and/or variability in institutional and physician practice.

4.2.2 Study Design

We will conduct a case-control study of women who are transfused during the peripartum period as cases, and a sample of women who are not transfused during the peripartum period as controls.

4.2.3. Study Population or Specimens for Analyses

The obstetrical services at the four sites serve low-income women (predominantly Black-African and Colored*) from Johannesburg (Soweto), Durban and Cape Town (two hospitals). The four sites have major second-tier or tertiary obstetric services and participated in the recently completed REDS-III OH pilot study. The patients do reflect a generally urban, high HIV prevalence population in South Africa. This affords broad representation both by population as well as obstetric pathology, with management of uncomplicated deliveries as well as complex referrals from primary-level hospitals and mobile obstetric units.

*Colored in South Africa denotes a specific mixed-race population group.

4.2.3.1 Inclusion Criteria

All peripartum obstetric patients with an index hospitalization at Chris-Hani Baragwanath Hospital [CHB] (Johannesburg), King Edward VIII Hospital [KEH] (Durban), Mowbray Maternity Hospital [MMH] and Groote Schuur Hospital [GSH](both Cape Town) during the enrollment period.

a) Transfused Peripartum Women (cases; n=1200): Women who are transfused (any allogeneic red cells, platelets or plasma) in the peripartum period (as per definitions 4.2.6.2). Although red cells represent the overwhelming majority of transfusions, for completeness we shall also collect data on women transfused with only platelets or plasma. Peripartum for the purposes of the study is defined as being within 48hrs of delivery (pre-, during or post). Under Aim 1, we will only collect data on women who are at least 26 weeks gestation; delivery in obstetric patients less than 24 to 26 weeks is managed by gynecology as abortion/miscarriage, e.g., complete-, incomplete abortion, etc. Antepartum transfusion data will be captured under Aim 3.

Data collection will include live births as well as stillborns and early neonatal deaths. Both normal vaginal delivery (NVD) and births by cesarean section (C/S) will similarly be included.

(b) Non-transfused Deliveries (controls; n=2400): Women who are admitted during the peripartum period, are at least 26 weeks gestation and are not transfused during the peripartum period (within 48hrs of delivery)

4.2.3.2 Exclusion Criteria

- Patients <18yrs old: any minor requires parental/guardian consent for participation in research in South Africa. Given the sensitivity surrounding capture of obstetric and HIV-related data, we have elected to exclude minors. From the pilot data, minors account for ~2.5% of patients who sustain OH and/or are transfused.
  Subjects that are unable or unwilling to provide informed consent to participate in the study
- Patients whose peripartum period falls outside the index hospitalization;
- Patients who are either transferred or discharged before consent and enrollment

4.2.4. Study Enrollment or Specimen Procurement

4.2.4.1 Screening/Recruitment/Specimen Acquisition

The methods of enrollment are derived from those used successfully in the REDS-III OH pilot study. (See Appendices I &J for flowcharts detailing study overview, and the screening/enrollment process). Research personnel will identify eligible subjects in the case group with peripartum transfusion, as follows:

Crosschecking with the transfusion service at each hospital for blood units issued to the obstetric service: The requesting ward/destination is documented on the blood requisition. Blood Bank staff will flag any transfusion requisition originating in the antenatal, labor and delivery wards as well as any obstetric clinic; these flagged requisitions will be compiled in a daily list. Research personnel will review requisitions on a daily basis to facilitate early identification of these cases. This was found to be the most successful means of identifying transfused patients in the OH pilot study. Prior to study initiation, the hospital blood bank staff will be educated about the study and the need to alert designated research personnel of all requisitions either originating from or destined to the obstetric wards.

Daily review of ward logs: The research nurse will conduct daily review of the ward admission logs, maternity, and delivery registers each morning to identify which patients have sustained transfusion. The admissions log contains an admitting diagnosis that can allude to complications that predispose to transfusion. An HIV/Prevention of mother-to-child transmission (PMTCT) register is also available for HIV data that may not be documented in the medical records.

Daily conversations with nursing and house-staff: This provides another mechanism for identification of patients who have been transfused. Our collaborators are all senior consultants in the obstetric services at their respective hospitals. Therefore, they are able to identify patients through bi-daily clinical ward rounds conducted through the antenatal, post-natal, and labor wards, in addition to the obstetric surgical service. Joint academic ward rounds also allows for identification of patients not directly under or collaborators' care. In addition, our collaborators supervise both obstetric registrars (cf. residents) and house-staff (senior house officers, interns, and junior consultants) facilitating communication. The nursing staff is another valuable resource, particularly for communicating patients with less transfusion that are otherwise managed without senior input. A large proportion of routine deliveries are conducted by nursing midwives and senior medical students.

Before initiating the study we plan to conduct a series of training seminars at the four clinical sites to familiarize the blood bank, obstetric-nursing, and medical staff with the intended study methods, the importance of their cooperation, and the significance of the potential findings.

Enrollment of the cases:
Following transfusion and when the patient is deemed sufficiently stable to provide consent, the research nurse will approach the patient to obtain full informed consent and conduct the study activities.

Selection of the Control group: A comparison group of peripartum patients who have not been transfused (controls) will be selected from among all non-transfused deliveries and enrolled concurrently with the transfused group. The research personnel will communicate frequently with the blood bank: prior to enrollment of controls, the research nurse will first confirm with the blood bank and ward staff that the patient has not been transfused in the peripartum period. Similarly, if a control is later transfused, the patient will then be converted

into a case. Although this is anticipated to be rare event, direct communication between the blood bank and research nurse avoids the potential for double enrolment of patients as both controls and later as cases.

Controls will be enrolled at a ratio of two controls to each case (see section 4.2.9.2). Using pilot data, we will enroll controls prospectively based on the expected number of cases at each of the clinical sites, with adjustment of the sampling fraction on a monthly basis to account for actual case enrollment. Therefore, a prescribed number of controls will be enrolled on a daily/weekly basis using probability sampling of all deliveries within each site.

4.2.4.2 Stratification or Randomization (if applicable)

Not applicable

4.2.5 Interventions

Not applicable- observational study only

4.2.6. Measurement

4.2.6.1 Schedule of Measurement

The enrollment period will proceed over a **24-month** period beginning in 2013 (see timeline). Based on preliminary data from the recently completed OH pilot study we anticipate the monthly deliveries to be 1800, 650, 375 and 1035 at CHB, KEH, GSH and MMH respectively. From the pilot data, we anticipate that the number of subjects per month that are transfused at the respective hospitals (CHB, KEH, GSH and MMH) to be 48, 20, 22 and 21 (total ~111 cases per month [1332 potential cases per year]). The study management system will also record the denominator of all births per week at each hospital.

Data acquisition will take place at the time of enrollment only, and will comprise (1) medical record review and data abstraction (2) administration of a clinical questionnaire, (3) laboratory measurement and (4) Follow-up determination of HIV status in Babies of HIV positive mothers. Given the cross-sectional design of Aim 1, all activities will occur only once. With the exception of laboratory investigation on cases, all activities will only proceed after informed consent has been obtained. The only data to be gathered after enrollment will be the HIV status of the babies of HIV positive mothers, which will be delayed by 6-8 weeks.

4.2.6.2 Definitions

Peripartum: For the purposes of the study, “peripartum” is defined as 48 hours prior, during or post-labor/delivery”

Antenatal: For the purposes of the study, “antenatal” or “prenatal” (used interchangeably) is defined as any stage of pregnancy prior to 48 hours of delivery (i.e. before the peripartum period)

Delivery: For the purposes of the study, delivery is confined to parturition at a minimum of 6 months (26w) gestation.

Obstetric Hemorrhage (OH): Peripartum hemorrhage as defined by the WHO as >500 mL blood loss for vaginal delivery or >1000 mL blood loss for caesarian section. We acknowledge that this definition may be restrictive; this has been echoed in recent discussion in the obstetric literature [22] (*see pitfalls section for further considerations surrounding use of this definition*)

Transfused: Having received any transfusion during the prescribed timeframe with any allogeneic blood product e.g. Red cells, platelets or plasma

Anemia (in pregnancy): hemoglobin less than 11g/dL in the first and third trimester and <10.5g/dL in the 2nd trimester

Booking: Presentation for antenatal care. Patients who are "unbooked" have not received antenatal care and are consequently at high risk for obstetric complications.

4.2.6.3 Assessment and Measurement Procedures

Research Personnel

The research personnel that will identify and enroll eligible subjects will be dedicated obstetric nurses employed by the study. Given the projected numbers of eligible subjects we plan to distribute the research nursing staff unequally between the hospitals (proportionate to the projected work-load). The same research personnel will be tasked with activities under all 3 study aims. The study activities (assessment and measurement procedures) comprise (1) medical record review and data abstraction (2) administration of a clinical questionnaire, (3) laboratory measurement and (4) follow-up determination of HIV status in Babies of HIV positive mothers

1. Medical Record Review and Data Abstraction

The research personnel will review the patient's medical record to abstract data that will be used to test our hypotheses under Aim 1. The data will be captured on the newly designed Peripartum Research Form (PRF). The PRF is a paper-based Teleform instrument allowing ease of data capture as well as automated data entry into the research database thereby minimizing transcription errors. The PRF is a modified version of the Obstetric Hemorrhage Audit Tool (OHAT); the latter was successfully piloted in the OH fast-track study and has been modified for the Phase II study to address deficiencies identified during the pilot study. The same PRF form will be used for both cases and controls.

The content for the PRF was developed in consultation with our SANBS and obstetric collaborators in South Africa, allowing capture of information, which is useful for assessing clinical practice as suggested by leading obstetric consultants working in South Africa. Specifically, the PRF has been designed to evaluate risk of peripartum blood transfusion with

particular attention to antecedent anemia, obstetric hemorrhage, HIV and/or the contribution of variability in physician practice to transfusion rates. The PRF contains sections on demographic information, hospital data, pregnancy and delivery (both current as well as past obstetric history), HIV status and treatment, obstetric hemorrhage and transfusion.

The research nurse will use all available resources (e.g. medical records, admissions log, delivery register and blood bank requisitions) to complete the PRF. The medical records provide the most complete documentation of clinical events; however other resources may be needed to complete the forms, particularly where the data of interest are not readily available from the medical charts alone. For example, the Direct Antiglobulin Test (DAT) may be obtained directly from the blood bank and HIV data may be aggregated centrally in a treatment log.

All PRF forms will be stored in a secure location in the hospital until transferred to SANBS; eventually, the de-identified data will be stored in a secure database at BSRI in San Francisco and RTI in Rockville. RTI will assist with training of the research personnel so as to ensure uniformity in application of the PRF.

2. Administration of a Clinical Questionnaire:

Following informed consent, the research nurse will administer a brief clinical questionnaire to the patient (limited interview), which is intended to capture information that is not expected to be readily available from the medical charts alone, yet would prove useful to test our hypotheses. The questions include a bleeding history to ascertain a potential role of coagulopathy for bleeding and consequent transfusion risk, compliance with medications, use of traditional medicines, previous transfusion etc. The questionnaire will be conducted in the patient's language of preference and is expected to take approximately 10 minutes to complete.

3. Laboratory Investigation

Laboratory measurement procedures will differ between cases and controls:

Cases: With respect to cases, we plan to obtain a waiver such that additional tubes of blood will be drawn on peripartum patients prior to transfusion (and consent). This is important because a number of our laboratory measures would be adversely affected if blood were to be drawn post-transfusion. The consent process will only be conducted after the patient is deemed sufficiently stable to provide informed consent.

Prior to transfusion, the ordering physician will collect the additional tubes of blood (not to exceed 40mL) using pre-assembled study packs that have been prepared by the Laboratory Services. These will be routed to the blood bank for storage pending informed consent.

If the patient later agrees to participate in the study, the tubes will be relayed to the laboratory for testing; if the patient declines to participate in the study, the tubes will be safely disposed.

In the event that blood has not been collected from Cases prior to transfusion, the research nurse will draw blood samples following consent/enrollment. Collection of bloods after transfusion is not optimal given the probable effect on some of the laboratory indices that are being evaluated in the study. However, we recognize that a proportion of patients may not have blood drawn prior to transfusion given clinical acuity and/or logistical challenges (high clinical burden with limited house staff). The timing of the blood collection in relation to the transfusion will be recorded so that data analysis can account for pre- versus post-transfusion phlebotomy. Bloods on Aim 1 cases that are drawn at time of enrollment/consent will be restricted to FBC and HIV indices (CD4 and viral load). Similar to pre-transfusion evaluation, HIV indices will only be collected in patients who are known to be HIV positive. Importantly, coagulation studies will not be collected on Cases who lack pre-transfusion bloods.

Controls: With respect to controls, it is unlikely that blood will have been obtained as part of routine care. Therefore, non-transfused patients who agree to participate in the study (to serve as controls) will undergo dedicated phlebotomy for the study. This will be explained in the informed consent process. An additional EDTA [purple top] tube will be collected on controls for determination of the FBC.

Test Panels:
The panel of tests that are routinely available as component of standard clinical management, as well as those tests proposed under REDS-III Aim 1 to meet the study objectives are detailed in **Figure 3** below. On a subset of enrolled subject (n=400), a portion of the blood specimens will be processed into PBMC and plasma aliquots and saved in a US-based repository to support future study of hematologic and obstetric diseases. Examples include studies of hepcidin and studies of residual HIV (proviral DNA and cell associated RNA). Testing of repository samples will be contingent on availability of funding and will be performed in the United States.

Figure 3. Routine Testing and Study Panels under Aim 1

	HIV Status	Routine Tests			Study Panel	
CASES	Pos		No.	Test performed	Tube type	Voume Required (mL)
		FBC	1	PI, PTT, D-dimers	Citrate (blue top)	4.5
		Pre-Transfusion Crossmatch	2	vWF Ag*†	Citrate (blue top)	4.5
			3	ABO group, DAT†	EDTA (purple top)	3
			4	CD4	EDTA (purple top)	3
			5	Viral load	EDTA (purple top)	3
			6	PBMC**§	CPT	4.5
			7	PBMC**§	CPT	4.5
					Approximate Total Volume	**27**
	Neg	FBC				
		Pre-Transfusion Crossmatch	1	PI, PTT, D-dimers	Citrate (blue top)	4.5
			2	vWF Ag*†	Citrate (blue top)	4.5
			3	ABO group, DAT†	EDTA (purple top)	3
			4	CD4	EDTA (purple top)	3
			5	Viral Load	EDTA (purple top)	3
			6	PBMC**§	CPT	4.5
			7	PBMC**§	CPT	4.5
					Approximate Total Volume	**27**
	HIV Status	**Routine Tests**			**Study Panel**	
CONTROLS	**Pos**	None	**No.**	**Test performed**	**Tube type**	**Voume Required (mL)**
			1	PI, PTT, D-dimers	Citrate (blue top)	4.5
			2	vWF Ag*†	Citrate (blue top)	4.5
			3	FBC, ABO group, DAT†	EDTA (purple top)	3
			4	CD4	EDTA (purple top)	3
			5	Viral load	EDTA (purple top)	3
			6	PBMC**§	CPT	4.5
			7	PBMC**§	CPT	4.5
					Approximate Total Volume	**27**
	Neg	None				
			1	PI, PTT, D-dimers	Citrate (blue top)	4.5
			2	vWF Ag*†	Citrate (blue top)	4.5
			3	FBC, ABO group, DAT†	EDTA (purple top)	3
			4	PBMC**§	CPT	4.5
			5	PBMC**§	CPT	4.5
					Approximate Total Volume	**21**

Abbreviations

PT: Prothrombin Time

APTT: Activated Thromboplastin Time

vWF: von Willebrand factor antigen

FBC: Full Blood Count (≈CBC)

DAT: Direct Antiglobulin Test

*Limited number of subjects (50 subjects from each group to a total of n=200)

**Limited number of subjects (100 per group to a total of n=400)

† Testing for vWF Ag and ABO will be confined to patients at CHB only

§ collection of repository samples will be confined to CHB and KEH only

Tests that are routinely available prior to transfusion

The following are expected to be available on the majority of patients who are transfused; however, in the setting of uncontrolled OH, a subset of patients will receive un-crossmatched blood (emergency Group O Rh negative blood), pending formal type and screening.

(i) Pre-transfusion crossmatch: This will include determination of ABO and Rh.
(ii)Full Blood Count (FBC~CBC); The pilot study demonstrated that a FBC is not always readily available. Therefore, we shall perform a FBC on the pre-transfusion crossmatch sample should a dedicated FBC not be available. The pre-transfusion FBC will be used to evaluate transfusion thresholds (“triggers”) both across institutions as well as by cadre of transfusing physician.

Study Panel under Aim 1

The following tests will help to evaluate the contribution of coagulopathy, anemia and variability in transfusion triggers to the high rates of transfusion observed in the pilot study.

(i) PT and APTT: useful for evaluation of extrinsic (PT), intrinsic (APTT) and common coagulation pathways (both PT and APTT), in addition to liver biosynthetic function (PT).

(ii) D-Dimers: provides evidence of fibrinolysis and useful indicator of disseminated intravascular coagulation (DIC)
(iii) vWF (CHB only): This is useful to evaluate for von Willebrand's disease, the commonest primary coagulopathy. Although the study does not include a comprehensive evaluation of von Willebrand's disease, we will conduct limited screening of this coagulopathy. In addition, repository samples may be used for ancillary studies of vWD, if additional funding permits.
(iv) ABO (CHB only)*: this is necessary for interpretation of vWF
(v) FBC*
(vi) DAT: a proportion of HIV positive patients are reportedly DAT positive. While this is not thought to correlate with clinical hemolysis, it is deemed worthwhile to evaluate in this population
(vii) CD4 (HIV patients only)
(viii) Viral Load (HIV patients only)

*if unavailable through routine testing

Collection of a Sample for Testing and Repository Storage in the USA
Under Aim 1, we plan to collect dedicated samples (plasma and peripheral blood mononuclear cells [PBMCs]) on a limited number of subjects (n=400), which will be exported to the REDS-III Central Laboratory in the United States for indefinite storage in a peripartum transfusion repository. We will collect sufficient samples to represent each of the subject groups by HIV status (positive vs. negative) and study designation (case vs. control). This will ensure that there are 100 samples in each group (total n=400). These samples will be collected using a convenience sampling approach (first 100 subjects consecutively enrolled in each category) and will be confined to two of the hospitals (CHB and KEH) for logistical reasons. Informed consent will cover the collection and of these samples. Although the large majority of protocol testing will be done in South Africa, the repository will allow for downstream special testing available only in the United States. For example, contingent upon later funding, repository samples could be used to evaluate the contribution of low level residual HIV to development of anemia and/or bleeding risk, particularly HIV positive patients on ART. We may also evaluate hepcidin (a hormone that controls iron absorption) in patients with iron deficiency anemia. Notably, we will not perform genetic testing on the repository samples.

While we have budgeted for collection, processing and storage of the exported samples; testing will be deferred until funding is available.

Communication of test results: Abnormal, clinically relevant test results will be communicated back to the patient's attending physician and/or clinical care team. The laboratory service that will perform the laboratory testing for the study also performs routine clinical testing in the selected study hospitals.

4. Follow-up determination of HIV status in Babies of HIV positive mothers:

HIV status, using PCR, is routinely ascertained in South Africa on all babies of known HIV positive mothers at 6-8 weeks post delivery. While the study is not specifically powered to do so, recording the baby's HIV status will enable an preliminary evaluation of the effect of anemia, blood transfusion and/or obstetric hemorrhage on perinatal maternal to child transmission of HIV. Maternal consent will cover our requesting the baby's test result; we will also capture data on whether the mother has breastfed the baby to address potential confounding i.e. viral transmission via breast milk. At time of enrollment, the baby's laboratory number and clinic will be documented so as to facilitate the follow-up process. The research nurse will contact either the laboratory or pediatric clinic to obtain this follow-up result.

4.2.6.4 Specimen collection procedures

In Aim 1, phlebotomy and specimen collection in cases will occur prior to obtaining full informed consent. Testing, however, will be contingent upon full informed consent obtained after the patient has been transfused and deemed sufficiently stable to interact with the research nurse (this is expected to be within 12-36hrs of transfusion). A description of the tubes is included in figure 3. In the event that blood samples are not collected from Cases prior to transfusion, phlebotomy and specimen collection will occur following consent/enrollment. Aim 1 cases who have blood drawn at time of enrollment/consent, will only have FBC and HIV indices (viral load and CD4) collected (coagulation studies will be omitted given the effect of transfusion on coagulation). Like Cases who are drawn prior to transfusion, HIV indices will only be drawn on patients who are known to be HIV positive. In addition to the clinical tests, we will collect Cell Preparation Tubes with Sodium Citrate (CPT) for PBMC processing. The CPT tubes will be used for repository storage and testing that is unavailable in South Africa. The total volume to be collected from each patient under Aims 1 will not exceed 40mL.

We will only collect and perform protocol laboratory testing on a 50% sample of controls in Aim 1, allowing a comparison of 1200 cases and 1200 controls. This decision has been deemed adequate to meet our secondary aims while minimizing the cost of laboratory testing. Study staff will be instructed to draw blood samples from every other control participant.

4.2.6.5 Special test procedures

Coagulation studies (INR, APTT and vWF) need to be processed soon after collection; therefore we plan to isolate and freeze the plasma component from the citrate tube (coagulation) samples on receipt at the blood bank. Separation and freezing will prevent the degradation of clotting factors and ensure reliability of the results. Testing, however, will only proceed following consent and enrollment. Research blood samples from Cases can be sent to the laboratory directly after consent/enrollment given that coagulation studies will not be included in the panel.

We plan to collect a dedicated sample (PBMCs and plasma) for repository storage in view of the potential for downstream study. These samples will be kept at room temperature while couriered to SANBS laboratories. The SANBS staff will then perform PBMC separation and aliquot PBMCs and plasma into freezer tubes for long-term storage according to protocols overseen by the REDS-III central laboratory. Samples will be shipped from SANBS to BSRI periodically throughout the study. The testing of repository samples will be contingent on acquisition of

additional funding. PBMC samples will not be collected on Cases who have not had pre-transfusion bloods collected.

4.2.7. Survey Considerations and OMB Requirements

Under administration of the clinical questionnaire, selected questions will be posed directly to the patient. Those questions will address clinical history that cannot be otherwise obtained from the medical records e.g. bleeding history, anemia symptoms and compliance with medication. We plan to apply for clinical exemption from OMB approval for this limited patient contact.

4.2.8. Data Management

Data Handling will be conducted as follows: the completed forms (PRF and clinical questionnaires) will be stored in a secure location at each hospital pending transfer to SANBS. Safe transfer will accompany a designated secure courier from the respective hospitals on a weekly basis to SANBS in Johannesburg. A data-entry clerk will be employed at SANBS, Johannesburg to perform aggregation and scanning of the completed forms following receipt at SANBS. They will also perform primary quality control on the data. The transcribed data will be transmitted thereafter to RTI and BSRI pending analysis. The original paper forms will be archived securely at SANBS headquarters in Johannesburg. This method was successful in the pilot study.

4.2.9. Statistical Considerations

4.2.9.1 Hypothesized outcome rate and smallest difference to detect w/high statistical power

From the Phase 1 OHAT data, the adjusted odds of being HIV positive is about 1.4 times higher in the women who were transfused compared to the women who were not transfused.

4.2.9.2 Sample size and power

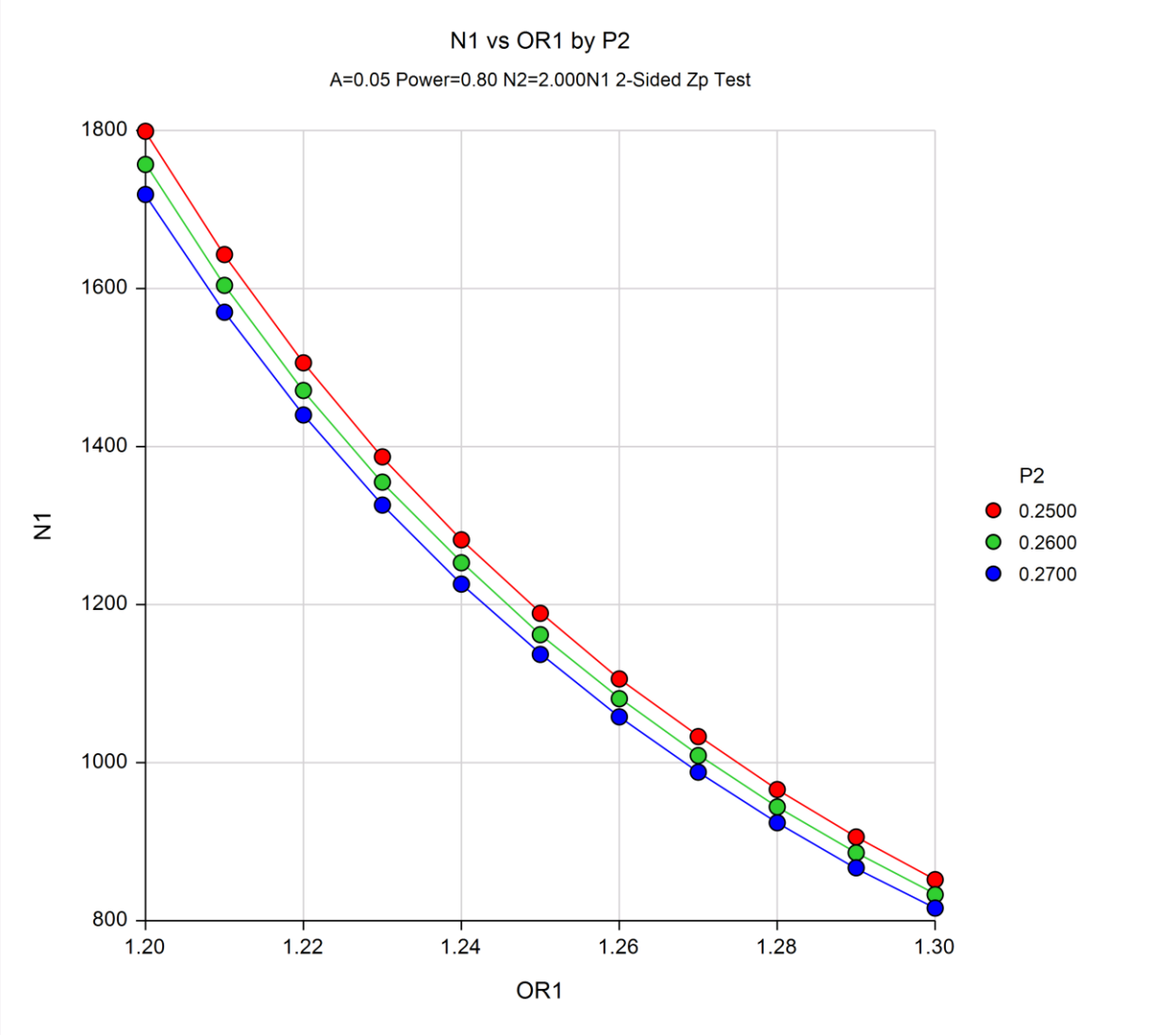
Note that all the calculations are based on alpha equal to 0.05, power equal to 0.8, and a two-sided test. The citation for the software used for these power calculations is Hintze, J. (2011). *PASS 11*. NCSS, LLC. Kaysville, Utah, USA. www.ncss.com.

Complications and Transfusion

For the comparison of the proportion of HIV positive women between those women who are transfused and those who are not transfused and based on the Phase 1 OHAT data, the proportion of women that were HIV positive in the transfused group was **0.35**, and the proportion of women that were HIV positive in the non-transfused group was **0.26**. We will use the 0.26 proportion for women there were HIV positive in the non-transfused group as a starting point for the power calculations. In a case control study where there are two women that were not transfused (controls) for each woman that was transfused (cases), we will need **1,189** cases and **2,378** controls (total number of women is 3,567) to achieve 80 percent power to detect an odds ratio of **1.25**. That is, the estimated odds of being HIV positive are **1.25** times higher in the

transfused group compared to non-transfused group. Under the alternative hypothesis, the HIV prevalence is assumed to be **0.29** in the transfused group and **0.26** in the non-transfused group. The test statistic used is the two-sided Z test with pooled variance. The significance level of the test was targeted at 0.05. Also, because the odds ratio treats the transfusion status and HIV status symmetrically, the estimated odds of being transfused are **1.25** times higher in the HIV positive group compared to the HIV negative group. Figure 1 shows the relationship between the odds ratio, sample size, and proportion of HIV positive women in the non-transfused group and is for a study that has two non-transfused women for each transfused woman. The odds ratio is on the horizontal axis; the sample size of transfused cases is on the vertical axis; and three curves that correspond to the proportion of HIV positive women in the non-transfused group on plotted. The proportions are 0.25 for the red line, 0.26 for the green line, and 0.27 for the blue line.

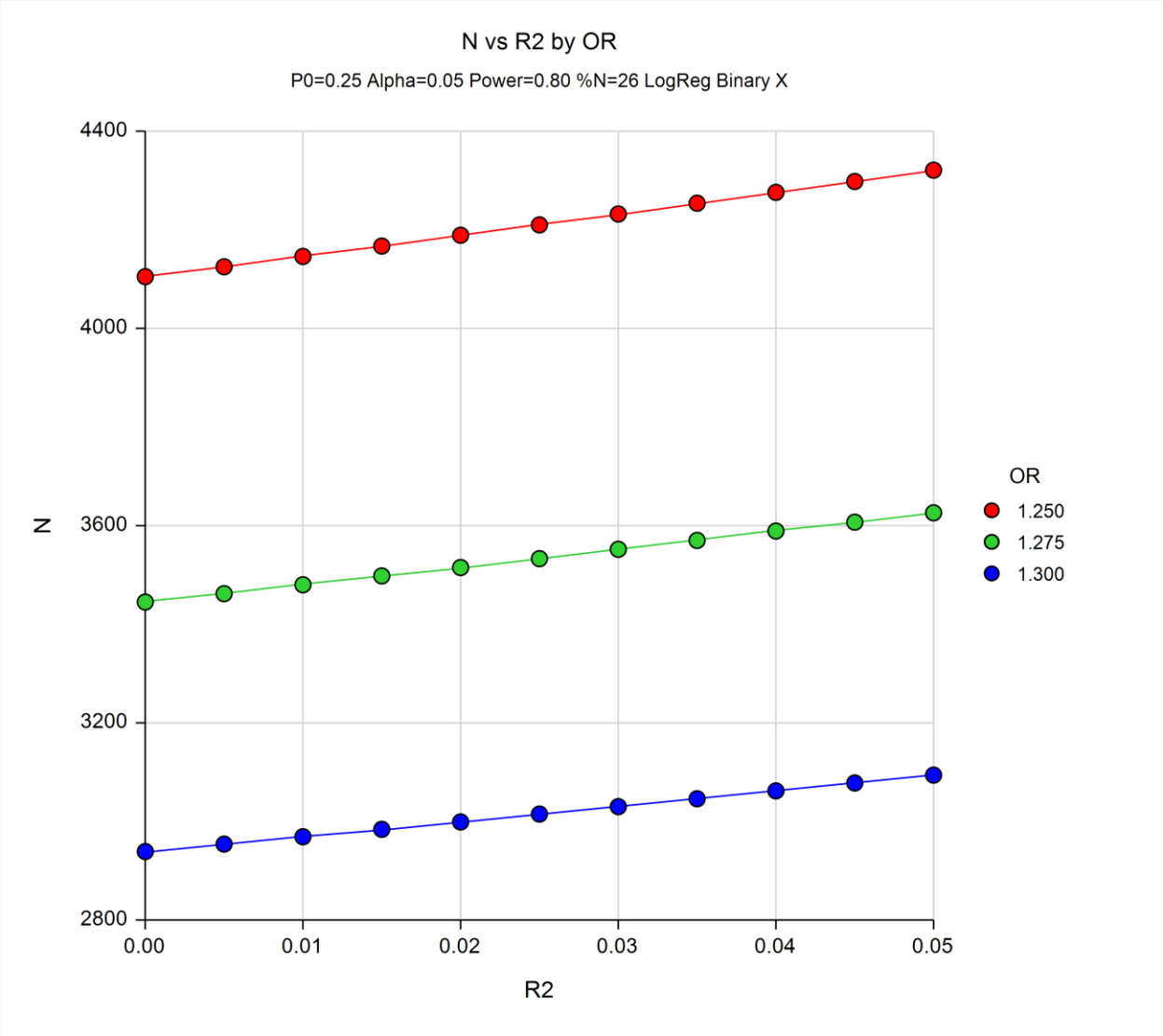
Figure 4a. Sample Sizes for the Comparison of HIV Prevalence between Transfused and Non Transfused Women



Sample Sizes for Logistic Model Regressing Transfusion

For the logistic regression model regressing transfusion status (transfused and non-transfused) on HIV status (HIV+ and HIV-) and other predictor variables, a sample size of 3,607 observations (of which 66% are in the HIV negative group and 33% are in the HIV positive group) achieves 80% power at a 0.05 significance level to detect a change in probability of transfusion from the baseline value of 0.25 to 0.30. This change corresponds to an odds ratio of 1.275. An adjustment was made since a multiple regression of the HIV status on obstetric hemorrhage, parity, mode of delivery, and advanced maternal age in the logistic regression obtained an R-Squared of 0.05. **Figure 4b** shows the linear relationship between R-squared and sample size. The horizontal axis is the R-squared value, and the vertical axis is the sample size. The line plotted in the figure represent the different odds ratios. The red line is for an odds ratio of 1.250; the green line is for an odds ratio of 1.275; and, the blue line is for an odds ratio of 1.300. For an odds ratio line, as the R-squared for the relationship between HIV status and the other covariates increases, the sample size increases.

Figure 4b. Relationship Between R-squared and Sample Size for Logistic Regression Model



4.2.9.3 Assessment and Measurement Procedures

We will use the PRF to capture data on the following domains: (i) patient demographics, (ii) current admission [“hospital”], (iii) pregnancy and delivery, (iv) HIV status and treatment, (v) obstetric hemorrhage and clinical complications (vi) transfusion (vii) coagulopathy (viii) transfusion thresholds, (ix) baby HIV status and maternal laboratory results.

4.2.9.4 Analytic Approach (primary, secondary and subgroup analyses)

The primary analysis will be a comparison of HIV status between women who were transfused (cases) and women who were not transfused (controls) using a chi-squared test. Secondary analysis will compare other potential associations with transfusion, namely obstetric hemorrhage, admission hemoglobin, coagulation laboratory tests and physician of record between cases and

controls. Obstetric hemorrhage is expected to be a major predictor of transfusion, but we have demonstrated in our pilot study that HIV status and admission hemoglobin were independent predictors after controlling for obstetric hemorrhage. Also, we will examine differences in antenatal anemia, bleeding risk (as determined by data capture using the PRF, supported by laboratory indices that could predict bleeding e.g. PT, platelets and hemoglobin), and variability in transfusion practice by institution and physician. The appropriate chi-squared test or two-sample t-test will be used to assess the associations. Finally, in order to control for potential predictor variables, analyses will regress, using logistic regression, transfusion status on HIV status, obstetric hemorrhage, demographics, parity, admitting hospital, and any other relevant variables identified in the bivariate analysis. Interaction terms will be examined to determine their significance. Significant interaction terms will be included in the final model. Model diagnostics will be reviewed to ensure the adequacy of model fit.

4.2.9.5 Pitfalls, Bias, Confounding, Limitations and Alternative Approaches

Pitfalls: We anticipate similar logistical difficulties to those encountered in the pilot study e.g. workflow, communication between the obstetric staff and research personnel with early identification of eligible patients, variability in record keeping/ missing data etc.

The definitions are inherently variable. For example, while we plan to use a standard definition of OH we recognize that the estimation of blood loss is notoriously inaccurate and non-reproducible. Aim 1 also uses a definition of peripartum that is restricted to deliveries after 26 weeks. While antenatal bleeding will escape detection through aim 1, we will be able to capture this in aim 3.

Identification and enrollment of all patients who are transfused
Preliminary audit data from the OH pilot study indicate that we were reasonably successful in capturing the majority of patients who were transfused in the peripartum period. However, given limited staffing over a two-year enrollment period, this may pose a challenge.

Collection of research blood samples prior to transfusion
Reliance on the clinical house staff for collection of research blood samples prior to transfusion is challenging. In the absence of compensation, this may be met with variable compliance, given a high patient caseload and clinical acuity, coupled with logical challenges imposed by a low resource setting. Therefore, while collection of bloods prior to transfusion is the preferred approach, we have allowed for a contingency mechanism to collect research bloods after transfusion. Although collection at time of enrollment risks changes to the indices that will be evaluated, with the exception of coagulation studies (which will be omitted), this is preferable to the sampling bias that would result if compliance for pre-transfusion collection is moderate or even low. Data analysis will take account of the time of collection in relation to transfusion enabling some quantification of the transfusion effect.

In parallel, we will still encourage -the preferred- collection of blood prior to transfusion and will not inform the house-staff of a contingency approach that could prompt reliance on deferred collection.

Selection bias: We have selected the same four major obstetric transfusion services employed in the pilot study. The clinical sites (with the exception of MMH and GSH) are geographically removed from each other and serve diverse populations, affording broad representation. Provision of services to a predominantly urban population potentially limits generalizability to transfusion and obstetric practice in rural settings. Similar to the pilot study, it would be logistically impossible to conduct this Phase 2 in remote settings. There is also a bias toward a lower socioeconomic group in view of the population served by these hospitals; this is, however, the population of foremost interest.

HIV seroconversion in pregnancy: given the high background prevalence of HIV in South Africa, rates of HIV seroconversion during pregnancy may be as high as 5-8%. This has the potential for misclassification of patients should the patient's HIV status be based on a single antenatal test result. However, current obstetric guidelines recommend testing at booking, 32 weeks gestation and during labor. We plan to capture all available HIV test results on the patient to ensure accuracy of the patient's HIV classification.

4.3. Aim 2 Protocol

This section will address protocol activities for Aim 2.

4.3.1 Objectives

4.3.1.1 Primary

Specific Aim 2a: To investigate the causes of antenatal anemia in both HIV positive and negative patients who are referred to the antenatal clinic (AAC) at Chris Hani-Baragwanath (CHB) Hospital.

Hypothesis: Compared to HIV negatives, HIV positive women will have an increased prevalence of anemia of chronic disease but similar prevalence of anemia due to iron and B12 deficiency.

4.3.1.2 Secondary

Specific Aim 2b: To determine whether there is a difference in the response to oral iron therapy (in those patients who have iron deficiency anemia) between HIV positive and HIV negative patients (after controlling for treatment regimens)

Hypothesis: HIV positive women with iron deficiency anemia will have a poorer hemoglobin response to oral iron therapy as compared to HIV negative women with iron deficiency anemia.

4.3.2 Study Design

Specific aim 2a: We will perform a cross-sectional evaluation of patients who are referred to the antenatal clinic at CHB during the enrolment period using, medical chart review and data abstraction, administration of clinical questionnaires and laboratory investigation to assess etiology for underlying anemia.

Specific aim 2b: We will conduct a limited prospective cohort study of women with documented iron deficiency anemia to determine whether a standard iron therapy regimen results in a different response in HIV positive iron deficient pregnant patients from contemporaneous controls (HIV negative iron deficient, pregnant patients). The patients enrolled under Aim 2b are a subset of those enrolled under Aim 2a.

4.3.3. Study Population or Specimens for Analyses

The obstetrical services at Chris Hani-Baragwanath (CHB) Hospital serves low-income women (predominantly Black-African and Colored*) from Johannesburg (Soweto). The patients reflect a generally urban, high HIV prevalence population in South Africa.

While all four sites will participate in the study activities outlined in Aims 1 and 3, only CHB, because of its unique antepartum anemia clinic, will participate in Aim 2.

*Colored in South Africa denotes a specific, mixed-race population group.

4.3.3.1 Inclusion Criteria

Aim 2(a) Patients who are referred to the specialist antenatal anemia clinic (AAC) at CHB during the study enrollment period.

Patients who are referred to the clinic will have already met at least one of the hemoglobin or clinical criteria for referral to the antenatal anemia clinic. The hemoglobin and clinical criteria are listed in the table below:

Inclusion criteria for Aim 2a participants:

Hemoglobin level	• <8g/dl: any stage of pregnancy (there is no minimal gestational age for referral to the AAC)
	• <10g/dl: if the patient's gestational age is >36 weeks
	• <9g/dl: if HIV positive, prior to initiation of ARVs
	• Decrease in hemoglobin (particularly if on Fe supplementation and/or antiretroviral therapy e.g. 11.8, 10 ,9.5, 9.2g/dL on sequential visits to the peripheral clinic)
	• < 10g/dl: all patients who are not responding to oral iron therapy
Clinical criteria	• Signs of severe anemia (CCF [congestive cardiac failure], dyspnea, etc.)
	• Specific hematological conditions predisposing to anemia such as sickle cell disease, thalassemia, ITP etc.

Aim 2(b): Patients who are enrolled under Aim 2a, are determined to have iron deficiency anemia (see definitions) based on clinical and laboratory evaluation, and are managed accordingly with iron-supplementation (oral and/or intravenous iron therapy). Many women arrive at the anemia clinic taking iron supplements prescribed by their primary care physicians, but in most cases of confirmed iron deficiency this therapy is deemed inadequate and is intensified by the anemia clinic. Previous iron therapy will be treated as a potential confounder in our analysis (see below).

4.3.3.2 Exclusion Criteria

- Patients <18yrs old: any minor requires parental/guardian consent for participation in research in South Africa. Given the sensitivity surrounding capture of obstetric and HIV-related data, we have elected to exclude minors.
- Subjects that are unable or unwilling to provide informed consent to participate in the study
- Patients who do not meet criteria for referral to the antenatal specialist anemia clinic as outlined in the inclusion criteria

4.3.4. Study Enrollment or Specimen Procurement

4.3.4.1 Screening/Recruitment/Specimen Acquisition

Patients who are newly referred to the antenatal anemia clinic will be approached regarding enrollment at first presentation to the clinic. The study will be explained to the potential subjects and written informed consent will be obtained.

4.3.4.2 Stratification or Randomization (if applicable)

Not applicable

4.3.5 Interventions

In the antenatal anemia clinic, the standard intervention for iron deficiency anemia is as follows:

Ferrous sulfate 325 mg (65 mg elemental iron) TID
Ascorbic acid 100 mg TID
Folic acid 5 mg QD

This will serve as our predictor variable for Aim 2b, and deviations from standard practice will be recorded. However, the intervention is chosen on a clinical basis and not modified for research purposes.

4.3.6. Measurement

4.3.6.1 Schedule of Measurement

Aim 2 will begin 3 months after Aims 1 and 3 to allow for acclimatization to the study activities and will proceed for 21 months, which includes both enrollment (to a minimum target of 500 subjects) and limited follow-up (aim 2b).

Dr. Jenny Hull, our clinical collaborator at CHB and head of the antenatal anemia clinic at CHB, is notified of all newly referred patients affording ease of workflow. The designated research nurse will enroll patients into the study while the patients are awaiting evaluation by Dr. Hull or her associate in the clinic. Patients typically wait several hours for the clinic physician allowing for ample time for the enrollment process, administration of the clinical questionnaire and initiation of the medical chart review. The latter will be completed after the clinic physician has evaluated the patient. The blood draw and laboratory investigation will precede the physician's assessment. Blood is routinely collected as part of the clinic visit; therefore we will leverage this opportunity to collect additional tubes of blood for the study.

Aim 2a is cross-sectional in design and measurements will be performed only on the index referral visit to the clinic.

Aim 2b will include prospective follow-up to determine if there is a difference in response to iron therapy by HIV status. The follow-up interval is determined by severity of anemia and the gestational age of the patient at time of referral (patients with advanced GA are monitored more closely). The first two AAC visits are between 1 and 2 weeks apart. Thereafter patients are

followed from weekly to monthly (interval 1-4 weeks) until time of delivery or until they meet criteria for clinical discharge (attainment of a Hemoglobin ≥ 10g/dl).

From discussion with Dr. Hull, we anticipate there being approximately 8 new patients attending the HIV positive anemia clinic per week and approximately 5 new patients attending the HIV negative anemia clinic per week. These half-day clinics are run on a Thursday and Friday for HIV negative and positive patients respectively.

Under Aim 2(b), we anticipate that 50% of patients will have iron deficiency anemia. This will enable us to meet enrollment within 1 year of enrollment. Attrition is not anticipated to be a major problem given regular clinic follow-up, component of clinical care.

The panel of tests performed specifically for the study, will be completed at first visit to the AAC. All follow-up testing (Aim 2b only) will be borne by the AAC as component of routine management, which includes monitoring patients with follow-up FBC and indices obtained on the ADVIA instrument.

4.3.6.2 Definitions

Antenatal: For the purposes of the study, “antenatal” is defined as any stage of pregnancy prior to 48 hours of delivery (i.e. before the peripartum period)

Anemia (in pregnancy): hemoglobin less than 11g/dL in the first and third trimester and <10.5g/dL in the 2nd trimester

Iron deficiency anemia: in the absence of a gold standard (bone marrow biopsy with special iron stain to assess stores), we will define iron deficiency anemia as the presence of a microcytic hypochromic anemia and a soluble transferrin receptor of ≥12mg/L with or without a ferritin < 12ng mL. Ferritin, an acute phase reactant, can be increased in reactive and disease states, artificially normalizing levels in a patient with iron deficiency anemia.

Although our definition of iron deficiency is primarily based on testing of the soluble transferrin receptor, our evaluation of iron deficiency will also include ferritin, serum iron levels, transferrin levels and the Total Iron Binding Capacity (TIBC). While we will collect these data to support the diagnosis of iron deficiency anemia, these variables are subjected to comparatively greater pregnancy-associated changes thereby rendering the soluble transferrin receptor a preferred index measure of iron deficiency for the study. We have also elected to collect these other data as they are routinely used in South Africa for the evaluation of iron deficiency.

Physiological response to iron supplementation: For the purposes of the study, an optimal response to iron supplementation is defined as a weekly gain in hemoglobin and MCV of 0.7g/dL and 3fL respectively.

Booking: Presentation for antenatal care. Patients who are “unbooked” have not received antenatal care and are consequently at high risk for obstetric complications.

4.3.6.3 Assessment and Measurement Procedures

Similar to Aim 1, activities related to measurement under Aim 2 include (1) medical chart review and data abstraction; (2) administration of a clinical questionnaire; and (3) laboratory investigation.

(1) Medical Chart Review and Data Abstraction
Medical chart review and data abstraction will begin after having obtained informed consent and completion of administration of the clinical questionnaire. Completion of the chart review and data abstraction will require review of the physician's notes from the index assessment. Therefore the medical chart review will be completed after the clinic evaluation.

The research personnel will collect the data using the newly designed Antenatal Anemia Form (AAF-see Appendix B). The AAF, a Teleform paper-based instrument, is designed to capture demographic, comorbid disease, obstetric and hematologic data in an attempt to glean etiology, antecedent risk factors and management of anemia in this population. The form also includes space for laboratory results documented at the referring clinic.

All AAF forms will be stored in a secure location in the hospital until transferred to SANBS; eventually, the de-identified data will be stored in a secure database at BSRI in San Francisco and RTI in Rockville.

Under Aim 2b, we will collect follow-up data on patients with iron deficiency anemia at each clinic visit until the patient is either discharged from the clinic or delivers her baby. The data that will be collected will include routine laboratory testing results (FBC), medication, compliance with medication, underlying diagnoses and documentation of complications. These data are routinely collected at the antenatal anemia clinic.

(2) Administration of a Clinical Questionnaire:
Following informed consent, the research nurse will administer a brief clinical questionnaire, which is intended to capture information that is not expected to be readily available from the medical charts alone, yet would prove useful to evaluate factors that may have contributed to the patient's anemia. The questions include a bleeding history to ascertain a potential role of coagulopathy for bleeding and anemia, compliance with medications, previous history of anemia and management, signs and symptoms of anemia and questions surrounding the patient's HIV disease if applicable. The questionnaire will be conducted in the patient's language of preference and is expected to take approximately 10 minutes to complete.

(3) Laboratory Investigation:
Laboratory measurements will include (a) documentation of test results from the referring clinic; (b) documentation of results from testing that is performed routinely at the AAC; (c) laboratory testing that will be conducted specifically for the purposes of the study; and (d) collection of

samples for export to the United States either for repository storage and testing that is not routinely available in South Africa. Collectively, the laboratory measurements are intended to improve characterization of anemia. We will collect specimens on all subjects.

The tests that will be performed either routinely or for the study are detailed in **Figure 5**.

Figure 5. Routine Testing and Study Panels under Aim 2

	Tube No.	Tests Performed	Tube Type	Volume required (mL)
HIV Positive	1	Se ferritin, Se iron, TIBC. Se haptoglobin, LDH, LFTs (AST. ALT bilirubin and albumin), CRP, Urea & creatinine	Clotted (gel) (yellow top)	5
	2	Soluble transferrin receptor	Clotted (gel) (yellow top)	3
	3	Red cell folate	EDTA (purple top)	3
	4	CD4	EDTA (purple top)	3
	5	Viral load	EDTA (purple top)	3
	6	PI, PTT	Citrate (blue top)	4.5
	7	PBMC	CPT	4.5
	8	PBMC	CPT	4.5
			Approximate Total Volume	26
	Tube No.	**Tests**	**Tube Type**	**Volume required (mL)**
HIV Negative	1	Se ferritin, Se iron, TIBC. Se haptoglobin, LDH, LFTs (AST. ALT bilirubin and albumin), CRP, Urea & creatinine	Clotted (gel) (yellow top)	5
	2	Soluble transferrin receptor	Clotted (gel) (yellow top)	3
	3	Red cell folate	EDTA (purple top)	3
	4	PI, PTT	Citrate (blue top)	4.5
	5	PBMC	CPT	4.5
	6	PBMC	CPT	4.5
			Approximate Total Volume	24.5

Abbreviations

PT: Prothrombin Time

APTT: Activated Thromboplastin Time

FBC: Full Blood Count (≈CBC)

DAT: Direct Antiglobulin Test

RPI: Reticulocyte Production Index

M,C& S: microscopy, culture and sensitivity

*Limited number of subjects (50 subjects from each group to a total of n=200)

**Limited number of subjects (100 per group to a total of n=400)

(a) Documentation of results from blood tests performed at peripheral clinics

Results from an FBC or Hb (HemoCue) from the referring clinic will be documented on the AAC.

However, a formal FBC from the referring clinic is not always available. While this is not critical to the study (an FBC is routinely performed on the ADVIA on initial presentation to the AAC), the absence of a formal FBC may account for inappropriate referral i.e. the Hb obtained at the peripheral clinic may not correlate with a “normal” Hb noted on presentation to the AAC.

(b) Laboratory tests that are routinely performed at the AAC

The following tests are routinely performed as part of the initial assessment at the AAC; the results will be captured for the study.

(i) FBC on the ADVIA, which include:

- Red cell indices (incl. MCH, Hb content, RBC volume, RCW etc.)
- Red cell morphology
- Reticulocyte count
- RPI

- Reticulocyte hemoglobin content (CHr)

(ii) Serum B12 on first visit
(iii) Urine dipstix, microscopy, culture and sensitivity: the urine testing may help to evaluate the patient for hemolysis (presence of urobilinogen and hemoglobinuria on dipstix; red blood cells on microscopy), renal insufficiency, infection and asymptomatic bacteruria, all of which may contribute the patient's anemia.

(c) Laboratory testing for the study:
In addition to documentation of test result from routine testing, we also plan to collect tubes of blood specifically for the purposes of the study. The clinic nurse will collect the blood at time of the routine blood draw after evaluation by the physician.

(i) Iron studies: serum ferritin, soluble transferrin receptor, serum iron and TIBC
(ii) Red cell folate
(iii) C-reactive protein (CRP)
(iv) Prothrombin time (PT): in addition evaluation of the common and extrinsic coagulation pathways, PT provides a good index of liver biosynthetic function
(v) Indices of hemolysis (haptoglobin, LDH): these will be supported by other indices tested e.g. bilirubin, urine dipstix and microscopy
(vi) Renal function tests (urea and creatinine): renal insufficiency is known to contribute to anemia in HIV disease
(vii) Liver function tests (AST/ALT, bilirubin and albumin): liver dysfunction will contribute to coagulopathy and anemia through multiple mechanisms e.g. impaired production of clotting factors, decreased absorption of vitamin B12 etc.
(viii) CD4 (HIV only)
(ix) Viral Load (HIV only)

(d) Collection of a Sample for Testing and Repository Storage in the USA
Similar to Aim 1, we plan to collect dedicated samples for export to the United States for storage in an antenatal anemia repository. We will collect samples on a limited number of subjects that are representative of both HIV positive and HIV negative patients (n=100 from each group). Consent will allow for downstream study related to anemia and HIV in the obstetric population. As stated under Aim 1, the repository samples afford an invaluable opportunity to address a host of novel research questions. Some examples of potential use include an evaluation of the contribution of (i) hepcidin levels in relation to HIV-associated anemia; (ii) low level residual HIV to development of anemia and hematopoietic failure and (iii) viral co-infection to development of anemia in HIV; the latter includes conventional -yet untested- agents e.g. parvovirus B19. Notably, we will not perform genetic testing on the repository samples.

While we have budgeted for collection, processing and storage of the repository samples; actual testing will be deferred until funding is available.

Communication of test results:

Similar to Aim 1 abnormal test results (non-exported samples) will be shared directly with the clinic staff and may benefit the patient's management. Those results will be available on the hospital laboratory system at CHB that is routinely used by the clinic staff.

4.3.6.4 Specimen collection procedures

The clinic nurse will collect additional tubes of blood during routine phlebotomy performed at the initial referral visit. The additional tubes of blood will be used to support the study objectives and will be used to characterize the patients' underlying anemia and HIV disease (if HIV positive). The panel will also include CPT tubes for PBMC processing. The CPT tubes will yield PBMCs and plasma both for repository storage as well as testing that is unavailable in South Africa e.g. hepcidin levels. The total volume to be collected from each patient will not exceed 40mL.

4.3.6.5 Special test procedures

We plan to collect a dedicated sample (PBMCs and plasma) for repository storage in view of the potential for downstream study. These samples will be processed separately, subjected to centrifugation shortly after collection with aliquoting and freezing. The samples will be centrifuged on-site at laboratories either on site or in close proximity to the hospitals. The frozen samples will be batch couriered to SANBS headquarters and for long-term storage and ultimately shipped to the United States for further testing. Samples will be shipped from SANBS to BSRI periodically throughout the study.

4.3.7. Survey Considerations and OMB Requirements

Selected questions will be posed directly to the patient (administration of a clinical questionnaire). Those questions will address clinical history that cannot be otherwise obtained from the medical records e.g. bleeding history, anemia symptoms and compliance with medication. We plan to apply for an OMB clinical exemption to allow for this limited patient contact.

4.3.8. Data Management

Data Handling will be conducted as follows: the completed forms (AAF and clinical questionnaires) will be stored in a secure location at each hospital pending transfer to SANBS. Safe transfer will accompany a designated secure courier from the respective hospitals on a weekly basis to SANBS in Johannesburg. A data-entry clerk will be employed at SANBS, Johannesburg to perform aggregation and scanning of the completed forms following receipt at SANBS. They will also perform primary quality control on the data. The transcribed data will be transmitted thereafter to RTI and BSRI pending analysis. The original paper forms will be archived securely at SANBS headquarters in Johannesburg. This method was successful in the pilot study.

4.3.9. Statistical Considerations

4.3.9.1 Hypothesized outcome rate and smallest difference to detect w/high statistical power

Aim 2a: In a study with equal numbers of HIV positive and negative women, a group sample sizes of 258 for the HIV negative women and 258 for the HIV positive women (total sample size 516) achieves 80% power to detect a prevalence of anemia of chronic disease of 24% in HIV+ versus 14% in HIV- women (an odds ratio of 1.9).

Aim 2b: Given a sample size of 220 subjects, we will be have 80% power to detect a difference of 0.4 g/dL in physiological response between HIV positive and negative iron deficient subjects.

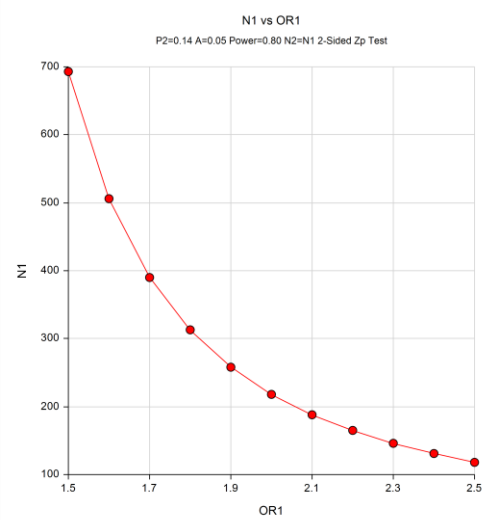
4.3.9.2 Sample size and power

Aim 2a: HIV Status and Anemia Due to Chronic Disease

In a study with equal numbers of HIV positive and negative pregnant women, group sample sizes of 258 for the HIV negative women and 258 for the HIV positive women (total sample size 516) achieves 80% power to detect an odds ratio of 1.9 for the association between HIV status and anemia of chronic disease. The proportion of HIV positive women who have anemia of chronic disease is assumed to be 0.14 under the null hypothesis and 0.24 under the alternative hypothesis. The proportion of HIV negative women who have anemia of chronic disease is assumed to be 0.14. The test statistic used is the two-sided Fisher's Exact test. The significance level of the test was targeted at 0.05.

Figure 6a shows the required sample sizes to meet the analytic conditions for equal size groups of women. The horizontal axis shows the odds ratio. The vertical axis shows the required sample size per group.

Figure 6a. Sample Sizes for Comparing Proportion of Women with Chronic Disease by HIV status.

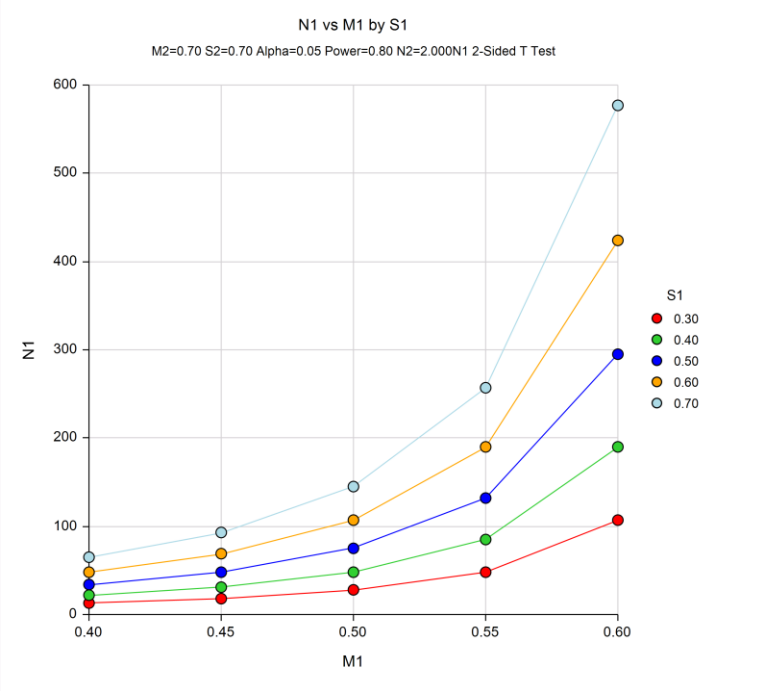


Aim 2b: HIV Status and Iron Deficiency Anemia

In a study where there are two iron deficient women who are HIV negative for each iron deficient woman that is HIV positive, we need **75** iron deficient women who are HIV positive and **150** iron deficient women who are HIV negative (total number of women **225**) to achieve 80% power to detect a difference of **-0.2** g/dL between the null hypothesis that the change both HIV negative and HIV positive group means is 0.7 g/dL and the alternative hypothesis that the mean of the HIV positive group is **0.5** g/dL with estimated HIV negative and HIV positive groups standard deviations of **0.5** and with a significance level (alpha) of 0.05 using a two-sided two-sample t-test. The standard deviation was selected to be the same size as the HIV positive group difference, a conservative estimate for the standard deviation. On average women present at the anemia clinic with a hemoglobin of 8.0 gm/dL, and HIV negatives are expected to have an increase to 0.7 g/dL on iron therapy on return visit whereas we expect HIV positives to have an increase of 0.5 g/dL.

Figure 6b shows the required sample sizes for the HIV positive women to meet the analytic conditions when there are two HIV negative women for every HIV positive woman. The horizontal axis shows mean for the HIV positive women. The vertical axis shows the required sample size for the group of HIV positive women. The light blue line is for a standard deviation of 0.7. The gold line is for a standard deviation of 0.6. The blue line is for a standard deviation of 0.5. The green line is for a standard deviation of 0.4. The red line is for a standard deviation of 0.3.

Figure 6b. Sample Sizes for Comparing Change in Hemoglobin of Women by HIV Status



4.3.9.3 Assessment and Measurement Procedures

We will use the AAF to capture data on the following domains: (i) patient demographics, (ii) current admission ["hospital"], (iii) pregnancy, (iv) HIV status and treatment, (v) transfusion (vi) coagulopathy (vii) transfusion thresholds, (viii) maternal laboratory results, (ix) antenatal anemia and management. The AAF will also capture laboratory test results for all enrolled patients as well as follow-up results under aim 2b.

4.3.9.4 Analytic Approach (primary, secondary and subgroup analyses)

Study Aim 2a

Aim 2a is a cross-sectional study comparing anemia diagnoses in HIV positive versus negative pregnant women. First, the study population will be described in terms of demographics and obstetric characteristics of the patient population presenting to the antenatal anemia clinic. The primary analysis will compare prevalence of anemia diagnoses in the HIV-positive group compared HIV-negative group. Additional analyses will include hemoglobin, complete blood count indices, other laboratory data, and obstetric data including obstetric hemorrhage.

The primary analysis will be a comparison of the proportion of women with anemia of chronic disease in HIV positive women (cases) to HIV negative women (control) using a chi-squared test. In addition, similar analysis will be conducted controlling for admission hemoglobin level, treatment regime, and compliance to the treatment regime. Secondary analysis will compare other potential associations with anemia due to chronic disease, namely admission hemoglobin, treatment regime, compliance to treatment regime, complete blood count indices, other

laboratory data between cases and controls. The appropriate chi-squared test or two-sample t-test will be used to assess the associations. Finally, in order to control for potential confounding, analyses will regress, using logistic regression, anemia due to chronic disease on HIV status, treatment regime, compliance to treatment regime, demographics, parity, and any other relevant variables identified in the bivariate analysis. Interaction terms will be examined to determine their significance. Significant interaction terms will be included in the final model. Model diagnostics will be reviewed to ensure the adequacy of model fit.

Study Aim 2b

Study aim 2b will compare the change in hemoglobin in response to iron supplementation between the HIV-positive and HIV-negative women. An average 0.5 g/dL per week increase in hemoglobin for HIV positive women compared to an average 0.7 g/dL for HIV negative women would be clinically relevant difference for this analysis. The primary analysis will be a comparison of the change in hemoglobin level at first return visit in HIV positive women to HIV negative women using a two-sample t-test. In addition, similar analysis will be conducted controlling for initial hemoglobin level, treatment regime, compliance to the treatment regime, previous iron therapy and time since good compliance with the treatment regime. Secondary analysis will compare other potential associations with hemoglobin level, namely initial hemoglobin, complete blood count indices and other laboratory data between cases and controls. The appropriate chi-squared test or two-sample t-test will be used to assess the associations. Finally, in order to control for potential confounding, analyses will regress, using multiple regression, hemoglobin level on HIV status, initial hemoglobin level, treatment regime, compliance to treatment regime, previous iron therapy, time since good compliance with treatment regime, demographics, parity and any other relevant variables identified in the bivariate analysis. Interaction terms will be examined to determine their significance. Significant interaction terms will be included in the final model. Model diagnostics will be reviewed to ensure the adequacy of model fit. Finally, we only expect to have one post-treatment measurement for each woman, but since time to return visit may be variable we shall include time as a covariate in each analysis. In the event that we have more than one measurement per woman, we will treat the multiple measurements as clustered within woman in a repeated measures analysis.

4.3.9.5 Pitfalls, Bias, Confounding, Limitations and Alternative Approaches

Pitfalls: We anticipate similar logistical difficulties to those encountered in the pilot study e.g. variability in record keeping/ missing data etc.

Enrollment and follow-up: Based on communication with Dr. Hull, we expect there to be sufficient patients to meet the enrollment target. Under Aim 2b, we expect that 50% of patients will have iron deficiency anemia. This will enable us to meet enrollment within 1 year. Attrition is not anticipated to be a major problem given regular clinic follow-up, component of clinical care.

Interpretation of results can be difficult in pregnancy and need to be compared to controls that are matched for population of study and gestational age. A low Hemoglobin level alone may be

due to the physiological anemia of pregnancy as a consequence of an increase in plasma volume relative to red cells (hemodilution). This is why the red cell indices and morphology need to be evaluated individually. This is done routinely and will be continued for the study both in the initial assessment at the AAC as well as component of the evaluation of the response to therapy.

The definition of iron deficiency anemia: We have already alluded to some of the challenges both in definition and concomitant diagnosis, particularly in pregnancy. Given the overlay of comorbid megaloblastic anemia, a microcytic anemia may appear compensated by laboratory definitions.

Selection bias: Provision of services to a predominantly urban population potentially limits generalizability to obstetric practice in rural settings. There is also a bias toward a lower socioeconomic group in view of the population served by this hospital; this is, however, the population of foremost interest.

Compliance: compliance with taking medication, particularly iron supplementation, is often poor when prescribed at peripheral clinics. Compliance of patients attending the AAC is reportedly better due to the more intensive education and follow-up. While this may limit the extent to which the findings can be generalized to a peripheral/non-specialist setting, the difference between HIV positive and negative patients should be apparent given application to the same management to both groups at the AAC.

Confounding effect of prior treatment and supplementation: All pregnant women are routinely prescribed iron supplements at the peripheral clinics (once daily 200mg Fe sulphate containing 65mg elemental iron) in the 3rd trimester as well as folic acid (5mg per day). Although the above, standard treatment is not always followed at the peripheral clinics, the majority of patients are expected to be on supplementation at time of evaluation. This will be treated as potential confounder during the data analysis phase.

Confounding by variability in treatment regimes: For example, alternative iron preparations may be prescribed during periods when Fe sulphate is unavailable. A combination tablet of 200mg ferrous fumarate and 200µg folic acid may be supplemented instead. Furthermore, patients who do not demonstrate an acceptable response to oral iron therapy or are intolerant of the treatment (e.g. due to side effects) may be considered for intravenous iron. These patients routinely have laboratory iron studies prior to initiating therapy. This source of confounding will be managed either by exclusion (rare alternative treatments) or by treating treatment as a covariate (e.g. dosage of iron).

4.4. Aim 3 Protocol

This section will address protocol activities for Aim 3.

4.4.1 Objectives

4.4.1.1 Primary

Specific Aim 3: To characterize reasons for transfusion among patients who are transfused prior to the peripartum period.

We will conduct a cross-sectional record abstraction of women who are transfused prior to delivery. This descriptive study has no hypothesis as we expect antenatal transfusion to be an infrequent event.

4.4.1.2 Secondary

None.

4.4.2 Study Design

We will perform a cross-sectional, descriptive evaluation of patients who are transfused prior to delivery.

4.4.3. Study Population or Specimens for Analyses

We will survey the same population described under Aim 1. The obstetrical services at the four sites serve low-income women (predominantly Black-African and Colored*) from Johannesburg (Soweto), Durban and Cape Town (two hospitals). The four sites have major second-tier or tertiary obstetric services and participated in the recently completed REDS-III OH study. The patients do reflect a generally urban, high HIV prevalence population in South Africa. This affords broad representation both by population as well as obstetric pathology, with management of uncomplicated deliveries as well as complex referrals from primary-level hospitals and mobile obstetric units.

*Colored in South Africa denotes a specific, mixed-race population group.

4.4.3.1 Inclusion Criteria

All pregnant women who are transfused at Chris-Hani Baragwanath Hospital [CHB] (Johannesburg), King Edward VIII Hospital [KEH] (Durban), Mowbray Maternity Hospital [MMH] and Groote Schuur Hospital [GSH](both Cape Town) during the enrollment period. We shall include both: i) women with viable pregnancies (> 26 weeks gestation) who are transfused > 48 hours prior to delivery; and ii) pregnant women who are transfused before 26 weeks independent of whether the transfusion is associated with delivery of a non-viable pregnancy such as ectopic pregnancy and spontaneous abortion

A subset of patients who are transfused at CHB may have attended the antenatal anemia clinic (under Aim 2) either as a consequence of evaluation at the AAC or by virtue of admission to CHB via a different mechanism with co-incidental prior attendance at the AAC. However, most patients who will be transfused in the antenatal period are expected to bypass the clinic entirely. We plan to be broadly inclusive of all women who receive transfusion during the antenatal period and enrollment into more than one Aim is permitted

4.4.3.2 Exclusion Criteria

- Patients <18 yrs old: any minor requires parental/guardian consent for participation in research in South Africa. Given the sensitivity surrounding capture of obstetric and HIV-related data, we have elected to exclude minors.
- Subjects that are unable or unwilling to provide informed consent to participate in the study
- Patients with viable pregnancies (≥ 26 weeks gestation) who are transfused within 48hrs of delivery (will be enrolled in Aim 1).

4.4.4. Study Enrollment or Specimen Procurement

4.4.4.1 Screening/Recruitment/Specimen Acquisition

Screening and recruitment includes and extends approaches successfully employed in the OH pilot study (albeit that the latter was focused on peripartum OH and/or transfusion). In contrast to both the OH pilot study and Phase 2 Aim 1, a large proportion of patients in Aim 3 may be transfused in gynecological wards given that obstetric complications prior to viability (~24-26weeks) are managed by gynecology. This would include ectopic pregnancies and diagnoses in the abortion spectrum, e.g., threatened abortion, complete/incomplete abortion, etc.

Communication from Blood Bank
Blood Bank staff will flag any transfusion requisition originating in the antenatal ward or obstetric clinic; these flagged requisitions will be compiled in a daily list. Research personnel will review requisitions on a daily basis to facilitate early identification of these cases. This was found to be the most successful means of identifying transfused patients in the OH pilot study.

Communication with the Clinical Nursing and Medical House staff
Research personnel will consult the obstetric and blood bank staff so as to identify patients who were transfused in the obstetric and gynecology wards. Prior to study initiation, the obstetric medical and nursing house staff will be educated as to the intent of and significance of the research. We will ask that clinical staff notify research personnel of any patient that undergoes transfusion in the antenatal period.

4.4.4.2 Stratification or Randomization (if applicable)

Not applicable

4.4.5 Interventions

Not applicable

4.4.6. Measurement

4.4.6.1 Schedule of Measurement

The enrollment period will proceed over a **24 month** period beginning in 2013 (see timeline).

Aim 3 is cross-sectional design: following informed consent, the research nurse will conduct medical chart review and data abstraction. Al activities are conducted at a single time point; there is no follow-up under Aim 3.

4.4.6.2 Definitions

Peripartum: For the purposes of the study, "peripartum" is defined as 48 hours prior, during or post-labor/delivery"

Antenatal: For the purposes of the study, "antenatal" is defined as any stage of pregnancy prior to 48 hours of delivery (i.e. before the peripartum period) of a viable (≥26 weeks gestation) fetus

Obstetric Hemorrhage (OH): Peripartum hemorrhage as defined by the WHO as >500 mL blood loss for vaginal delivery or >1000 mL blood loss for caesarian section. We acknowledge that this definition may be restrictive; this has been echoed in recent discussion in the obstetric literature [22] (*see pitfalls section for further considerations surrounding use of this definition*)

Transfused: Having received any transfusion during the prescribed timeframe with any allogeneic blood product e.g. Red cells, platelets or plasma

Booking: Presentation for antenatal care. Patients who are "unbooked" have not received antenatal care and are consequently at high risk for obstetric complications.

4.4.6.3 Assessment and Measurement Procedures

Aim 3 adopts a similar approach to that used in the REDS-III OH Pilot Study: measurement is confined to medical chart review and data abstraction. In contrast to Aims 1 and 2, there is no clinical contact (administration of questionnaire) and no laboratory investigation.

Medical Chart Review and Data Abstraction

After obtaining informed consent, the research nurse will complete the data abstraction/chart review using the Antenatal Transfusion Form (ATF). Similar to the forms used in Aims 1 and 2 (PRF and AAF respectively), the ATF is a paper-based Teleform instrument that was developed in consultation with our SANBS and obstetric collaborators in South Africa, optimizing capture of information that will support the aims of the study. The form incudes sections on (i) demographics, (ii) hospital data, (iii) pregnancy data, (iv) HIV status and treatment, (v) bleeding in current pregnancy and (vi) blood transfusion.

RTI will also assist with training of the research personnel so as to ensure uniformity in application of the instrument. The ATF forms will be stored in a secure location in the hospital until transferred to SANBS; eventually, the de-identified data will be stored in a secure database at BSRI in San Francisco and RTI in Rockville.

4.4.6.4 Specimen collection procedures

None

4.4.6.5 Special test procedures

Not applicable

4.4.7. Survey Considerations and OMB Requirements

Not applicable

4.4.8. Data Management

Data Handling will be conducted as follows: the completed forms (ATF) will be stored in a secure location at each hospital pending transfer to SANBS. Safe transfer will accompany a designated secure courier from the respective hospitals on a weekly basis to SANBS in Johannesburg. A data-entry clerk will be employed at SANBS, Johannesburg to perform aggregation and scanning of the completed forms following receipt at SANBS. They will also perform primary quality control on the data. The transcribed data will be transmitted thereafter to RTI and BSRI pending analysis. The original paper forms will be archived securely at SANBS headquarters in Johannesburg. This method was successful in the pilot study.

4.4.9. Statistical Considerations

4.4.9.1 Hypothesized outcome rate and smallest difference to detect w/high statistical power

Aim 3 is primarily descriptive.

4.4.9.2 Sample size and power

Given the paucity of data on antenatal blood transfusion, Aim 3, is a pilot study, does not have a hypothesis nor a sample size. We plan to enroll all eligible patients under this Aim. We will perform exploratory analysis to evaluate risk factors for transfusion among patients who are transfused prior to the peripartum period.

4.4.9.3 Assessment and Measurement Procedures

We will use the ATF to capture data on the following domains: (i) demographics, (ii) hospital data, (iii) pregnancy data, (iv) HIV status and treatment, (v) bleeding in current pregnancy and (vi) transfusion

4.4.9.4 Analytic Approach (primary, secondary and subgroup analyses)

Aim 3 is descriptive only. We will examine the epidemiology of antenatal transfusion. There is paucity of data pertaining to antenatal transfusion. Analysis of data will include tabulation of summary statistics e.g. patient demographics, pre and post- transfusion hemoglobin levels, transfusion thresholds, frequency of defined indications, product type transfused, HIV prevalence, co-morbidity, adverse responses to transfusion and other key parameters. We will evaluate this as aggregate data as well as within each institution to determine variability in the complication rates of transfusion.

We intend to compile the analyses into at least two or three manuscripts on transfusion practice in the antenatal obstetric population in South Africa.

4.4.9.5 Pitfalls, Bias, Confounding, Limitations and Alternative Approaches

Pitfalls: We anticipate similar logistical difficulties to those encountered in the pilot study e.g. work-flow, communication between the obstetric staff and research personnel with early identification of eligible patients, variability in record keeping/ missing data etc.

Identification of all patients who are transfused during pregnancy
Preliminary audit data from the OH pilot study indicate that we were reasonably successful in capturing the majority of patients who were transfused in the peripartum period. However, transfusion in the antenatal period is more varied. We anticipate that transfusion is prescribed to antenatal patients in three possible settings: (1) following evaluation at the antenatal anemia clinic [Dr. Hull has indicated that this is rare], (2) following evaluation at another antenatal clinic (non-anemia clinic e.g. medical) and (3) during an antenatal admission, where severity of anemia has resulted in the patient being admitted directly to the ward for severity of symptoms.

Patients admitted or transfused as outpatients from the antenatal anemia clinic (CHB only) are easily captured. Those who bypass the clinic (all four hospitals) pose a greater challenge and we will need to rely on active surveillance by the research personnel for communication with the blood bank and ward staff to identify eligible patients. This will be supported by pre-study education to emphasize the importance of notification of the research staff. Finally, as in Aim 1, all transfusion requisitions that originate in the antenatal clinics, obstetric and gynecology wards will be flagged by the blood bank during the study enrollment period.

In addition, a number of patients who are transfused in pregnancy, particularly for acute anemia (e.g. ectopic pregnancy and spontaneous abortion), are managed at primary level hospitals and

may not be captured in our study. Therefore, the selected hospitals, by virtue of location as well as the level of care, may under-represent the burden of disease.

Selection bias: we have selected the same four major obstetric transfusion services that were used in the REDS-III OH pilot study. All four sites have large patient volumes. The clinical sites (with the exception of MMH and GSH) are geographically removed from each other and serve diverse populations, affording broad representation. Provision of services to a predominantly urban population potentially limits generalizability to transfusion and obstetric practice in rural settings. Similar to the pilot study, it would be logistically impossible to conduct this Phase 2 in remote settings. There is also a bias toward a lower socioeconomic group in view of the population served by these hospitals; this is, however, the population of foremost interest.

Enrollment under multiple aims: There is the possibility that some women at CHB will be enrolled under multiple aims. For example, a patient enrolled under Aim 2 at the AAC may subsequently be transfused under Aim 3 and even Aim 1 if later transfused in the antenatal and peripartum period respectively. Enrollment into more than one Aim is permitted.

4.5. Human Subjects (applies to all Aims)

Procedures & risks: Data will be abstracted from medical records, brief clinical questionnaires will be administered to patients, and phlebotomy (<= 40 mL) will be performed for research laboratory testing.

There is a risk of lost confidentiality and social stigmatization due to the abstraction of medical record information, particularly that related to HIV infection and other sensitive medical diagnoses. There is also an information risk secondary to communication of the abnormal research test results.

Physical risk is limited to a single venous sample to be obtained from patients under aims 1 and 2. The risk incurred from venous sampling is negligible and includes infection, nerve irritation, pain and hematoma.

Recruitment, consent and protection against risks: Subjects will be recruited either on the hospital wards at the time they are clinically stable, or in the anemia clinic. **Full informed consent will be obtained** (see consent forms in Appendices F, G, and H) prior to implementation of study procedures. Only a coded subject identifier number will be used for data transcribed onto study forms which will be stored in locked cabinets. Data safeguards will include a coded database, secure computers, and limited number of staff with access to personal information. Only abnormal, clinically relevant research test results will be communicated back to the patient's physician and/or clinical care team. Research personnel will be trained nurses skilled in phlebotomy. The study protocol will be approved by ethical committees in South Africa (SANBS and each participating hospital ethics committee) as well as at UCSF prior to study initiation.

We anticipate that the majority of patients will speak at least one of four languages: English, Afrikaans, Xhosa and Zulu. The consent process will be conducted in English or the patient's primary language by multilingual research nurses or by hospital translators in cases where they do not speak the patient's language. The English language consent form will record the language in which the consent was administered.

We will request an approval for deferred consent to draw blood for testing prior to transfusion (before obtaining full informed consent). Should the patient later decline to participate in the study, those tubes of blood will be discarded. Laboratory testing will be contingent on obtaining informed consent. Aim 1 Cases, who have not had blood samples drawn prior to transfusion, will undergo phlebotomy and sample collection after consent and enrollment.

Potential benefits to subjects: There is no direct benefit to the subject.

Importance of knowledge to be gained: The potential gains in insight into transfusion practice in the obstetric population, blood utilization, antenatal anemia and obstetric hemorrhage, and perinatal HIV prevalence and treatment in South Africa outweigh the potential risks to the subjects.

Women and minorities: Because the focus is on transfusion and anemia in pregnancy, only women will be enrolled. There are no inclusion or exclusion criteria based on race, but we expect that the majority of our participants will be of Black or Colored race because of the patient populations of the four hospitals

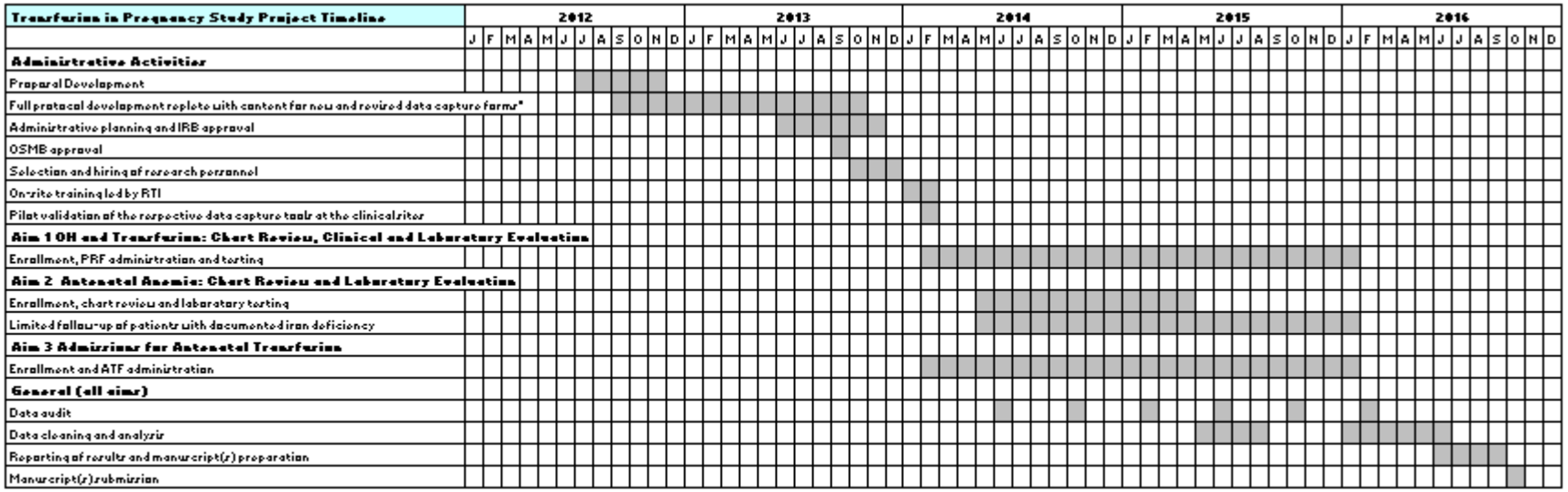
Children: In South Africa, women aged 14 or older are able to consent to medical care and treatment without parental consent. This includes therapeutic abortion and management of obstetric complications. However, any minor (age <18yrs) requires parental consent to participate in research. Owing to the sensitivity surrounding HIV status and pregnancy, we have elected to exclude minors from the study. From the pilot study, minors accounted for a very low proportion of the total number of patients (<2.5%).

However, following maternal consent we do intend to capture the HIV status of babies, born to know HIV infected mothers. The HIV status on the babies is routinely obtained between 6-8 weeks i.e. the babies will not be tested for the purposes of the study, rather their data will be captured.

4.6. Timeline

Please refer to Figure 7 below. Aims 1 and 3 will begin concurrently given the predominantly cross-sectional design. Aim 2 will begin 3 months after Aims 1 and 3, to allow for appropriate scale-up in activities. We will restrict minimal prospective follow-up to a subset of patients in aim 2 with confirmed iron deficiency to evaluate responses to oral iron supplementation. A long planning phase has been allotted, particularly for the IRB approval process. From recent experience with the pilot OH study, this can incur significant delay. We expect to begin the enrollment of the Phase 2 studies in February 2014. The formal start-date will be preceded by on-site training lead by RTI, which will include a short pilot validation of the newly designed data capture instruments used in aims 2 and 3, in addition to the revised PRF used for aim 1. The formal data collection phase is anticipated to begin in February 2014; with 111 cases per month (patients with peripartum transfusion) expected to be eligible for enrollment (aim 1), we expect to observe 932 possible cases per year (assuming 30% non-enrollment). We will be able to reach a target enrollment of 1200 cases and 2400 controls (total 3600 enrolled subjects) across the 4 sites within an 18-month period (end enrollment and data collection August 2015). We have extended to this to 24 months to compensate for unanticipated shortfall or logistical challenges. Aim 2 will begin 3 months later than Aim 1: preliminary data from Dr Hull indicate that ≈13-15 new patients attend the antenatal anemia clinics per week (~50 per month). This will enable evaluation of 500 subjects with antenatal anemia over 21 months of enrollment and follow-up. We plan to enroll for 1 year or to a minimum target of 500 subjects. Under aim 2b, we expect a high proportion of patients who attend the AAC to have documented iron deficiency anemia. This will allow us to meet target enrollment of 224 cases of iron deficiency anemia (see sample size calculation) within 1 year. The number of patients who fall under Aim 3 is unknown; we will conduct data capture under Aim 3 for the full 24 months (in parallel with Aim 1). From discussions with Dr Hull, few patients who attend the antenatal anemia clinic are transfused, but a greater number of patients bypass the clinic and are admitted for transfusion.

Figure 7. Study Timeline



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6. Appendices

Part A: Peripartum Research Form

This form needs to be completed for all patients consenting for Aim 1.

STUDY ID: [AFFIX LABEL HERE] **Today's Date:** ☐☐/☐☐/☐☐☐☐
Day Month Year

Section 1 – Demographics

1. Date of birth: ☐☐/☐☐/☐☐☐☐
Day Month Year

2. Height ☐☐☐ cm

3. Weight at booking kg

4a. Residence for the last 12 months

Country

City

Province ☐☐ Postal Code ☐☐☐☐

4b. Second residency if patient lived in more than one in the past 12 months

Country

City

Province ☐☐ Postal Code ☐☐☐☐

97. Did not live in more than one place.

5. Nationality
01. South African
02. Africa national

03. Other:
97. Don't Know

6. Race / ethnic origin: (Choose one)
01. Black
02. White
03. Coloured
04. Asian

05. Other: _____
97. Don't Know

7. Gravidity
(Number of times pregnant)
☐☐
97. Don't Know

8. Parity (Number of pregnancies carried to viability i.e. through 26 weeks gestation)
☐☐
97. Don't Know

Section 2 - Hospital Data

1. Was the patient referred for:

01. T**ransfusion**

02. Other
03. None [SKIP TO Q3]

2. Referring Institution (if applicable)

3. Highest level of care during most recent hospital stay:
01. Ward
02. High Care
03. ICU

4. Alive at discharge?
01. Yes
02. No

5. Admission Date:

☐☐/☐☐/☐☐☐☐

Day Month Year

6. Discharge Date:

/ /

Day Month Year

7. Date of Delivery:

/ /

Day Month Year

8. Time of Delivery: ☐☐: HH

(24 HOUR TIME, e.g. 21:53)

Section 3 - Pregnancy and Delivery

PREVIOUS *Pregnancy*

1. Previous cesarean sections?

01. Yes
02. No [SKIP TO Q2]
03. Unknown [SKIP TO Q2]

1a. How many cesarean sections? __ __

2. Delivery complications with PREVIOUS pregnancies?

01. Yes
02. No [SKIP TO Q3]
03. Unknown [SKIP TO Q3]

2a. If yes, what type? (Mark all that apply.)

01. Antepartum hemorrhage
02. Postpartum hemorrhage
03. Blood transfusion
04. Induction of labor
05. Gestation diabetes
06. Gestational proteinuric hypertension
07. TB
08. Anemia
09. Malaria
10. Urinary Tract Infections
11. Other infections

12. Other_____
13. Unknown

CURRENT *Pregnancy*

3. Booking status at time of admission

01. Booked
02. Unbooked [SKIP TO Q8]

4. Date of booking:

/ /

Day Month Year

5. Number of visits to the antenatal clinic in current pregnancy:

__ __ 97. Unknown

6. (CHB only) Was the patient ever seen at the antenatal anemia clinic in current pregnancy?

01. Yes
02. No
03. Unknown/Not CHB Hospital

7. Was a hemoglobin ≤10g/dL documented at any antenatal visit?

01. Yes
02. No [SKIP TO Q8]
03. Unknown [SKIP TO Q8]

7a. Note the lowest recorded hemoglobin in current pregnancy:

__ __. __ g/dL

7b. Date and time that it was obtained:

/ / __ __:__ __

Day Month Year (24:00 time)

8. Complications during THIS pregnancy?

01. Yes
02. No [SKIP TO Q9]
03. Unknown [SKIP TO Q9]

8a. What type? (Mark all that apply.)

01. Malposition/abnormal lie
02. Diabetes gestational
03. Multiple pregnancy
04. Threatened abortion
05. Intrauterine death
06. Placenta praevia
07. Gestational proteinuric hypertension
08. Other: _____

9a. Comorbid disease during THIS pregnancy (Mark all that apply.)

01. Pre-existing Diabetes from before pregnancy
02. Hypertension
03. Cardiac Disease
04. Renal Disease
05. Syphilis or laboratory evidence of WR
06. Hepatitis B Virus
07. TB

08. Other
_____

09. None during this pregnancy

9b. List all medications patient was taking during this pregnancy:

Antibiotics
01. Cotrimoxazole (Bactrim)
02. Cephalosporin eg Keflex (cephalexin), cefuroxime
03. Amoxil (amoxicillin)
04. Augmentin (amoxicillin clavulanic acid)
05. Penicillin
06. Piperacilin

Anti TB Drugs
07. Rifafour
08. INH (isoniazid)
09. Pyridoxine

Anti hypertensives
10. Aldomet (methyl dopa)
11. Adalat (nifedipine)
12. Other : specify

Labor and Delivery

10. Mode of delivery:

01. NVD
02. Cesarean Section
03. Vacuum
04. Forceps
05. BBA

11. Estimated blood loss at delivery:

__ ____ __ml 97. Unknown

12. Gestational Age at Delivery

Weeks: ___ ___ # Days: ___

13. Delivery in the current pregnancy

01. Spontaneous [SKIP TO Q14]
02. Induced

13a. If labour was induced, what medications were used for induction?

01. Oral prostaglandin
02. Vaginal prostaglandin
03. Oxytocics
04. Catheter Bulb Induction
05. **Other**_____

14. Were there complications during labor and delivery?

01. Yes
02. No [SKIP TO Q15]
03. Unknown [SKIP TO Q15]

14a. If Yes, what complications
(Mark all that apply)
01. Chorioamnionitis
02. Placental abruption ("abruption placentae")
03. Prolonged rupture of membranes
04. Prolonged labour
05. Pre-eclampsia
06. Assisted delivery e.g. vacuum or forceps
07. Vaginal breech delivery
08. Episiotomy
09. Vaginal tears (first, second, third, fourth degree tear)

Post-delivery

15. Were there complications post-delivery?
01. Yes
02. No [SKIP TO Q16]
03. Unknown [SKIP TO Q16]

15a. Complications following delivery (Mark all that apply; this excludes pre-existing conditions)

Organ Dysfunction:
01. Symptomatic anaemia (e.g. palpitations, dizziness shortness of breath)
02. Cardiovascular e.g. cardiac arrest, cardiopulmonary resuscitation
03. Respiratory e.g. intubation or ventilation
04. Renal e.g. documented renal failure, dialysis
05. Coagulation/hematologic e.g. DIC
06. Hepatic e.g. jaundice, HELLP syndrome

07. Neurologic e.g. coma, stroke

Sepsis:

08. Local e.g. C/S wound infection, endometritis
09. Systemic e.g. pneumonia

10. **Other**_____
11. Unknown

Baby

16. Birth Outcome

01. Stillbirth
02. Live birth
03. Early Neonatal Death

17. Baby's first admission after delivery:

01. Admission to neonatal ICU
02. Pediatric ward
03. Directly to mother/no complications

18. Baby's birth weight:

____ ___ ___ ___ g

19. Baby's APGAR scores

Immediate: __ __ **5 minutes:** __ __

20. Baby's laboratory number:

__ __ __ __ __ __ __ __

21. At which clinic will the baby be followed-up?

_____

97. Unknown

22. Is the mother breastfeeding/intending to breastfeed?

01. Breastfeeding
02. Intending to breastfeed
03. Not breastfeeding
04. Unknown

Section 4 - HIV Status & Treatment

1. HIV Status of the patient

1a. At booking	○ HIV +	○ HIV-	○ Unknown
Date of test:			
1b. At Delivery Admissions	○ HIV +	○ HIV-	○ Unknown
Date of test:			
1c. Other tests during pregnancy	○ HIV +	○ HIV-	○ Unknown
Date of test:			
1d. Other tests during pregnancy	○ HIV +	○ HIV-	○ Unknown
Date of test:			

IF NEGATIVE OR UNKNOWN FOR ALL, SKIP TO SECTION 5.

2. Last CD4 count: *(e.g. 382)*

__ __ __ __ cells/mm3
97. Unknown

2a. Date of CD4 count from question 2:

/ /

Day Month Year
97. Unknown

3. Last Viral Load: *(example: 12,000)*

__ __ __ __ __ __ copies/ml

97. Unknown

3a. Date of viral load in question 3:

/ /

Day Month Year
97. Unknown

4. Was patient on ART prior to this pregnancy?

01. Yes
02. No [SKIP TO Q5]
97. Unknown [SKIP TO Q5]

4a. Start Date for ART

/ /

Day Month Year

97. Unknown

4b. Which ART drugs was the patient taking prior to pregnancy? (Mark all that apply)

01. AZT – Zidovudine/ Azidothymidine
02. ddI – Didanosine
03. 3TC – Lamivudine
04. D4T – Stavudine
05. ABC – Abacavir
06. TDF – Tenofovir
07. FTC - Emtricitabine
08. NVP – Nevirapine
09. EFV – Efavirenz

10. ETV - Etravirine
11. ATV – Atazanavir
12. LPV/r – Lopinavir/Ritonavir
13. RAL - Raltegravir
14. SQV - Saquinavir
15. IDV - Indinavir
16. FDC- Fixed Dose Combination- w/ AZT
17. FDC- Fixed Dose Combination – w/o AZT

18. Other: _____
19. Unknown

5. Was patient on PMTCT during this pregnancy?

01. Yes
02. No [SKIP TO Q6]
97. Unknown [SKIP TO Q6]

5a. Start Date for PMTCT

/ /

Day Month Year

97. Unknown

5b. Which PMTCT drugs was the patient taking? (Mark all that apply)

01. AZT – Zidovudine/Azidothymidine
02. ddI – Didanosine
03. 3TC – Lamivudine
04. D4T – Stavudine
05. ABC – Abacavir
06. TDF – Tenofovir
07. FTC - Emtricitabine
08. NVP – Nevirapine
09. EFV – Efavirenz
10. ETV - Etravirine
11. ATV – Atazanavir
12. LPV/r – Lopinavir/Ritonavir
13. RAL - Raltegravir
14. SQV - Saquinavir
15. IDV - Indinavir
16. FDC- Fixed Dose Combination- w/ AZT
17. FDC- Fixed Dose Combination – w/o AZT

18. Other: _____
19. Unknown

5c. If patient was taking AZT, when did she begin and end?

Started AZT: / /

Day Month Year

97. Unknown
99. Not taking AZT

Ended AZT: / /

Day Month Year

97. Unknown
99. Not taking AZT

6. Was the patient HIV positive during the preceding pregnancy?

01. Yes
02. No [SKIP TO Section 5]
03. Not applicable/Primigravida [SKIP TO Section 5]

6a. If yes, was PMTCT used in that pregnancy?

01. Yes
02. No
97. Unknown

6b. Was ART used during previous pregnancy?

01. Yes
02. No
97. Unknown

Section 5 - Obstetric Hemorrhage

Note: *OH is defined by a* ***total*** *of 500 ml* ***blood loss*** *Normal Vaginal Delivery (NVD) or1000 ml following caesarean section within 48 hours of delivery.*

1. Estimated TOTAL Blood Loss within 48 hours of NVD or caesarean:

__ __ __ __ ml

97. Unknown

2. Did the patient have OH?

01. Yes
02. No [SKIP TO Q5]
97. Unknown [SKIP TO Q5]

[Complete questions 3 & 4 ONLY if the patient sustained an Obstetric Hemorrhage.]

3. List all contributing causes for the patient's Obstetric Hemorrhage?
(Mark ALL that apply.)

01. Abruption with gestation hypertension
02. Abruption without gestational hypertension

03. Praevia
04. Retained placenta
05. Morbidly adherent placenta (accreta, percreta, increta)
06. Ruptured uterus with previous C/S
07. Ruptured uterus without previous C/S
08. Inverted uterus
09. Associated fibroids or other uterine abnormality
10. Uterine atony
11. Vaginal/cervical laceration
12. Bleeding during C/S
13. Bleeding after C/S
14. Bleeding during hysterectomy
15. Bleeding during surgical procedure other than C/S or hysterectomy
16. Antepartum hemorrhage not specified
17. Postpartum hemorrhage not specified

18. Other (please list)

_____

_____

19. Unknown

4. OH Management (Mark all that apply.)

Pharmacological

01. Oxytocin
02. Ergometrine/ syntometrine
03. Misoprostol
04. Protaglandin F2 alpha
05. Tranexamic acid (cyclokapron)
06. Other Pharmacological

Operative

07. Emergency C/S
08. Examination under anesthesia
09. Suturing cervical tears
10. Suturing vaginal tears
11. Hysterectomy
12. Laparotomy
13. Internal iliac artery ligation
14. Ligation of uterine vessels
15. Brace sutures e.g. B-Lynch sutures
16. Removal of retained products
17. Uterine tourniquet

Other

18. Blood Transfusion
19. Abdominal packing
20. Vaginal packing
21. Interventional radiology e.g. uterine artery embolization
22. Ballon tamponade
23. Condom tamponade
24. Glove tamponade
25. Other
26. Unknown

4a. If 'Operative Management' options were checked, how long was the patient in theatre?

Hours: __ __ Minutes: __ __

99. Not Applicable

5. Was the patient on any of the following anticoagulants at time of delivery?

01. Warfarin
02. Aspirin
03 Clexane

04 Other _____

05. No, the patient was not on any of the listed anticoagulants.

Section 6 – Transfusion

Note: *This section applies to transfusion of any blood product, which includes whole blood, packed red blood cells, platelets, plasma and/or cryoprecipitate*

1a. Was the patient transfused?

01. Yes
02. No [SKIP TO SECTION 7.]

1b. Were any of the units transfused as emergency blood (uncrossmatched) RBCs?

01. Yes
02. No

1c. Physician that ordered the blood and rank:

_____

_____

1d. What was the highest level with which the decision to transfuse was discussed?

01. Intern
02. Medical Officer
03. Registrar
04. Obstetric/Gynecological Consultant
05. Critical Care
06. Anesthetist
07. Unknown

1e. Patient Transfused at:

01. Referring Institution [SKIP TO Q2]

02. Current Institution
03. Both

1f. If at the current institution, where was the patient when the blood transfusion was started?

01. Antenatal
02. Labour
03. Post-natal
04. Theatre
05. Casualty
06. Medical
07. Surgical
08. ICU
09. Outpatient

10. Other _____
11. Unknown

2. LAST hemoglobin measurement prior to 1st Transfusion

__ __ . __ g/dL 97. Unknown

2a. Date: / /

Day Month Year

Time: H H: H H (24:00 time)

3. Hemoglobin method used:

01. FBC
02. Blood Gas
03. Point of Care (e.g. finger stick)

04. Other _____
05. Not Done
06. Unknown

4. MCV: __ __ __ fl
97. Unknown

5. MCH: __ __ **.** __ pg
97. Unknown

6. Platelets: __ __ __ (mcL or x 10^9/L)
97. Unknown

7. Vital signs at the time of hemoglobin measurement prior to 1st Transfusion:

7a. Heart Rate: __ __ __ **per minute**
97. Unknown

7b. Blood pressure: __ __ __ **/** __ __ __
97. Unknown

7c. Respiratory rate: __ __ **per minute**
97. Unknown

7d. Temperature: __ __**ºC**
97. Unknown

8. First hemoglobin measurement AFTER last Transfusion

__ __ . __ g/dL 97. Unknown

8a. Date: / /

Day Month Year

Time: H H: H H (24:00 time)

9. Hemoglobin method used:

01. FBC
02. Blood Gas
03. Point of Care (e.g. finger stick)

04. Other _____
05. Not Done
06. Unknown

10. Vital signs at the time of hemoglobin measurement AFTER last Transfusion:

10a. Heart Rate: __ __ __ **per minute**
97. Unknown

10b. Blood pressure:

__ __ __ **/** __ __ __
97. Unknown

10c. Respiratory rate: __ __ **per minute**
97. Unknown

10d. Temperature: __ __ **ºC**
97. Unknown

11. What was the medical rationale for/ diagnosis requiring blood transfusion?
(*Please be specific as possible: provide the underlying cause.)* Mark **ALL** that apply.

01. Obstetric hemorrhage
02. Surgical (e.g. C/S, hysterectomy or laparotomy)
03. Chronic anemia
04. Iron deficiency
05. Vitamin B12 deficiency
06. Folate deficiency
07. Thalassemia
08. Sickle cell anemia
09. Hemoglobinopathy or enzyme disorder-other
10. Chronic anemia; cause not identified
11. Chronic anemia; drug related (e.g. AZT)
12. TB
13. Unknown
14. Other

12a. Had the patient been identified as anemic during current pregnancy?

01. Yes
02. No [SKIP TO Q13]
03. Unknown [SKIP TO Q13]

12b. When was the patient first identified as being anemic?

01. Booking visit
02. Antenatal clinic follow-up visit
03. Admission for delivery
04. Other
05. Unknown

13. Hematinic Therapy during Pregnancy
(Mark all that apply)

01. None
02. Iron, Oral
03. Iron, Parenteral
04. Folate
05. Vitamin B12
06. Antenatal blood transfusion
07. Other
08. Unknown

14. Direct Antiglobulin test (Direct Coombs Test)

01. Positive
02. Negative
03. Unknown

15. Transfusion reaction?

01. Yes
02. No [SKIP TO Q17]

16. Type of Reaction

01. Febrile non-hemolytic
02. Allergic
03. Anaphylactic
04. Acute hemolytic
05. Delayed hemolytic
06. TRALI
07. TACO
08. Septic
09. Unknown

Section 7 – NOTES:

Note: Please print clearly in all capital letters.

CONTINUE TO Question 17 on next page, if patient was transfused.

17. Components Transfused
If not in chart, obtain this from blood bank.

Date/Time 1		**Type 1**	**BUI 1**
Day Month Year	*24 HOUR TIME (e.g. 21:53)*	○ Red Cells ○ Platelets ○ FFP (fresh/ frozen plasma) ○ Bioplasma/ FDP(fresh dried plasma) ○ Cryo	
Date 2	**Time 2**	**Type 2**	**BUI 2**
/ / : Day Month Year		○ Red Cells ○ Platelets ○ FFP (fresh/ frozen plasma) ○ Bioplasma/ FDP(fresh dried plasma) ○ Cryo	
Date 3	**Time 3**	**Type 3**	**BUI 3**
/ / : Day Month Year		○ Red Cells ○ Platelets ○ FFP (fresh/ frozen plasma) ○ Bioplasma/ FDP(fresh dried plasma) ○ Cryo	
Date 4	**Time 4**	**Type 4**	**BUI 4**
/ / : Day Month Year		○ Red Cells ○ Platelets ○ FFP (fresh/ frozen plasma) ○ Bioplasma/ FDP(fresh dried plasma) ○ Cryo	
Date 5	**Time 5**	**Type 5**	**BUI 5**
/ / : Day Month Year		○ Red Cells ○ Platelets ○ ○ FFP (fresh/ frozen plasma) ○ Bioplasma/ FDP(fresh dried plasma) ○ Cryo	
Date 6	**Time 6**	**Type 6**	**BUI 6**

/ / Day Month Year	:	○ Red Cells ○ Platelets ○ ○ FFP (fresh/ frozen plasma) ○ Bioplasma/ FDP(fresh dried plasma) ○ Cryo	
Date 7	**Time 7**	**Type 7**	**BUI 7**
/ / Day Month Year	:	○ Red Cells ○ Platelets ○ FFP (fresh/ frozen plasma) ○ Bioplasma/ FDP(fresh dried plasma) ○ Cryo	

*In the event of **massive transfusion**, please list under NOTES (Section 7 above) the date, time, component type, and BUI for each additional component.*

END OF FORM.

TIP STUDY-AIM 1:
HIV FOLLOW-UP PERIPARTUM RESEARCH FORM (PRF)

This form needs to be completed 4-8 weeks after delivery for all Aim 1 participants testing HIV positive.

STUDY ID*: [AFFIX LABEL HERE] **Mother's Delivery Date*:** / /

**Apply label/complete this top section during enrolment.* Day Month Year

Section 8 –Follow-up and Laboratory Test Results

Note: This section needs to be completed 4-8 weeks after delivery

1. Today's Date: / /

Day Month Year

2. What is the HIV status on the baby as determined by PCR at 4-8 weeks?

01. HIV Positive
02. HIV Negative
03. Unknown

3. If HIV positive, did the mother breastfeed?

01. Yes
02. No
03. Unknown

Section 9 – NOTES:

Note: Please print clearly in all capital letters.

END OF FORM

Part B: Antenatal Anemia Form

This form needs to be completed on all pregnant patients who are referred to the Antenatal Anaemia Clinic at Chris Hani Baragwanath Hospital.

STUDY ID: [AFFIX LABEL HERE] **Today's Date:** / /
Day Month Year

Section 1 - Demographics

1. Date of birth: / /
Day Month Year

2. Height ☐☐☐ cm

97. Don't Know

3. Weight at booking ☐☐☐ kg

4a. Residence for the last 12 months

Country ☐

City ☐

Province ☐☐ Postal Code ☐☐☐☐

4b. Second residency if patient lived in more than one in the past 12 months

Country ☐

City ☐

Province ☐☐ Postal Code ☐☐☐☐

97. Did not live in more than one place.

5. Nationality
01. South African
02. Africa national

03. Other:
97. Don't Know

6. Race / ethnic origin: (Choose one)
01. Black
02. White
03. Coloured
04. Asian

05. Other: _____
97. Don't Know

7. Gravidity
(Number of times pregnant)

XX

97. Don't Know

8. Parity (Number of pregnancies carried to viability i.e. through 26 weeks gestation)

XX

97. Don't Know

Section 2 – Referring Clinic Data

1. How was the patient referred?

01. Clinic
02. Hospital [SKIP to Section 3]
03. General Practitioner (GP) [SKIP to Sect 3]
04. Other [SKIP to Sect 3]
↳ _____
05. Unknown [SKIP to Sect 3]

1a. If referred from the clinic, what is the name of the referring clinic?

☐

1b. Number of visits to the referring antenatal clinic:

__ __

2. Last Hemoglobin at referring clinic:

__ __ . __ g/dL 97. Unknown

2a. Date it was obtained:

/ /

Day Month Year

2b. Hemoglobin method used:

07. FBC
08. Point of Care (e.g. finger stick)
09. Other → _____
10. Not Done
11. Unknown

3. What treatment was the patient taking as prescribed by the <u>referring clinic</u>?

Treatment	**<u>Total dosage per day</u>**	**<u>Date started</u>**
a. Iron sulphate	☐☐☐ __ __ g	☐☐ ☐☐ ☐☐☐☐ Day Month Year
b. Folic acid	___ ___ ___ µg	/ / Day Month Year
c. Vitamin C:	___ ___ ___ mg	/ / Day Month Year
d. Vitamin A:	___ ___ ___ mg	/ / Day Month Year
e. Vitamin B12:	___ ___ ___ mg	/ / Day Month Year
f. Thiamine:	___ ___ ___ mg	/ / Day Month Year
g. Other iron supplement (specify): _____	___ ___ ___ mg	/ / Day Month Year

4. Side effects from supplementation?

01. Yes
02. No
03. Unknown

5. Was the patient reported to be compliant with medication at the referring clinic?

01. Yes
02. No
03. Unknown

Section 3 – Evaluation at the Antenatal Anaemia Clinic at CHB

1. Date referred for anaemia: / /

Day Month Year

2. First specialist antenatal anaemia clinic visit date: / /

Day Month Year

3. Gestational age at time of first visit: # Weeks: ___ ___ # Days: ___ ___

4. Reason for referral (Mark all):

01. Hb<8g/dl: any stage of pregnancy
02. Hb<10g/dl: if the patient's gestational age is >36 weeks
03. Hb<9g/dl: HIV positive (not on ARVs)
04. Hb: Downward trend in hemoglobin
05. Hb: Patient not responding to oral iron therapy
06. Low Hb
07. Signs of severe anaemia (e.g. heart failure, CCF, dyspnea, etc.)
08. Specific hematological conditions (specify) } → [ ]
09. Other (specify)
10. Unknown

5. What is the tentative diagnosis for the underlying anaemia at the AAC?

Check **ALL** that apply

01. Antenatal hemorrhage [SKIP TO Q6.]
02. Chronic anaemia

5a. If Chronic anaemia, please be as specific as possible. *(i.e. Rather than anaemia, bleeding or hemorrhage, provide the underlying cause)*

(Mark all that apply.)

01. Iron deficiency
02. Vitamin B12 deficiency
03. Folate deficiency
04. Thalassemia
05. Sickle cell anaemia
06. Hemoglobinopathy or enzyme disorder-other
07. Infection
08. HIV – ARVs
09. HIV- infection
10. Medication other
11. Anaemia of chronic disease

12. Other _____
13. Unknown

6. What treatment was the patient taking as prescribed by the AAC?

Treatment	Total dosage per day	Date started
a. Iron sulphate	☐☐☐ mg	☐☐ ☐☐ ☐☐☐☐ Day Month Year
b. Folic acid	___ ___ ___ µg	/ / Day Month Year
c. Vitamin C:	___ ___ ___ mg	/ / Day Month Year
d. Vitamin A:	___ ___ ___ mg	/ / Day Month Year
e. Vitamin B12:	___ ___ ___ mg	/ / Day Month Year
f. Thiamine:	___ ___ ___ mg	/ / Day Month Year
g. Other iron supplement (specify): _____	___ ___ ___ mg	/ / Day Month Year

6a. Has the patient been reported to be compliant with hematinic supplementation?

01. Yes
02. No
03. Unknown

7. Does the patient report side effects from the haematinics while at the clinic?

01. Yes
02. No [SKIP TO Section 4]
03. Unknown [SKIP TO Section 4]

7a. What side effects were experienced? (Mark all that apply.)

01. Vomiting
02. Heartburn
03. Constipation
04. Diarrhea
05. Other: _____

Section 4 - Pregnancy and Comorbid Medical History

PREVIOUS Pregnancy

1. Previous caesarean sections?

01. Yes
02. No [SKIP TO Q2a]
03. Unknown [SKIP TO Q2a]

1a. How many cesarean sections?

___ ___

2. Delivery complications with PREVIOUS pregnancies?

01. Yes
02. No [SKIP TO Q3]
03. Unknown [SKIP TO Q3]

2a. If yes, what type? (Mark all that apply.)

01. Antepartum hemorrhage
02. Postpartum hemorrhage
03. Blood transfusion
04. Induction of labour
05. Gestation diabetes
06. Gestational proteinuric hypertension
07. TB
08. Antenatal anaemia
09. Malaria
10. Urinary Tract Infections
11. Other infections

12. Other: _____
13. Unknown

3. Please answer the following questions about previous pregnancy history:

	Number (enter zero if none)
a. Number of pregnancies delivered at < 35 weeks	
b. Number of Still births	
c. Number of miscarriages	

CURRENT Pregnancy

4. LMP: / /

Day Month Year

97. Unknown

5. Estimated Date of Delivery:

/ /

Day Month Year
97. Unknown

6. Booking Date:

/ /

Day Month Year
97. Unknown

7. Complications during THIS pregnancy?

01. Yes
02. No [SKIP TO Q8.]
01. Unknown [SKIP TO Q8.]

7a. What complications were experienced during THIS pregnancy? (Mark all that apply)

01. Malposition/abnormal lie
02. Multiple pregnancy
03. Threatened abortion
04. Intrauterine death
05. Placenta praevia
06. Antenatal anaemia
07. Diabetes gestational
08. Pre-existing Diabetes from before pregnancy.
09. Malposition:
    - Breech
    - Transverse
10. Gestational proteinuric hypertension
11. IUGR:
    - Suspected
    - Confirmed
12. Decreased liquor
13. Placenta Praevia→ Grade ____
14. Hypertension
15. Cardiac Disease
16. Renal Disease
17. Intrauterine Growth Retardation (IUGR)
18. Syphilis or laboratory evidence of WR
19. Hepatitis B Virus
20. Chorioamnionitis
21. TB-completed treatment this pregnancy
22. TB-currently on treatment
23. TB suspected not yet confirmed or treated
24. Malaria
25. Other: _____

8. Has the patient experienced antepartum hemorrhage (APH) or threatened miscarriage during the current pregnancy?

01. Yes
02. No [SKIP TO Q9]
03. Unknown [SKIP TO Q9]

8b. What was the primary cause of the Antepartum Hemorrhage?

01. Placenta Previa
02. Threatened abortion
03. Cervicitis
04. Antepartum hemorrhage not specified
05. Other _____
06. Unknown

9. List all medications patient was taking during this pregnancy:

01. Cotrimoxazole (Bactrim)
02. Cephalosporin eg Keflex (cephalexin), cefuroxime
03. Amoxil (amoxicillin)
04. Augmentin (amoxicillin clavulanic acid)
05. Penicillin
06. Piperacilin

Anti TB Drugs

07. Rifafour
08. INH (isoniazid)
09. Pyridoxine

Anti hypertensives

10. Aldomet (methyl dopa)
11. Adalat (nifedipine)
12. Other: specify:

Vital signs at initial visit to AAC

10. Heart Rate: _____ _____ _____
(Beats per minute)
97. Unknown

11. Blood pressure:

___ ___ ___ **/** _____ _____ _____
97. Unknown

12. Respiratory rate: _____ _____ _____
(per minute)
97. Unknown

13. Temperature: ____ ____**.** ____ ^{o}C
97. Unknown

Section 5 - HIV Status and Treatment

1a. HIV Status of the patient

	Status			If known, date of test:
1a. At booking	○HIV +	○HIV-	○Unknown	
1b. Delivery Admissions	○HIV+	○HIV-	○Unknown	
1c. Other tests during pregnancy	○HIV+	○HIV-	○Unknown	
1d. Other tests during pregnancy	○HIV +	○HIV-	○Unknown	

IF NEGATIVE OR UNKNOWN FOR ALL, SKIP TO SECTION 6.

2. Last CD4 count if known (e.g. 382*)*

___ ___ ___ ___ cells/mm3
97. Unknown

2a. Date of CD4 count from Q2 if known:

/ /
Day Month Year
97. Unknown

3. Last Viral Load: *(example: 12,000)*

__ __ __ __ __ __ copies/ml
97. Unknown

3a. Date of viral load in question 3:

/ /
Day Month Year
97. Unknown

4. Was patient on ART prior to this pregnancy?
01. Yes
02. No [SKIP TO Q5]
97. Unknown [SKIP TO Q5]

4a. Start Date for ART

/ /
Day Month Year
97. Unknown

4b. Which ART drugs was the patient taking <u>prior</u> to pregnancy? (Mark all that apply)

01. AZT – Zidovudine/Azidothymidine
02. DDL – Didanosine
03. 3TC – Lamivudine
04. D4T – Stavudine
05. ABC – Abacavir
06. TDF – Tenofovir

07. FTC – Emtricitabine
08. NVP – Nevirapine
09. EFV – Efavirenz
10. ETV – Etravine
11. ATV – Atazanavir
12. LPV/r- - Lopinavir/Ritonavir
13. RAL – Raltegravir
14. SQV – Saquinavir
15. IDV – Indinavir
16. FDC – Fixed Dose Combination Combination – w/ AZT
17. FDC- Fixed Dose Combination - w/o AZT

16. Other: _____
17. Unknown

5. Is patient on ART/ PMTCT during this pregnancy?

01. Yes
02. No [SKIP TO Q6]
97. Unknown [SKIP TO Q6]

5a. Start Date for ART/PMTCT

/ /
Day Month Year

5b. Which ART/PMTCT drugs is the patient taking? (Mark all that apply)

01. AZT – Zidovudine/Azidothymidine
02. ddI – Didanosine
03. 3TC – Lamivudine
04. D4T – Stavudine
05. ABC – Abacavir
06. TDF – Tenofovir
07. FTC - Emtricitabine
08. NVP – Nevirapine
09. EFV – Efavirenz
10. ETV - Etravirine
11. ATV – Atazanavir
12. LPV/r – Lopinavir/Ritonavir
13. RAL - Raltegravir
14. SQV - Saquinavir
15. IDV - Indinavir
16. FDC- Fixed Dose Combination– w/ AZT
17. FDC- Fixed Dose Combination - w/o AZT
18.

19. Other: _____

20. Unknown

5c. If patient was taking AZT, when did she begin and end?

Started AZT: / /

Day Month Year

97. Unknown

99. Not taking AZT

Ended AZT: / /

Day Month Year

97. Unknown
99. Not taking AZT

6. Was the patient HIV positive during the preceding pregnancy?

1. Yes
2. No [SKIP TO Section 6]
3. Not applicable/Primigravida [SKIP TO Section 6]

6a. If yes, was PMTCT used in that pregnancy?

01. Yes
02. No
97. Unknown

6b. Was ART used during previous pregnancy?

01. Yes
02. No
97. Unknown

Section 6 –Transfusion

1. Has the patient been transfused during the current pregnancy?

01. Yes
02. No [SKIP TO END]
03. Unknown [SKIP TO END]

2. If Yes, date of transfusion:

/ /

Day Month Year

3. Number of units transfused: __ __

4. Where was the patient when the blood transfusion was started?

01. Antenatal
02. Gynaecology
03. Theatre
04. Casualty
05. Medical
06. Surgical
07. ICU
08. Outpatient

09. Other _____

10. Unknown

Section 7 – NOTES:

Note: Please print clearly in all capital letters.

End of Form

Part C: Antenatal Transfusion Form

ANTENATAL TRANSFUSION FORM (ATF)

Complete this form on all pregnant patients who are:

- *transfused prior to the peripartum period (48hrs of delivery) in patients ≥26weeks, or*
- *transfused for any reason in patients <26weeks gestation.*

STUDY ID: [AFFIX LABEL HERE] **Today's Date:** / / Day Month Year

Section 1 – Demographics

1. Date of birth: ☐☐ / ☐☐ / ☐☐☐☐ Day Month Year

2. Height ☐☐☐ cm

3. Weight at booking kg

4a. Residence for the last 12 months

Country ☐

City ☐

Province ☐☐ Postal Code ☐☐☐☐

4b. Second residency if patient lived in more than one in the past 12 months

Country ☐

City ☐

Province ☐☐ Postal Code ☐☐☐☐

97. Did not live in more than one place.

5. Nationality

01. South African
02. Africa national
03. Other: ☐
97. Don't Know

6. Race / ethnic origin: (Choose one)

01. Black
02. White
03. Coloured
04. Asian
05. Other: ☐
97. Don't Know

7. Gravidity

(Number of times pregnant)

☐☐

97. Don't Know

8. Parity (Number of pregnancies carried to viability i.e. through 26 weeks gestation)

☐☐

97. Don't Know

Section 2 - Hospital Data

1. Was the patient referred for transfusion?

01. Yes
02. No [SKIP TO Q3]

2. Referring Institution (if applicable)

3. Was the patient admitted to hospital?

01. Yes
02. No [SKIP TO Section 3]

4. Admission Date: ☐☐ / ☐☐ / ☐☐☐☐
Day Month Year

5. Discharge Date: ☐☐ / ☐☐ / ☐☐☐☐
Day Month Year

6. Highest Level of Care at current institution

01. Ward
02. High Care
03. ICU

7. Alive at discharge?

01. Yes
02. No

Section 3 – Pregnancy

PREVIOUS *Pregnancy*

1. Previous caesarian sections?

01. Yes
02. No [SKIP TO Q2]
03. Unknown [SKIP TO Q2]

1a. How many cesarean sections? XX

2. Delivery complications with PREVIOUS pregnancies?

01. Yes
02. No [SKIP TO Q3]
03. Unknown [SKIP TO Q3]

2a. If yes, what type? (Mark all that apply.)

01. Antepartum hemorrhage
02. Postpartum hemorrhage
03. Blood transfusion
04. Induction of labor
05. Gestation diabetes
06. Gestational proteinuric hypertension
07. TB
08. Anemia
09. Malaria
10. Urinary Tract Infections
11. Other infections

12. Other_____
13. Unknown

3. Was a hemoglobin <10g/dL documented at any time during a previous pregnancy?

01. Yes
02. No
03. Unknown

CURRENT *Pregnancy*

4. Gestational age at time of transfusion:

Weeks: ___ ___ # Days: ___

5. Estimated Date of Delivery:

/ /
Day Month Year

6. Booking status at time of admission:
01.Booked
02. Unbooked [SKIP TO Q10]

6a. Date of booking: / /
Day Month Year

7. Number of visits to the antenatal clinic:

_____
○ 97. Unknown

8. Was the patient ever seen at the antenatal anemia clinic (CHB only)?

01. Yes
02. No
03. Unknown

9. Was a hemoglobin ≤10g/dL documented at any antenatal visit?
01. Yes
02. No
03. Unknown

9b. List all medications patient was taking during this pregnancy:
01. Cotrimoxazole (Bactrim)
02. Cephalosporin eg Keflex (cephalexin), cefuroxime
03. Amoxil (amoxicillin)

04. Augmentin (amoxicillin clavulanic acid)
05. Penicillin
06. Piperacilin

Anti TB Drugs
07. Rifafour
08. INH (isoniazid)
09. Pyridoxine

Anti hypertensives
10. Aldomet (methyl dopa)
11. Adalat (nifedipine)
12. Other, specify:

10. Complications during THIS pregnancy?

01. Yes
02. No [SKIP TO Q11]
03. Unknown [SKIP TO Q11]

10a. If yes, what complications were there during this pregnancy? (Mark all that apply.)

01. Malposition/abnormal lie
02. Diabetes gestational
03. Gestational proteinuric hypertension
04. Multiple pregnancy
05. Threatened abortion
06. Intrauterine death
07. Placenta praevia
08. Chorioamnionitis
09. Syphilis or laboratory evidence of WR
10. Other →_____
11. Unknown

Admission

11. Were there complications during this admission?

01. Yes
02. No [SKIP TO Section 4]
03. Unknown [SKIP TO Section 4]

11a. If yes, what complications during this admission? (Mark all that apply. This excludes pre-existing conditions)

Organ Dysfunction
01. Symptomatic anaemia (e.g. palpitations, dizziness shortness of breath)
02. Cardiovascular e.g. cardiac arrest, cardiopulmonary resuscitation, Cardiac failure
03. Respiratory e.g. intubation or ventilation
04. Renal e.g. documented renal failure, dialysis
05. Coagulation/hematologic e.g. DIC
06. Hepatic e.g. jaundice, HELLP syndrome
07. Neurologic e.g. coma, stroke

Sepsis
08. Local e.g. endometritis
09. Systemic e.g. pneumonia

10. Other → _____
11. Unknown

Section 4 - HIV Status and Treatment

1a. HIV Status of patient:

1a. At booking	○ HIV +	○ HIV-	○ Unknown
Date of test:			
1b. At Delivery Admissions	○ HIV +	○ HIV-	○ Unknown
Date of test:			
1c. Other tests during pregnancy	○ HIV +	○ HIV-	○ Unknown
Date of test:			
1d. Other tests during pregnancy	○ HIV +	○ HIV-	○ Unknown
Date of test:			

IF NEGATIVE OR UNKNOWN FOR ALL, SKIP TO SECTION 5.

2. Last CD4 count: *(e.g. 382)*

__ __ __ __ cells/mm3
97. Unknown

2a. Date of CD4 count from question 2:

/ /

Day Month Year
97. Unknown

3. Last Viral Load: *(example: 12,000)*

__ __ __ __ __ __ copies/ml

97. Unknown

3a. Date of viral load in question 3:

/ /

Day Month Year

97. Unknown

4. Was patient on ART prior to this pregnancy?

03. Yes
04. No [SKIP TO Q5]
97. Unknown [SKIP TO Q5]

4a. Start Date for ART

/ /

Day Month Year

97. Unknown

4b. Which ART drugs was the patient taking prior to pregnancy? (Mark all that apply)

01. AZT – Zidovudine/ Azidothymidine
02. ddI – Didanosine
03. 3TC – Lamivudine
04. D4T – Stavudine
05. ABC – Abacavir
06. TDF – Tenofovir
07. FTC - Emtricitabine
08. NVP – Nevirapine
09. EFV – Efavirenz
10. ETV - Etravirine
11. ATV – Atazanavir
12. LPV/r – Lopinavir/Ritonavir
13. RAL - Raltegravir
14. SQV - Saquinavir
15. IDV - Indinavir
16. FDC- Fixed Dose Combination– w/ AZT
17. FDC- Fixed Dose Combination - w/o AZT

18. Other: _____
19. Unknown

5. Was patient on PMTCT during this pregnancy?

03. Yes
04. No [SKIP TO Q6]
97. Unknown [SKIP TO Q6]

5a. Start Date for PMTCT

☐☐ / ☐☐ / ☐☐☐☐

Day Month Year

○ Unknown

5b. Which PMTCT drugs is the patient taking? (Mark all that apply)

01. AZT – Zidovudine/Azidothymidine
02. ddI – Didanosine
03. 3TC – Lamivudine
04. D4T – Stavudine
05. ABC – Abacavir
06. TDF – Tenofovir
07. FTC - Emtricitabine
08. NVP – Nevirapine
09. EFV – Efavirenz
10. ETV - Etravirine
11. ATV – Atazanavir
12. LPV/r – Lopinavir/Ritonavir
13. RAL - Raltegravir
14. SQV - Saquinavir
15. IDV - Indinavir
16. FDC- Fixed Dose Combination– w/ AZT
17. FDC- Fixed Dose Combination - w/o AZT

18. Other: _____
19. Unknown

6. Was the patient HIV positive during the preceding pregnancy?

04. Yes
05. No [SKIP TO Section 5]
06. Not applicable/Primigravida [SKIP TO Section 5]

6a. Was PMTCT used in that pregnancy?

03. Yes
04. No
97. Unknown

6b. Was ART used during previous pregnancy?

03. Yes
04. No
97. Unknown

Section 5 –Bleeding in Current Pregnancy

1a. Was there bleeding during this pregnancy?

01. Yes
02. No [SKIP TO SECTION 6]
03. Unknown [SKIP TO SECTION 6]

1b. If Yes, what was the estimated blood loss?

___ ___ ___ ml

2. What was the cause of the hemorrhage? (Mark all that apply)

01. Placental abruption
02. Placenta Previa
03. Abruptio Placentae
04. Vasa Previa
05. Neoplasm (specify e.g. chorangioma)
    ↳ _____
06. Ectopic pregnancy/Extra uterine pregnancy
07. Threatened abortion
08. Incomplete abortion
09. Complete abortion
10. Cervicitis
11. Bleeding during surgical procedure (If checked, specify type of procedure.)
    ↳
    - Elective abortion
    - Dilation and curettage
    - Salpingectomy
    - Salpingoopherectomy
    - Hysterectomy
    - Hysterotomy
    - Anaesthesia for non-pregnancy related condition e.g. appendicitis, drainage of abscess
12. Antepartum hemorrhage not specified
13. Other_____
14. Unknown

3. APH Management (Mark all that apply)

APH

01. Expectant/conservative
02. Induction of Labor
03. Caesarian section
04. Antibiotics (for cervicitis)

Miscarriage

05. Oxytocin
06. Misoptrostol
07. Hysterotomy
08. Hysterectomy
09. Evacuation of uterus under anaesthesia
10. MVA (Manual Vacuum extraction) side ward, analgaesia
11. Antibiotics

Ectopic

12. Laparotomy
13. Salpingectomy

Section 6 - Transfusion

Note: *Please applies to transfusion of any blood product, which includes whole blood, packed red blood cells, platelets, plasma and/or cryoprecipitate*

Prior to the current transfusion:

1. Had the patient been transfused before the current transfusion?

01. Yes
02. No [SKIP TO Q2]
03. Unknown [SKIP TO Q2]

1a. **If Yes, when?** (Mark all that apply)

01. During current pregnancy
02. During previous pregnancy
03. For a non-obstetric reason

2. Had the patient been identified as anemic during current pregnancy?

01. Yes
02. No [SKIP TO Q3]
03. Unknown [SKIP TO Q3]

2a. If yes, when was the patient first identified as being anemic?

01. Booking visit
02. Antenatal clinic follow-up visit
03. Antenatal specialist anemia clinic

04. Medical clinic
05. Admission
06. Other

3. Was the patient on hematinic therapy during pregnancy?

01. Yes
02. No
03. Unknown

3a. What type?

01. Iron, Oral
02. Iron, Parental
03. Folate
04. Vitamin B12

05. Other→ _____
06. Unknown

3b. Was the patient reported to be compliant with hematinic therapy?

01. Yes
02. No
03. Unknown

Current transfusion:

4. Was the patient transfused?

01. Yes
02. No [IF NO, SKIP TO END]

5. What was the medical rationale and/or diagnosis requiring blood transfusion?
Check **ALL** that apply (*Please be specific as possible: rather than anemia, bleeding or hemorrhage, provide the underlying cause)*

01. Obstetric hemorrhage
02. Surgical (e.g. C/S, hysterectomy or laparotomy)
03. Chronic anemia
04. Anaesthetic related (required for transfusion)
05. Other→ _____
06. Unknown

5. If Chronic anemia was selected above, specify which type:

01. Iron deficiency
02. Vitamin B12 deficiency
03. Folate deficiency
04. Thalassemia
05. Sickle cell anemia
06. Hemoglobinopathy or enzyme disorder-other
07. Malaria

6. Were any of the units transfused as emergency blood (uncrossmatched) RBCs?

01. Yes
02. No

7. Physician that ordered the blood and rank:

_____

_____

8. What was the highest level with which the decision to transfuse was discussed?

01. Intern
02. Medical Officer
03. Registrar
04. Obstetric/Gynecological Consultant
05. Anaesthetist
06. Critical Care
07. Unknown

9. Patient transfused at:

01. Referring Institution [SKIP TO Q11]
02. Current Institution
03. Both

10. If at the current institution, where was the patient when the blood transfusion was started?

01. Antenatal
02. Labour
03. Post-natal
04. Theatre'
05. Casualty
06. Medical
07. Surgical
08. ICU
09. Outpatient
10. Other _____
11. Unknown

11. <u>LAST hemoglobin measurement PRIOR to 1st Transfusion</u>

__ __ . __ g/dL 97. Unknown

11a. Hb hemoglobin method used:

07. FBC
08. Blood
09. Gas
10. Point of Care (e.g. finger stick)

11. Other→ _____
12. Not Done
13. Unknown

11b. If an FBC was used, were the results obtained prior to transfusion?

01. Yes
02. No
03. Unknown

12. *Measurements* at the time of hemoglobin measurement prior to 1st transfusion:

12a. MCV: __ __ __ fl

12b. MCH: __ __ **.** __ pg

12c. Platelets: __ __ __ **,** __ __ __
(mcL or x 10^9/L)

13. *Vital signs* at the time of hemoglobin measurement prior to 1st transfusion:

13a. Heart Rate: __ __ __ **per minute**

13b. Blood pressure: __ __ __ **/** __ __

13c. Respiratory rate: __ __ **per minute**

13d. Temperature: __ __**ºC**

14. First hemoglobin measurement AFTER last transfusion:

__ __ . __ g/dL 97. Unknown

14a. Hb hemoglobin method used:

01. FBC
02. Blood
03. Gas
04. Point of Care (e.g. finger stick)
05. Other _____
06. Not Done
07. Unknown

15. *Vital signs* at the time of hemoglobin measurement AFTER last transfusion:

15a. Heart Rate: __ __ __ **per minute**

15b. Blood pressure: __ __ __ **/** __ __

15c. Respiratory rate: __ __ **per minute**

15d. Temperature: __ __**ºC**

15. Direct Antiglobulin Test (Direct Coombs Test)

01. Positive
02. Negative
03. Unknown

15a. Was there a Transfusion Reaction?

01. Yes (GO TO Q15b)
02. No [SKIP TO Q16]
03. Unknown

15b. Type of Reaction

01. febrile non-hemolytic
02. allergic
03. anaphylactic
04. acute hemolytic
05. delayed hemolytic
06. TRALI
07. TACO
08. Septic
09. Unknown

Section 7 – NOTES:

Note: Please print clearly in all capital letters.

CONTINUE TO Question 16 on next page, if patient was transfused.

16. Components Transfused

Date/Time 1		**Type 1**	**BUI 1**
/ / Day Month Year	. *24 HOUR TIME (e.g. 21:53)*	○ Red Cells ○ Platelets ○ FFP (fresh/ frozen plasma) ○ Bioplasma/ FDP(fresh dried plasma) ○ Cryo	
Date 2	**Time 2**	**Type 2**	**BUI 2**
/ / Day Month Year	:	○ Red Cells ○ Platelets ○ Plasma ○ FFP (fresh/ frozen plasma) ○ Bioplasma/ FDP(fresh dried plasma) ○ Cryo	
Date 3	**Time 3**	**Type 3**	**BUI 3**
/ / Day Month Year	:	○ Red Cells ○ Platelets ○ FFP (fresh/ frozen plasma) ○ Bioplasma/ FDP(fresh dried plasma) ○ Cryo	
Date 4	**Time 4**	**Type 4**	**BUI 4**
/ / Day Month Year	:	○ Red Cells ○ Platelets ○ FFP (fresh/ frozen plasma) ○ Bioplasma/ FDP(fresh dried plasma) ○ Cryo	
Date 5	**Time 5**	**Type 5**	**BUI 5**
/ / Day Month Year	:	○ Red Cells ○ Platelets ○ FFP (fresh/ frozen plasma) ○ Bioplasma/ FDP(fresh dried plasma) ○ Cryo	
Date 6	**Time 6**	**Type 6**	**BUI 6**

/ / Day Month Year	:	○ Red Cells ○ Platelets ○ FFP (fresh/ frozen plasma) ○ Bioplasma/ FDP(fresh dried plasma) ○ Cryo	
Date 7	**Time 7**	**Type 7**	**BUI 7**
/ / Day Month Year	:	○ Red Cells ○ Platelets ○ FFP (fresh/ frozen plasma) ○ Bioplasma/ FDP(fresh dried plasma) ○ Cryo	

*In the event of **massive transfusion**, please list on reverse under NOTES the date, time, component type, and BUI for each additional component.*

END OF FORM

Part D:TIP Aim 1 Consent Form

REDS-III TIP Consent Form: Aim 1

Department of Obstetrics and Gynecology, Chris Hani-Baragwanath Maternity Hospital/King Edward Hospital/Mowbray Maternity Hospital/Groote Schuur Hospital

Study Title: REDS-III Transfusion in Pregnancy Study

Researchers at the South African National Blood Service and University of California San Francisco in collaboration with Dr ____ at the Department of Obstetrics and Gynecology -Chris Hani-Baragwanath Maternity Hospital/King Edward Hospital/Mowbray Maternity Hospital/Groote Schuur Hospital wish to invite you to participate in a medical research study. A research nurse will explain this study to you.

Medical research includes only people who choose to take part. Take your time to make your decision about participating. You may discuss your decision with your family and friends and with your health care team. If you have any questions, you may ask the research nurses.

You are being invited to take part in this study because you are/were recently admitted to Chris Hani-Baragwanath Maternity/King Edward VIII/Mowbray Maternity/Groote Schuur Hospital for the birth of your baby. A study is currently being done to compare differences between patients who have blood transfusion around time of delivery, with those that do not. Both patients who have transfusion as well as those who have not been transfused are needed for the study. The information obtained is expected to improve the quality of care that the hospital delivers.

Why is this research being done?

The researchers are doing this research to understand why women need to be transfused during childbirth in South Africa. They wish to understand whether it is due to anemia (low blood), HIV infection, problems with blood clotting or other reasons.

Who is paying for this research?

The National Heart Lung and Blood Institute (a branch of the National Institute of Health) in the United States pays for the study, which includes part of the research nurse's salaries. The study researchers are based at University of California, San Francisco (USA) and the South African National Blood Service (South Africa).

How many people will take part in this research?

A total of 3600 patients will take part in the study. That number includes 1200 patients who have been transfused and 2400 patients who have not been transfused.

What will happen if I agree to participate?

- Blood sample. If you were transfused around the time of delivery, blood that was already collected from you will be tested. If you were either not transfused or were transfused but did

not have blood drawn beforehand, we will draw 40mL of blood (approximately 2 ½ table spoons) by needlestick from a vein in your arm.

- The blood sample will be tested for anemia (low or thin blood), blood clotting ability and other medical tests. Abnormal test results will be shared directly with your doctor. In addition some of that blood will be stored for future research on pregnancy and blood disorders. That blood will be stored in what is called a "sample repository" which will be located in San Francisco (United States of America).

- We also will collect and save information from your medical record, including things like results of physical examinations, diagnostic tests, medical questionnaires and histories, diagnoses, and treatments. We will look at information about clotting and bleeding, the body's immune response, reasons for anemia [weak blood), pregnancy, and HIV [if applicable]. Information that we learn from the specimens and the medical record data may be shared broadly in coded form (i.e. without information that could identify you). Those data will be analyzed and kept in a database at Research Triangle International (RTI) in the United States.

- The research nurse will also ask you some questions about your medical history. This should take approximately 10 minutes of your time. You are not obliged to answer any of the questions.

- If you are HIV positive, the research nurse will contact the clinic in 4-8 weeks time to obtain your baby's HIV status. Your baby's name or information that could identify your baby will not be recorded.

- We may share your specimens and certain medical information about you (for example, diagnosis, blood pressure, age) with other scientists, but we will not give them your name, address, phone number, or any other information that would identify you. The National Heart, Lung, and Blood Institute (NHLBI) in the USA pays for this research and will obtain information from this clinical study under the authority granted by USA regulation 42 USC 285b. No future research or analysis, other than specified in the protocol, will be performed on specimens without additional consent being obtained from the ethics committee of the University of the Witwatersrand. Reports about any <u>research</u> will not be given to you or your doctor. However, we will share abnormal test results that impact your care with your doctor. It will be up to your doctor as to which results are discussed with you.
- Your specimens will be kept *indefinitely.* If you decide later that you do not want your specimens and information to be used for future research, you can notify the investigator in writing at the South African National Blood Service and we will destroy any remaining identifiable specimens and information if they are no longer needed for your care. However, if any research has already been done using portions of your specimens, the data will be kept and analyzed as part of those research studies.

What risks are involved with this research?

If you are selected to have blood drawn, there may be some bruising and discomfort at time of the blood test. There is a minimal risk of nerve irritation and bruising at the site of blood sampling. There is a small risk of infection yet this is very rare.

Confidentiality: Participating in research may involve a loss of privacy, but information about you will be handled as confidentially as possible. Study data will be physically and electronically secured. As with any use of electronic means to store data, there is a risk of breach of data security. Your name will not be used in any published reports from research performed using your specimen. The manager of the sample repository and select staff members will have access to information about you but they will not release any identifying information about you to researchers using your specimens. The Ethical Committees may see information about you to check on the research.

What happens if I am injured because I took part in this study?

It is important that you tell your study doctor, _____ *[investigator's name(s)]*, if you feel that you have been injured because of taking part in this study. You can tell the doctor in person or call him/her at _____ *[telephone number]*. If you are injured as a result of being in this study, the (*hospital site name*) will provide necessary medical treatment.

What are the benefits of participating in this research?

There is probably no benefit to you from participating in this research, although there could be a benefit to your health from the additional laboratory tests. These tests may help to explain why you needed or did not need transfusion around time of delivery. However, we hope there will be a benefit to other pregnant women in South Africa if we learn something that will contribute to the understanding of bleeding and transfusion during pregnancy.

What financial issues should I consider before participating in the study?

You will not be charged for participating in research. You will not be paid for participating in research. If the data or any new products, tests or discoveries that result from this research have potential commercial value, you will not share in any financial benefits. (***site name***) may receive payment from researchers requesting specimens in order to cover the costs of collecting and storing the specimens.

What alternatives do I have?

If you choose not to participate, you will receive medical treatment just like any other maternity patient. Your blood sample will not be used for research.

What are my rights if I take part in this study?

Taking part in this study is your choice. You may choose either to take part or not to take part in the study. No matter what decision you make, there will be no penalty to you and you will not lose any of your regular benefits. Leaving the study will not affect your medical care. You can still get your medical care from our institution.

In the case of injury resulting from this study, you do not lose any of your legal rights to seek payment by signing this form.

Who can answer my questions about the study?

You can talk with the study researcher about any questions, concerns or complaints you have about this study. Contact the study researcher(s) _____ *[name(s)]* at _____ *[telephone number(s)]*.

If you wish to ask questions about the study or your rights as a research participant to someone other than the research nurses or if you wish to voice any problems or concerns you may have about the study, please call the Dr Tshilidzi Muthivhi (South African National Blood Service) at _____.

Consent

If you have any questions about this study, please talk to the study doctor or nurse. No matter what you decide to do, it will not affect your care.

You have been given copies of this consent form and the Experimental Subject's Bill of Rights to keep.

PARTICIPATION IN RESEARCH IS VOLUNTARY. You have the right to decline to participate or to withdraw at any point in this study without penalty or loss of benefits to which you are otherwise entitled.

If you wish to participate in this study, you should sign below.

Date	Subject's Signature for Consent
Date	Person Obtaining Consent
Date	Witness – Only required if the participant is a non-English speaker

AND/OR:

Date	Legally Authorized Representative

Date Person Obtaining Consent

The consent was obtained in _____(note the language in which the process was conducted) and understood by the patient.

Part E: TIP Aim 2 Consent Form

REDS-III TIP Consent Form: Aim 2

Department of Obstetrics and Gynecology, Chris Hani-Baragwanath Maternity Hospital/King Edward Hospital/Mowbray Maternity Hospital/Groote Schuur Hospital

Study Title: REDS-III Transfusion in Pregnancy Study

Researchers at the South African National Blood Service and University of California San Francisco in collaboration with _____ (Physician) at the Department of Obstetrics and Gynecology -Chris Hani-Baragwanath Hospital wish to invite you to participate in a medical research study. A research nurse will explain this study to you.

Medical research includes only people who choose to take part. Take your time to make your decision about participating. You may discuss your decision with your family and friends and with your health care team. If you have any questions, you may ask the research nurses.

You are being invited to take part in this study because you were recently referred to the antenatal anemia clinic at Chris Hani-Baragwanath Maternity Hospital. A study is currently being done to understand why patients have anemia ("weak blood") in pregnancy and how they respond to treatment. The information obtained is expected to improve the quality of care that the hospital delivers.

Why is this research being done?

The researchers are doing this research to understand more about anemia (weak blood) in pregnancy. Specifically, they wish to understand whether there are any risk factors for anemia (weak blood) that could be prevented. They also wish to know how different patients respond to treatment for anemia (weak blood). This research could help doctors to improve the prevention and treatment of anemia (weak blood) in pregnant women in South Africa

Who is paying for this research?

The National Heart Lung and Blood Institute (a branch of the National Institute of Health) in the United States pays for the study, which includes part of the research nurse's salaries. The study researchers are based at University of California, San Francisco (USA) and the South African National Blood Service (South Africa).

How many people will take part in this research?

A total of 500 patients will take part in the study. We will also follow-up patients who have iron deficiency as the cause of their anemia.

What will happen if I agree to participate?

- Blood sample. At the time that the nurse collects blood for the clinic, he or she will draw an extra 40mL of blood (approximately 2 ½ table spoons)

- The blood sample will be tested for anemia (low or thin blood), vitamins, iron and other medical tests that could explain more about why you have anemia. Abnormal test results will be shared directly with your doctor. In addition some of that blood will be stored for future research on pregnancy and blood disorders. That blood will be stored in what is called a "sample repository" which will be located in San Francisco (United States of America).

- The research nurse will also ask you some questions about your medical history. This should take approximately 10 minutes of your time. You are not obliged to answer any of the questions.
- We also will collect and save information from your medical record, including results of physical examinations, diagnostic tests, medical questionnaires and histories, diagnoses, and treatments. We do not know for sure if your specimens or medical record will be used, but they might be used in research about anemia in pregnancy or the body's immune response. The medical record data may be shared broadly in coded form (i.e. without information that could identify you). Those data will be analyzed and kept in a database at Research Triangle International (RTI) in the United States.
- We may share your specimens and certain medical information about you (for example, diagnosis, blood pressure, age) with other scientists, but we will not give them your name, address, phone number, or any other information that would identify you. The National Heart, Lung, and Blood Institute (NHLBI) in the USA pays for this research and will obtain information from this clinical study under the authority granted by USA regulation 42 USC 285b. No future research or analysis, other than specified in the protocol, will be performed on specimens without additional consent being obtained from the ethics committee of the University of the Witwatersrand. Reports about any research will not be given to you or your doctor. However, we will share abnormal test results that impact your care with your doctor. It will be up to your doctor as to which results are discussed with you.
- Your specimens will be kept indefinitely. If you decide later that you do not want your specimens and information to be used for future research, you can notify the investigator in writing at the South African National Blood Service and we will destroy any remaining identifiable specimens and information if they are no longer needed for your care. However, if any research has already been done using portions of your specimens, the data will be kept and analyzed as part of those research studies.

- Patients who are shown to be iron deficient may have additional data collected during follow-up visits to the antenatal anemia clinic.

What risks are involved with this research?

There may be some bruising and discomfort at time of the blood test. There is a minimal risk of nerve irritation and bruising at the site of blood sampling. There is a small risk of infection yet this is very rare.

Confidentiality: Participating in research may involve a loss of privacy, but information about you will be handled as confidentially as possible. Study data will be physically and electronically secured. As with any use of electronic means to store data, there is a risk of breach of data security. Your name will not be used in any published reports from research performed using your specimen. The manager of the sample repository and select staff members will have access to information about you but they will not release any identifying information about you to researchers using your specimen. The Ethical Committees may see information about you to check on the research.

What happens if I am injured because I took part in this study?

It is important that you tell your study doctor, _____ *[investigator's name(s)]*, if you feel that you have been injured because of taking part in this study. You can tell the doctor in person or call him/her at _____ *[telephone number]*.

Treatment for Injury: If you are injured as a result of being in this study, Chris-Hani Baragwanath Hospital will provide necessary medical treatment.

What are the benefits of participating in this research?

There is probably no benefit to you from participating in this research, although there could be a benefit to your health from the additional laboratory tests. These tests may help to explain why you have anemia as it could help the doctors to decide on how best to treat you. We hope there will be a benefit to other pregnant women in South Africa if we learn something that will contribute to the understanding of anemia during pregnancy.

What financial issues should I consider before participating in the study?

You will not be charged for participating in research. You will not be paid for participating in research. If the data or any new products, tests or discoveries that result from this research have potential commercial value, you will not share in any financial benefits. Chris Hani Baragwanath Hospital may receive payment from researchers requesting specimens in order to cover the costs of collecting and storing the specimens.

What alternatives do I have?

If you choose not to participate, you will receive medical treatment just like any other maternity patient. Your blood sample will not be used for research.

What are my rights if I take part in this study?

Taking part in this study is your choice. You may choose either to take part or not to take part in the study. No matter what decision you make, there will be no penalty to you and you will not lose any of your regular benefits. Leaving the study will not affect your medical care. You can still get your medical care from our institution.

In the case of injury resulting from this study, you do not lose any of your legal rights to seek payment by signing this form.

Who can answer my questions about the study?

You can talk with the study research nurse about any questions, concerns or complaints you have about this study. Contact the study researcher(s) _____ *[name(s)]* at _____ *[telephone number(s)]*.

If you wish to ask questions about the study or your rights as a research participant to someone other than the research nurses or if you wish to voice any problems or concerns you may have about the study, please call the Dr Tshilidzi Muthivhi (South African National Blood Service) at _____.

Consent

If you have any questions about this study, please talk to the study doctor or nurse. No matter what you decide to do, it will not affect your care.

You have been given copies of this consent form and the Experimental Subject's Bill of Rights to keep.

PARTICIPATION IN RESEARCH IS VOLUNTARY. You have the right to decline to participate or to withdraw at any point in this study without penalty or loss of benefits to which you are otherwise entitled.

If you wish to participate in this study, you should sign below.

_____

Date Subject's Signature for Consent

_____

Date Person Obtaining Consent

_____ _____

Date Witness – Only required if the participant is a non-English speaker

AND/OR:

_____

Date Legally Authorized Representative

_____

Date

The consent was obtained in _____(note the language in which the process was conducted) and understood by the patient.

Part F: TIP Aim 3 Consent Form

REDS-III TIP Consent Form: Aim 3

Department of Obstetrics and Gynecology, Chris Hani-Baragwanath Maternity Hospital/King Edward Hospital/Mowbray Maternity Hospital/Groote Schuur Hospital

Study Title: REDS-III Transfusion in Pregnancy Study

Researchers at the South African National Blood Service and University of California San Francisco in collaboration with Dr _____ at the Department of Obstetrics and Gynecology -Chris Hani-Baragwanath Maternity Hospital/King Edward Hospital/Mowbray Maternity Hospital/Groote Schuur Hospital wish to invite you to participate in a medical research study. A research nurse will explain this study to you.

Medical research includes only people who choose to take part. Take your time to make your decision about participating. You may discuss your decision with your family and friends and with your health care team. If you have any questions, you may ask the research nurses.

You are being invited to take part in this study because you were recently admitted to the Chris Hani-Baragwanath Maternity/King Edward VIII/Mowbray Maternity/Groote Schuur Hospital and received a blood transfusion. A study is currently being done to understand the reasons why patients need blood transfusion during pregnancy. The information obtained is expected to improve the quality of care that the hospital delivers.

Why is this research being done?

The researchers are doing this research to understand why women need to be transfused during pregnancy in South Africa. They wish to understand whether it is due to anemia (low blood), HIV infection, problems with blood clotting or other reasons.

Who is paying for this research?

The National Heart Lung and Blood Institute (a branch of the National Institute of Health) in the United States pays for the study, which includes part of the research nurse's salaries. The study researchers are based at University of California, San Francisco (USA) and the South African National Blood Service (South Africa).

How many people will take part in this research?

We will collect information on all patients who have blood transfusion in pregnancy (not at delivery) for 2 years. The total number of patients is as yet unknown.

What will happen if I agree to participate?

- We will collect and save information from your medical record, including things like results of physical examinations, diagnostic tests, medical questionnaires and histories, diagnoses, treatments, etc. This information might be used in research about pregnancy, clotting and bleeding, the body's immune response, and reasons for anemia (weak blood).
- We may share certain medical information about you (for example, diagnosis, blood pressure, age) with other scientists, but we will not give them your name, address, phone number, or any other information that would identify you. The National Heart, Lung, and Blood Institute (NHLBI) in the USA pays for this research and will obtain information from this clinical study under the authority granted by USA regulation 42 USC 285b. Reports about any research will not be given to you or your doctor. Those data will be analyzed and kept in a database at Research Triangle International (RTI) in the United States.

What risks are involved with this research?

Confidentiality: Participating in research may involve a loss of privacy, but information about you will be handled as confidentially as possible. Study data will be physically and electronically secured. As with any use of electronic means to store data, there is a risk of breach of data security. Your name will NOT be used in any published reports from research performed. Select staff members will have access to information about you but they will not release any identifying information about you.

What happens if I am injured because I took part in this study?

There are no physical risks to you from participating in this study.

What are the benefits of participating in this research?

There is probably no benefit to you from participating in this research. However, we hope there will be a benefit to other pregnant women in South Africa if we learn something that will contribute to the understanding of bleeding and transfusion during pregnancy.

What financial issues should I consider before participating in the study?

You will not be charged for participating in research. You will not be paid for participating in research. If the data or any new products, tests or discoveries that result from this research have potential commercial value, you will not share in any financial benefits. **(Site name)** may receive payment from researchers requesting specimens in order to cover the costs of collecting and storing the specimens.

What alternatives do I have?

If you choose not to participate, you will receive medical treatment just like any other maternity patient.

What are my rights if I take part in this study?

Taking part in this study is your choice. You may choose either to take part or not to take part in the study. No matter what decision you make, there will be no penalty to you and you will not lose any of your regular benefits. Leaving the study will not affect your medical care. You can still get your medical care from our institution.

Who can answer my questions about the study?

You can talk with the study research nurse about any questions, concerns or complaints you have about this study. Contact the study researcher(s) _____ *[name(s)]* at _____ *[telephone number(s)]*.

If you wish to ask questions about the study or your rights as a research participant to someone other than the researchers or if you wish to voice any problems or concerns you may have about the study, please call the Dr Tshilidzi Muthivhi (South African National Blood Service) at _____.

Consent

If you have any questions about this study, please talk to the study doctor or nurse. No matter what you decide to do, it will not affect your care.

You have been given copies of this consent form and the Experimental Subject's Bill of Rights to keep.

PARTICIPATION IN RESEARCH IS VOLUNTARY. You have the right to decline to participate or to withdraw at any point in this study without penalty or loss of benefits to which you are otherwise entitled.

If you wish to participate in this study, you should sign below.

Date	Subject's Signature for Consent
Date	Person Obtaining Consent
Date	Witness – Only required if the participant is a non-English speaker

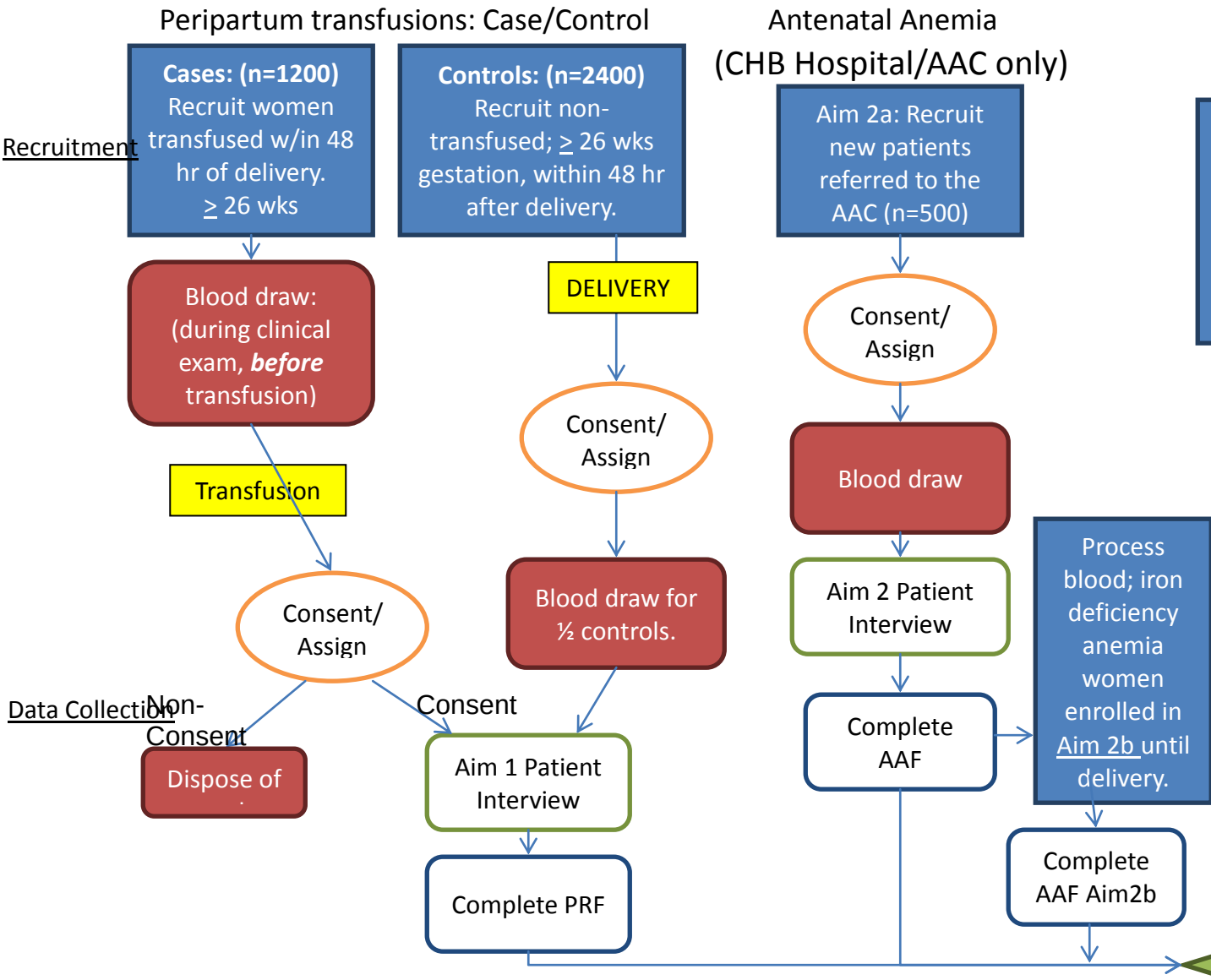
AND/OR:

Date Legally Authorized Representative

Date Person Obtaining Consent

The consent was obtained in _____(note the language in which the process was conducted) and understood by the patient.

Part G: Flow Chart- Overall Study Processes



*Must consent participant w/in 48hrs of delivery.

Part H: Flow Chart- Screening and Eligibility