

# **Chapter 1**

## **Study Overview**

### **1.1 Introduction**

The Epidemiology of Burkitt Lymphoma in East African Children or Minors (EMBLEM) Study Manual provides the detailed instructions for study conduct. The quality of the data depend very much on the knowledge and skills of the people collecting the data, it is therefore important that these procedures be followed closely to ensure success of this research effort.

This chapter provides an overview of the study background information and procedures that will be used to implement EMBLEM. Chapter 2 covers community mobilization procedures and details of how to gain local cooperation to facilitate enrollment of both cases and controls. Chapter 3 addresses case selection procedures while Chapters 4 and 5 provide details of control selection procedures for both health-center and population controls. The recruitment chapters provide specific details to be followed when enrolling case and control subjects and completing the data collection forms. Procedures for collecting and handling blood, saliva and biopsy tissue specimens are provided in Chapter 6. Chapter 7 details the data systems to be used in the conduct of EMBLEM. Chapter 8 contains the user guide for the biospecimen management system and Chapter 9 details the administrative procedures that will be implemented to monitor data quality and ensure that the study runs smoothly.

### **1.2 Background Information**

Burkitt lymphoma (BL) is an aggressive monoclonal B-cell malignancy that is rare (sporadic) worldwide, but is 100-fold more common (endemic) in equatorial Africa, particularly among children. Epstein-Barr virus (EBV) and malaria are epidemiologically linked to endemic BL in epidemiologic studies, but questions remain about role of EBV variants and the evidence for association with malaria is weak. EBV is ubiquitous, yet only few children develop BL, possibly because only a few EBV variants are pathogenically relevant. The association of BL with malaria is based on ecologic and non-comparative clinical studies. Two case-control studies have reported significant association of high anti-malarial antibodies with BL among children in Uganda and in Malawi, but selection bias (cases and controls came from dissimilar geographical areas) and reverse causality bias were limitations. Three studies were conducted in the 1960s and 70s to test association of carriage of malaria-resistance gene with BL, two of which reported a significant or marginal inverse association. These pioneering studies were small (240 cases all together) and looked at one polymorphism in one gene (sickle cell gene). Improvements in technologies to characterize genetic variation allow the EBV and malaria hypotheses to be examined with greater power by looking at genetic variation across multiple genes.

The Epidemiology of Burkitt lymphoma in East African Children or Minors (EMBLEM) is a case-control study of 1500 BL cases and 3000 age-, sex- and residence-frequency matched controls to be conducted in Uganda, Tanzania and Kenya (see Figure 1-1). Four separate cohorts (two in the main study and two in the sub study) will be enrolled (see Figure 1-2 for the basic study design). The cases will be enrolled from four hospitals in these regions of East Africa,

where malaria transmission is holoendemic and year round. The controls will be enrolled from general population attendees at Health Center II (HC-II) units where the cases originate. Another set of controls for each country will be enrolled directly from villages in the same geographic catchment areas. The primary study objectives are: 1) to test the hypothesis that genetic resistance to malaria is associated with a lower risk of BL, and 2) to use genome-wide association methods to discover genetic variation that may be associated with decreased or increased risk of BL. Because genetic variation conveys no information on actual exposure to malaria or EBV, in secondary analyses, we will use empiric epidemiological questionnaire and laboratory methods: a) to measure exposure to malaria and its association with BL, and b) to measure EBV variants and their association with BL. To examine issues related to bias and to obtain data to correct for deviations, we will also enroll 2250 population controls from 5% of the villages to obtain population distribution of key exposures variables. These data will be used to correct for differences in risk factor distribution in HC-II controls respective to the general population.

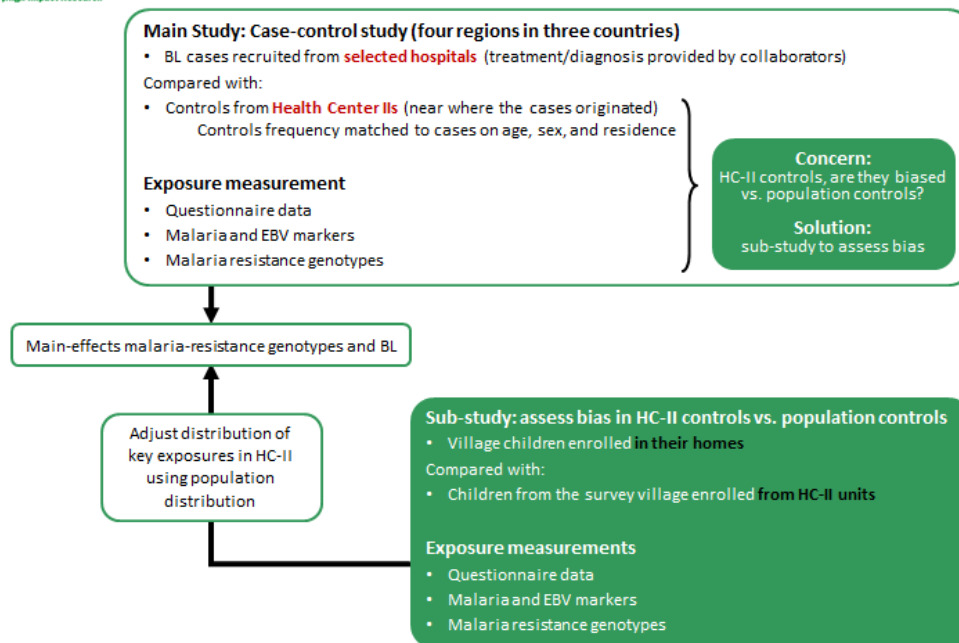
**Figure 1-1**  
**Location of EMBLEM study sites in East Africa (pink circles)**



**Figure 1-2**  
**EMBLEM basic study design**



## Basic Study Design for EMBLEM



### 1.3 Purpose of the Study

#### 1.3.1. Primary objectives

- 1) To evaluate the association between germline genetic polymorphisms and the risk of BL among 600 cases and 1500 controls in East Africa. Initial efforts will focus on polymorphisms in 25 genes, selected apriori because these polymorphisms associated with resistance to malaria and are prevalent in East Africa.
- 2) To conduct a genome-wide association study (GWAS) among 1,500 cases and 3,000 controls to discover genetic polymorphisms that may be associated with endemic BL.
- 3) To enroll a sample of population controls from 5% of the villages in the study area to determine population distribution of key exposures, including mild clinical malaria and malaria-resistance polymorphisms.

#### 1.3.2 Secondary objectives

- 1) To examine the association between malaria, measured by questionnaire and assays of malaria antigens and antibodies, and BL.
- 2) To examine the association between rare EBV variants and BL.
- 3) To examine, among controls, whether host genetic variants identified in objective #1 & 2 are associated with anti-malarial antibodies.

- 4) To examine, among controls, whether malaria antigenemia and anti-malaria antibodies are associated with EBV diversity.

#### **1.4 Overview of the Study Population**

The study will be conducted in four regions in three countries in East Africa: two in Northern Uganda, one in northern Tanzania and one in western Kenya. The catchment areas for each region are included in Appendix 1-1. Within each region, cases will be enrolled at selected hospitals with capacity to diagnose and treat BL and also to conduct high-quality epidemiologic research, and the controls will be enrolled from the parishes and villages where the cases originated. In Uganda, cases will be enrolled at a hospital in North-Central Uganda and at a hospital in northwestern Uganda. In Tanzania, cases will be enrolled at Hospital in northern Tanzania. In Kenya, cases will be enrolled at a hospital in western Kenya. These hospitals receive 90% or more of all the patients with BL in their respective regions. For each region, cases will be eligible for the study if they come from districts where 90% of the cases originate from, based on case admissions over the past 5 years. Case subjects will be patients enrolled at the hospital. Two sets of controls will be enrolled. The first set will be children who are attending parish-level Health Center II (HC-II) in the parish where cases originated. HC-II units serve a parish, which comprises 3-6 villages. HC-II controls will be frequency matched to the case by age ( $\pm 2$  years), residence (parish), and sex (male or female). Geographical delineation will be from historical BL case data from the hospital. Two HC-II controls will be enrolled for each case, frequency matched by age ( $\pm 2$  years), residence (parish), and sex (male or female). HC-II controls will be enrolled in two waves at each site. The first wave of controls will be selected using the age, sex, and residence distribution of historical case data and the second wave of controls will be selected to correct for deviations from the distributions in the actual case enrollment for EMBLEM.

The second set of controls (from the substudy) will be population controls enrolled from 12 villages randomly selected to be representative of the general study area. The population controls are being enrolled to evaluate the magnitude of bias that may be associated with enrolling controls from HC-II as opposed from villages at the home where children live. This substudy will compare village children enrolled in their households (population controls) with children from the same village enrolled from HC-II units serving the same village where population controls were recruited (HC-II controls for population controls). The substudy will evaluate the distribution of key study exposures, such the prevalence of malaria and EBV markers, malaria-resistance-genes among population versus HC-II children, and if there are distortions, use the population data to correct for distortions during analyses. Thus, we will randomly select about 30 households per village and invite all the children in those households to participate in the study for a sample size of 60-90 children per village. For comparison, children from the same village where population controls are obtained will be enrolled at HC-II units serving the same village.

Because the study is complex and covers large, non-contiguous geographic regions, study activities will be introduced to the field in phases, starting at one site and then moving to the next site after consolidating lessons learned from the initial sites. Phase I of the study will be implemented northcentral Uganda, where historical BL data have already been analyzed to

identify eligible parishes within a 100 mile radius of the hospital. Phase II will involve introduction of field work in northwestern Uganda. Analysis of historical BL data from that region will be used to determine the eligible parishes. Phase III will introduce field work in northern Tanzania. As with the other sites, historical analysis of BL data from the region will define the eligible parishes. Phase IV will introduces field work in western Kenya. Each phase will commence in 6-12 month intervals to allow for incorporation of adjustments based on the experience at initial. Activity will continue in all these sites until the sample size is attained, or the expected 5 year duration from the Phase I site.

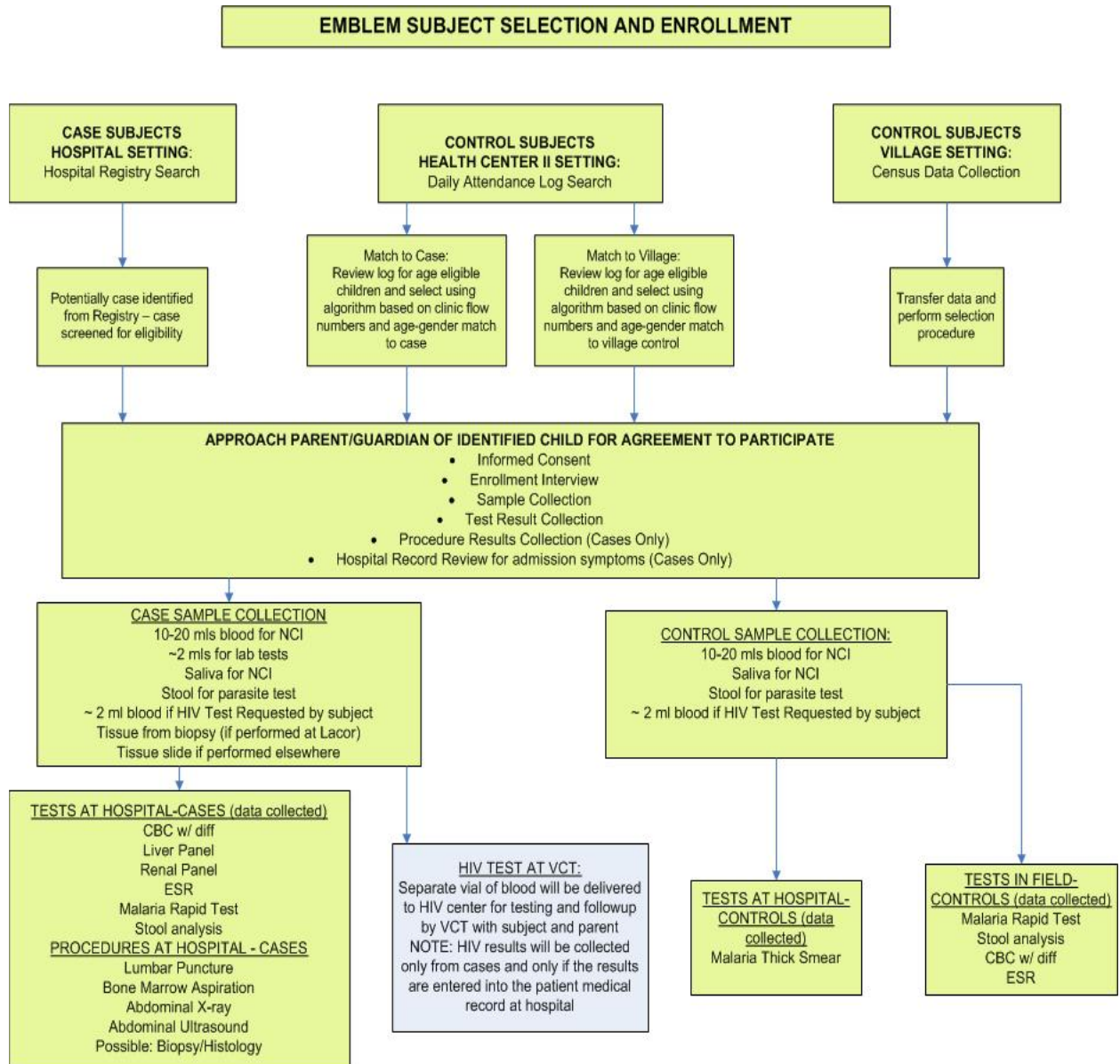
## **1.5 Data Collection Activities**

Data collected from subjects will include:

- Questionnaire data administered by an interviewer to elicit exposures related to birth and residence of the child, parentage and socioeconomic status, household exposures, and childhood illnesses and vaccination.
- Laboratory test results using samples collected from the subject.
- Physical examination measurements, including anatomic site involved for cases and height and weight for both cases and controls.

Figure 1-3 shows the overall study flow for subject selection and enrollment for both cases and controls. Table 1-1 gives an overview of the data collection activities related to each of the four study cohorts (Cases, HCII controls, Village controls at the HCII, and Village population controls).

**Figure 1-3**



**Table 1-1**  
**EMBLEM Data Collection Activities**

ACTIVITY	Cohort			
	Cases	Matched population controls	Pilot population controls	Pilot HCII Controls
Hospital Registry*	•			
HCII Registry^		•	•	
Census enumeration^^				•
Screening	•	•	•	
Enrollment	•	•	•	•
Informed Consent	•	•	•	•
Consent for release of tissue from outside institution	•			
Questionnaire	•	•	•	•
Physical Exam Measurements	•	•	•	•
Labs: Blood Draw+	•	•	•	•
CBC	•	•	•	•
Erythrocyte Sedimentation Rate	•	•	•	•
Liver/Renal Function Tests	•			
Malaria smear	•	•	•	•
Malaria rapid test	•	•	•	•
HIV serology†	•	•	•	•
Saliva Samples Collection	•	•	•	•
Stool Samples Collection/Microscopy	•	•	•	•
Bone Marrow Aspirate‡	•			
Lumbar Puncture‡	•			
Abdominal Ultrasound/xray‡	•			
Lung Xray‡	•			
Histology Report‡	•			

\*Hospital Registry occurs outside of EMBLEM as a concurrent activity. Presenting symptoms will be captured as part of the BL Registry database and accessed for patients enrolled in EMBLEM.

^HCII Registry is used as a mechanism for selecting appropriate controls from HCII on a given clinic day.

^^The census form will document the individuals enumerated in the villages from which the population controls will be randomly selected (serves as the screener for the population controls).

+In addition to performing lab tests, samples will be processed and aliquotted for storage and future testing (i.e. EBV genotyping assays; Immunohistology assessments; Antimalarial antibody assessments; Malaria susceptibility genotyping).

†Results from HIV Serology will only be reported to patients if consent obtained from participant. Those results will be communicated directly through the Ugandan Ministry of Health mechanism.

‡These evaluations are performed on an “as prescribed basis” outside of EMBLEM and data is provided for study patients.

## **1.6 Participating Institutions**

Below is a brief description of each of the participating institutions. Specific contact information is included in the chapter appendices. Appendix 1-2 displays the contact information for members of the NCI, Ohio State/Department of Pathology, Makerere College of Health Sciences, IMS and Westat project teams. Appendix 1-3 displays the contact information for members of the local project teams in Uganda. Contact information for Kenya, and Tanzania will be updated when the study starts field work in those locations.

### **1.6.1 National Cancer Institute**

The Infections and Immunoepidemiology Branch (IIB) of the Division of Cancer Epidemiology and Genetics, National Cancer Institute (NCI) studies the role of human viruses in the causation of cancer. IIB conducts epidemiological studies of HIV/AIDS and the impact of the epidemic on cancer risk. In addition, specific projects address the role of DNA and RNA tumor viruses in the pathogenesis of cancer. This study is being conducted under the direction of Dr. Sam Mbulaiteye (IIB) with Dr. James Goedert, M.D. (IIB), Ruth Pfeiffer, Ph.D. (Biostatistics Branch, NCI), Kishor Bhatiya, Ph.D. (AIDS Malignancy Program, NCI), Tom Pohida, M.Sc. (Division of Computational Bioscience, Center for Information Technology, NIH), and Steven Chanock, M.D. (Advance Technologies Center, NCI) as co-investigators.

### **1.6.2 UGANDA: St. Mary's Hospital (Lacor Hospital)**

St. Mary's Hospital (Lacor Hospital) is a 480-bed non-profit charitable institution (<http://www.lhospital.org>) located 6 km outside Gulu town, about 350 km north-northwest of Kampala, the capital city of Uganda. Gulu town is the largest town in northern Uganda, east of River Nile. Lacor is the third largest hospital in Uganda and receive an outpatient caseload of 280,000 in 2006/7. Based on 2006 figures, it has been estimated that approximately 150 BL cases are admitted to the hospital annually.

Local co-Principal Investigator:

Dr. Martin D. Ogwang, Medical Director, Lacor Hospital

### **1.6.3 UGANDA: Kuluva Hospital**

Kuluva Hospital is located 12 km from Arua town, about 500 km northwest of Kampala close to the border with Sudan and Democratic Republic of Congo. Kuluva Hospital has the reputation of being the best hospital in Arua and has along history of being the only hospital with facilities to diagnose and treat BL. The hospital was the site of all studies conducted in the West Nile district in Uganda in the 1960s.

Local co-Principal Investigator:

Dr. Patrick Kerchan, Medical Superintendant, Kuluva Hospital



#### **1.6.4 The African Field Epidemiology Network**

The African Field Epidemiology Network (AFENET) is a non-profit organization established in 2005 by U.S. [Centers for Disease Control and Prevention \(CDC\)](#) and the United States Agency for International Development (USAID) to help African nations enhance or develop their own applied epidemiology capacity (<http://www.afenet.net/>). The mission of AFENET is to improve the health of people in Africa by strengthening and expanding applied epidemiology and laboratory capacity on the African continent. AFENET pursues its objectives by working in partnership with Ministries of Health, non-government organizations, international agencies, private sector, and other public health agencies coordinated by a secretariat located in Kampala, Uganda.

AFENET is currently supporting more than ten projects in more than ten different countries in Africa, including Uganda, Tanzania, and Kenya. AFENET has capacity to provide technical and human resource support to ministries or local departments, establish, insert, or supervise monitoring, evaluation or surveillance activities, and to collect and manage data. We plan to establish a sub-contract with AFENET to implement our field activities. AFENET will implement its sub-contract by hiring competent field staff to do the field work, provide a local coordinating office at its Secretariat, and ensure timely delivery of supplies to the field and reports and data to NCI. AFENET provides epidemiological and laboratory support to government, academic and international agencies in Africa from its secretariat in Kampala, Uganda. AFENET will hire dedicated staff and will provide local support to the field teams through a subcontract with Westat International.

#### **1.6.5 Information Management Services, Inc. (IMS)**

IMS is responsible for overall study coordination, data systems design, management and analysis. IMS provides study management and computing services support to multiple studies funded by The National Institutes of Health (NIH). IMS employs approximately 200 staff consisting of programmers, statisticians, and biomedical specialists. IMS has offices in Rockville and Silver Spring, Maryland.

The IMS staff for EMBLEM includes Project Director Janis Beach and Study Managers Carol Giffen and Laurie Rich.

#### **1.6.6 Westat**

Westat is responsible for managing subcontracts with organizations supporting and conducting data collection for EMBLEM and for purchasing equipment needed for study laboratories and for transportation. The Westat project team includes Project Director Jeanne Rosenthal and Study Manager Janet Lawler-Heavner.

Westat is an employee-owned research company that serves agencies of the U.S. Government, as well as businesses, foundations, and state and local governments. Westat is well known for extensive skills and expertise in epidemiology, biomedical science, clinical trials, statistical survey design, survey methodology, data collection operations, and data management,

analysis and dissemination. Westat's headquarters are located in Rockville, Maryland with several satellite offices around the U.S. Westat also maintains international offices in China, Liberia, Costa Rica, Ethiopia, India, South Africa and Thailand. The professional staff of over 2,000 is enhanced by a large number of additional staff members involved in data management and processing activities at our Telephone Research Center and throughout our nationwide field interviewing operations.

#### **1.6.9 Ohio State University/AIDS Cancer Specimen Resource/Department of Pathology, Makerere College of Health Sciences**

We are collaborating with OSU/ACSR to evaluate histology at Lacor and to construct an endemic BL TMA for detailed study to avail it to U.S. researchers for morphological and molecular characterization. This opportunity led to the review of histopathology at Lacor and the subsequent plans to improve histopathology so that the tissues obtained during research can be studied using state-of-the-art morphology, immunohistochemistry, and molecular methods. The work at Ohio State University is funded through a budget supplement from the NCI Directors Office to the AIDS Cancer Specimen Resource, which will pay for the molecular pathology studies of Burkitt lymphoma.

Co-Investigator in charge of pathology: TBD

#### **1.6.10 Uganda Virus Research Institute (UVRI)/NIH/Uganda Project**

The Uganda Virus Research Institute (UVRI) was established in 1934 as a yellow fever research institute. In the 1960's, the mission of this institute was broadened to become a multi-disciplinary research institute into tropical virology. UVRI became a focal point of investigation of the HIV epidemic and currently support several research projects including CDC (HHV-8 and HIV), Walter Reed (HIV-1), MRC (HIV-1), NIH (vaccine development), and WHO/UNEPI (polio). Its laboratories and staff are able to support the tissue collection, processing and storage proposed in this study. The Team Leader of the NIH/Uganda Project will provide strategic help to the study, including receiving and forwarding capital equipment to local sites, facilitate review and approval of the protocol at local IRBs, and temporary storage, and shipping of biological samples to the NCI. The Virus Research Institute will also be responsible for storage of specimens during the study period.

#### **1.6.11 Kenya: Webuye District Hospital**

Webuye District Hospital is located in Bungoma County, in Western Province, ~about 500 km from Nairobi and about 70 km from Eldoret. It has a bed capacity of 220 beds. Investigators from Moi University, School of Medicine have successful on-going studies at Webuye District Hospital and several other groups have ongoing projects demonstrating the ability of these sites to conduct epidemiological investigations. There is an active Demographic Surveillance System (DSS) established in Webuye which can be a good source of information of disease indexing and other sociodemographic profiles in the region.

Local Co – Investigator,

Dr. Constance N Tenge, Paediatrician, Moi University, School of Medicine.

#### **1.6.12 Kenya: Homabay District Hospital**

Homabay District Hospital is located in Homabay County, in Nyanza Province. It is about 150km from Kisumu and about 220km from Eldoret. It has a bed capacity of about 250 beds. It is one of the hot spot for BL in the region. Several groups have ongoing projects in Homabay District Hospital demonstrating the ability of this site to conduct epidemiological investigations. It also hosts a medical training center

Local Co- Investigator.

Dr Constance Tenge, Paediatrician, Moi University, School of Medicine.

#### **1.6.13 Kenya: Research and Sponsored Project Office (RSPO)**

Established in 2003 under the Umbrella of Moi University and Moi Teaching and Referral Hospital (MU/MTRH). The role is to manage the financial and administrative activities of the donor funded projects. The office is located at the AMPATH building at MTRH, Eldoret, Kenya. RSPO has been subcontracted to manage EMBLEM finances in Kenya.

#### **1.6.14 Kenya Medical Research Institute**

The Kenya Medical Research Institute (KEMRI) is a state corporation established through the Science and Technology (Amendment) Act of 1979, as the national body responsible for carrying out health research in Kenya. KEMRI has grown from its humble beginning 27 years ago to become a regional leader in human health research. The Institute currently ranks as one of the leading centers of excellence in health research both in Africa as well as globally. KEMRI may be requested to offer permanent storage of research samples.

#### **1.6.15 Bugando Medical Center/ Shirati Hospital/ SHED Foundation**

The Tanzanian government, supported by Interchurch Mission Medical Assistance (IMA) World Health (Baltimore, Maryland), is pursuing an aggressive policy to decentralize Burkitt lymphoma treatment. Under this program, six faith-based hospitals in the Mwanza and Mara Regions in northern Tanzania receive drugs for treatment of BL. These regions are about 1200 km from Dar Es Salaam, the capital of Tanzania, where the tertiary cancer center, Ocean Road Cancer Institute (ORCI), is located. Together, these centers receive ~200 patients per year. The current study is targeting to enroll from Bugando Medical Center in Mwanza Region and Shirati Hospital/SHED in Mara Region. These hospitals treated ~1114 patients from 2001 to 2006.

Local Principal Investigator:

Dr. Esther Kawira, Medical Director Shirati Health, Education, and Development Foundation Shirati, Tanzania

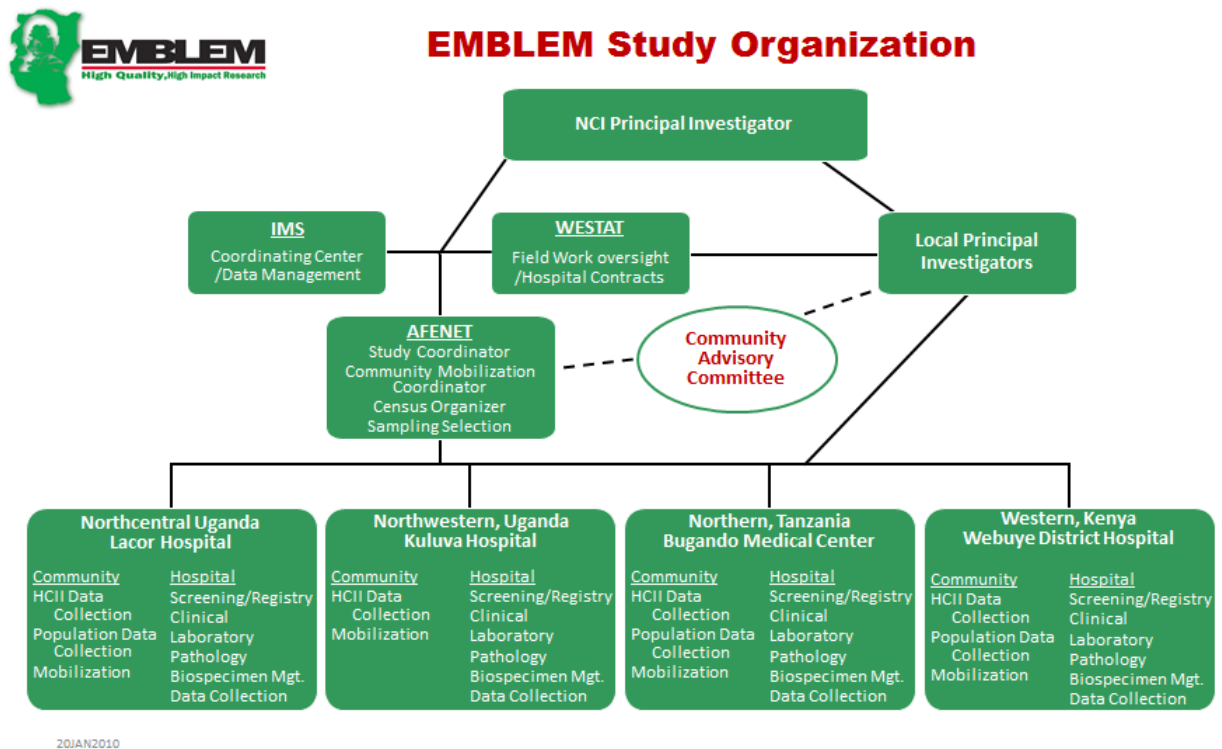
Local co-Principal Investigator:

Dr. Nestory Masalu, Head-Oncology Department Bugando Medical Centre

## EMBLEM Organization Structure

Figure 1-4 illustrates the Emblem study organization. In addition to oversight by the funding organization (NCI) and their US collaborators, study activities will be monitored by a Community Advisory Committee comprised of each country's local Principal Investigators and the staff from AFENET.

Figure 1-4



## 1.6 EMBLEM Enrollment Schedule

Table 1-2 shows the projected case attendance and case enrollment by site and accrual year.

**Table 1-2**  
**Study Enrollment Projections by Region**

	<b>North-Central Uganda</b>		<b>Northwestern Uganda</b>		<b>Northern Tanzania</b>		<b>Western Kenya</b>	
	Attend	Enroll	Attend	Enroll	Attend	Enroll	Attend	Enroll
Accrual Yr1	150	75	50*	25	NA		NA	
Accrual Yr2	150	98	100	65	125*	81	100*	65
Accrual Yr3	150	113	100	75	250	188	200	150
Accrual Yr4	150	135	100	90	250	225	200	180
<b>TOTAL</b>	<b>600</b>	<b>420</b>	<b>350</b>	<b>255</b>	<b>625</b>	<b>494</b>	<b>500</b>	<b>395</b>

Table notes:

We project that the study will accrue 409 cases by end of year 2 and 900 cases by end of year three, assuming 70% average enrollment.

\* Enrollment will be slow in the first year of the study at each site, but should improve thereafter.

Case enrollment in North-Central Uganda is expected to begin in mid-second quarter 2010. HCII controls will begin enrolling for North-Central Uganda in third quarter with population controls following by the end of that quarter. It is anticipated that each site will begin case enrollment approximately six months after the previous site is initiated.

## Appendix 1-1

### A. Districts in catchment area for Northern Uganda

District	No. of Counties	Sub counties	Parishes	Villages
ADJUMANI	1	6	35	176
AMOLATAR	1	4	35	345
AMURU	2	8	49	233
APAC	3	15	78	1125
ARUA	4	18	106	918
DOKOLO	1	5	32	356
GULU	3	15	66	324
KITGUM	2	19	90	698
KOBOKO	1	5	43	312
LIRA	4	19	118	1527
MOYO	2	8	25	223
NEBBI	3	19	84	1300
NYADRI	2	13	71	762
OYAM	1	7	39	804
PADER	2	18	71	1008
YUMBE	1	8	42	322

### B. Counties in catchment area for Western Kenya

HOSPITAL	PROVINCE	COUNTY
WEBUYE D.H	WESTERN	BUNGOMA
		BUSIA
		KAKAMEGA
		UASIN GISHU
		VIHIGA
HOMABAY D.H	NYANZA	KISUMU
		HOMABAY
		MIGORI
		KISII
		SIAYA
		NYAMIRA

C. Districts in catchment area for Northern Tanzania

<b>MWANZA</b>
Ukerewe
Magu
Mwanza
Kwimba
Sengerema
Geita
Missungwi
<b>MARA</b>
Musoma Rural
Musoma Urban
Bunda
Serengeti
Tarime

**Appendix 1-2**  
**Project Team in the United States**

Staff Name	Title	Location	Phone	Fax	Email
<b>National Cancer Institute</b>					
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## Collaborating Staff in Uganda

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## COMMUNITY MOBILIZATION CHAPTER

### 1.0 Introduction

Epidemiology of Burkitt Lymphoma in East African children and Minors (EMBLEM) is a case control study that will enroll 1500 BL cases and 3000 age, sex, residence frequency matched controls from four regions of East Africa namely north central and north western regions of Uganda, western Kenya, and Tanzania. BL cases will be enrolled from four regional Hospitals of Lacor and Kuluva Hospitals in northcentral and northwestern Uganda respectively, Bugando Medical Centre in northern Tanzania and Webuye District Hospital in Western Province and New Nyanza Provincial Hospital in Nyanza Province in Kenya. The study areas were chosen because endemic BL is fairly common in these areas.

### 1.2 Staffing

**Table 1: EMBLEM staff for community mobilization**

	Position	Functions	Required skills
1	Principal investigator	Overall authority	
2	Co-principal Investigators	Oversees and sanctions mobilization activities within the hospital. Link between the EMBLEM team and hospital staff	Administrative and public relation skills
2	Study coordinator	Delegated duty to sensitize community on EMBLEM	Public relation/communication skills
3	Survey manager	Responsible for planning and monitoring of survey activities	Public relation/communication skills
4	Community mobilizes	Mobilizes community, distributes study materials like posters, brochures and pamphlets	Community mobilization skill, Knowledge of local community
	Interviewers	Administers study questionnaires, and consents	Interviewing skill
5	Community Guides	Show direction to study staff during field activities	Knowledge of geographical layout of area, local comm
6	Interpreters	Interprets study materials into local languages	Fluency in both English and local languages spoken in study area



	Position	Functions	Required skills
7	Drivers	Transportation of study staff to study activity areas	Communication skills, knowledge of geography of study area, multi-talented

### 1.3 Preparing for the task

Before engaging in any mobilization activities the above staff will be selected through competitive interviews and trained. The training will equip the EMBLEM staff with;

- ☐ Effective communication skills
- ☐ Concepts of community mobilization,
- ☐ Working knowledge of the target community and the surrounding environment
- ☐ Above all the goals of EMBLEM

### 1.4 Objectives of community mobilization

- ☐ To inform the community in the study region about the BL study in Lacor and Kuluva Hospital in north central and wester Uganda respectively, Bugando medical centre in northern Tanzania, Webuye District Hospital in western province Kenya, and New Nyanza provincial Hospital in Nyanza Province , Kenya
- ☐ To secure full cooperation of the community in the study region through dissemination of correct knowledge about the study such that the community willingly participates in study related activities such as the Census, sero-survey, HC II activities, and referral of BL cases to the enrolment centres
- ☐ To encourage the community in the study region to timely refer BL cases to these hospitals where diagnostic and treatment expertise is availablle

### 1.5 Community mobilization tasks

#### 1.5.1 The EMBLEM team has the following tasks;

- ☐ Raise awareness among community and their leaders about Burkitt lymphoma and EMBLEM
- ☐ Conduct a stake holder's analysis and classification in the area

#### 1.5.2. Stake holders for EMBLEM

- ☐ Hospitals providing care to the children in the study area and their staff,
- ☐ Influential opinion leaders in the community, including priests,
- ☐ Community based organizations,
- ☐ School's local authorities
- ☐ The media.

Stakeholder's analysis will help EMBLEM team tap into the influence structure of the community, understand who is doing what and where, above all identify the decision makers in. The EMBLEM team will contact and influence key policy makers. During these contacts the team will emphasize the need for combined effort the local citizens and institutions to solve the problem of Burkitt lymphoma .The major institutional leaders targeted for influence will include, Resident District Commissioners, institutional Directors of hospitals, Local Council Chairpersons I-V, in charges of HC IIs, District Health Officers, Social and welfare workers, health workers, religious leaders, traditional and opinion leaders. It is the authority and power of these leaders that will give impetus

At every level of contact, the team will identify the most appropriate and effective approach through which to will articulate the problem of BL in the community by emphasizing the following;

### **1.5.3 Message to the stake holders**

- ☐ That BL is human and social tragedy
- ☐ The need for effective leadership for the community to understand that BL can be cured
- ☐ That BL can be diagnosed and treated at few centers in Uganda
- ☐ That treatment of BL at the hospitals will be free to the patient

### **1.6 Communities for EMBLEM**

For EMBLEM, three types of communities are envisaged: a) Hospital communities, b) Health center communities, and c) village, settled communities. These different settings bring different sets of challenges and opportunities that must be addressed or taken advantage of to protect the principal goals of the study. For EMBLEM, the principal field goals are three: a) enroll the specified number of cases for northern Uganda) within the specified period of time (60 months), b) enroll the specified matched controls and population controls as stipulated in the protocol, and c) perform

these enrollment activities with full and willing cooperation from general community and its leaders.

### **1.6.1 Authority to contact the community on behalf of EMBLEM**

Permission to contact the community for any study related activity is restricted and rests with the Principal Investigators in the country where the study is being conducted. Principal investigators are organized as the Steering Committee. This should be understood by everybody acting on behalf of EMBLEM so as to avoid sending conflicting information to the same community for the same activity. EMBLEM team has recruited staff with different skills and tasked them with different roles. The team leadership of this skill mix is bestowed upon the Study Coordinator who executes these duties on behalf of the Principal investigators. The Study Coordinator can delegate these responsibilities to another staff depending on the task at hand.

### **1.6.2 Delegation of authority from Local Co-PI to study coordinator**

In the Hospitals, the Co-Investigators can delegate the Study coordinator to do the following,

Write invitational letters to District leadership endorsed by the Co-PI. organize sensitization meetings for community leaders at all district levels, hold educational and planning meetings with community leaders at all district levels, make contact with the media such as radio talk shows, attend health related functions on behalf of the local Co-PI to promote EMBLEM, make presentations in academic circles in the interest of EMBLEM

<b>1.6.3 Administrative authority in study region</b> Ministry of Health	National	Minister
National Referral Hospital	National	Director
District Health office	District	Local Council V
Health Centre Four	County	Local Council IV
Health Centre Three	Sub- County	Local Council III
Health Centre Two	Parish	Local Council II

<b>1.6.3 Administrative authority in study region</b> Ministry of Health	National	Minister
Health Centre I (Individual)	Village	Local Council I

Paramount is obtaining permission from local authority's at all administrative levels, including districts, parishes and villages, where discrete study activities will be implemented. Requests for permission will always be accompanied by relevant general explanations of the objectives of the study, the study procedures, emphasizing how participant's rights are protected within the context of the study, safe handling of confidential data and hazardous material within the community, benefits of the study to the community. Questions raised by leaders will be answered in a timely manner. Leaders will also be given contact information where to send questions that arising after permission to conduct the study

#### **1.6.4 Tools for community mobilization**

The main tools that for community mobilization includes,

- ☐ BL tutorial,
- ☐ Educational meetings
- ☐ Sensitization workshops
- ☐ Formal and informal meetings
- ☐ Media; Print and electronic media
- ☐ T-shirts with printed messages about the study
- ☐ Educational articles in Weekly or Dailies newspapers

All these tools have one thing in common. They have been standardized to carry the same message about BL and the study.

#### **1.6.5 BL tutorial**

This important tool for community mobilization will carry the following messages; what Burkitt Lymphoma is, how and where it is diagnosed, possible causes of BL, suspected signs and symptoms of BL its distribution in Equatorial Africa, where one should go in case of suspected Burkitt Lymphoma, possible association of BLwith malaria, HIV and EB virus, where one can go for help in case of suspected BL, treatment of BL including institutions treating BL.

For wide coverage, this Tutorial will be translated in all the local languages spoken in the study region.

##### **1.6.5.1Tutorial audience;**

The main audience will be the literate community. In the study region these are; health workers in the hospitals providing care for children with BL, HC II where matched and population controls will be recruited, community leaders in the 12 survey villages and 88 villages where matched population controls will be obtained. Schools in the study region, caretakers of children admitted with BL. The tutorial also targets other caretakers of patients with other diseases in the hospital providing care for BL

#### **1.6.5.2 Tutorial use**

EMBLEM staff will distribute the written scripts will be distributed to be read in Hospitals, HC IIs, however the distribution will be limited to only those who are likely to understand the written word. The audio visual scripts will be shown to care takers of BL cases on TV screens using DVDs in BL wards, to participants of sensitization seminars, educational meetings using LCD. The audiovisual tutorial also will at times be used to attract the local community in the census villages, to attract people for sensitization meetings.

#### **1.6.5.3 Associated posters**

These posters carry the same messages like the tutorial but in addition some posters will carry messages in pictorial form describing the concept and process of obtaining consent from eligible cases of BL. Another one deals with in pictorial form misconceptions about why people seek treatment late yet another carries encouraging messages that BL can be cured. These messages compliments and reinforces the one carried in the tutorial

#### **1.6.5 Distribution of posters;**

These posters will be distributed in all BL activity areas in hospitals for enrollment of cases, HC II where f matched controls will be enrolled. They will also be distributed to community leaders during educational meetings. They will be displayed on walls in Laboratory, consent room and EMBLEM office. Community mobilizers, interpreters will be the main distribution agents

#### **1.6.6 Media**

There are 21 local FM radio stations, two English dailies and three vernacular weeklies in north central and north western Uganda. The radio stations have very wide coverage and good listenership. The local PIs will invite these media houses to provide coverage during important study activities like sensitization seminars, educational meetings, and community leaders meetings. Information gathered about the study by these media

agents will be aired over their radio stations and printed in the Newspapers. Access to these media houses is restricted. All information will be crosschecked by the local PI before broadcasting. In case of wrong information broadcasted about the study, the PI has the right to take the matter with the management of the media houses who will make amendments accordingly. With authority from the local PI, the study Coordinator may contact these media houses to arrange for talk shows or sending announcements to the community.

## **2.0 Obtaining support from the community**

### **2.1 Approaching district officials**

The district leaders are busy people. Any support from the community will come through them. At the district level, the study coordinator will prepare in a file folder a brief summary of the essential information about the study, what the study is about, its design, objectives, where the study is being done, why its being carried out, who are behind the study, its approvals and contractual partners, its benefits, risks, Such information will be used to approach the top leadership of the district who are busy people and do not have a lot of time. At times study coordinator will send invitation letters to the district leadership calling them for meetings. This may also be done through phone calls and e-mails

#### **2.1.3 Approaching village officials**

At the parish or village level, study coordinator will write to the Local Council one and his executives of the area for a meeting in a central place preferably in the premises of the hospital, health centre, primary school or church compound. During these meetings, the village leaders will be given clear information about Burkitt Lymphoma in general just like in the BL tutorial and the specific information about the study activity being planned in their area, why it is being planned, when it is being planned, who will carry out the activity. The leaders will be given contact of the study coordinator or any designated person to be contacted for nay change in plan or clarification

Armed with this information, the village leaders will return to their villages to sensitize their community about BL and EMBLEM. They can do this through a village meeting. After these sensitization meetings. The study coordinator will arrange another planning meeting in the parish or village targeting all the LC executives, opinion, and church, traditional and cultural leaders. This will now be a planning meeting during which the village leaders will identify date, time and venue for the upcoming study activity like

census. It is during these village meetings that the study Coordinator will obtain village consent to carry out activities like enrollment of population controls, census. During these meetings disagreements, conflicting interests will be solved.

### **3.0 Community Advisory Board:**

A group of persons within the study area appointed by the Principal Investigator and tasked with provision of open line of communication between EMBLEM study team and communities afflicted by Burkitt and Malaria. CAB will consist of six members per study region appointed by the principal investigators. A chairman will head the Advisory Board assisted by a deputy. A secretary will be responsible for taking all minutes of the board's proceedings. A liaison officer will be a direct link between the community and the board. These four core members will form the executive members of the board. Other members will act in their advisory capacity.

### **3.1 Membership**

Shall comprise of persons of diverse background including those resident within the study area, the following criteria could be used; a) persons respected by the community for their reputation and credibility b) parents of former patients with BL; these are people with experience of the disease, c) NGOs working within the study region with interest in malaria/HIV/Cancer d) Civil organization groups with portfolio to protect rights of persons. Membership of CAB will come from Religious groups, senior's citizens, women groups, cultural and opinion leaders

### **3.2 Roles of Community Advisory Board**

- ☐ Provide knowledge, information and local perspective of the community to the research agenda (Burkitt)
- ☐ Give general directions of ongoing emblem research
- ☐ Advise on cultural sensitivities (taboos) which may impact on study
- ☐ Together with local leadership provide a link between communities and the study
- ☐ Promote positive relationship between community and the research team
- ☐ Participating in evaluation and Monitoring of the study process
- ☐ Supporting study participant patient welfare and providing a link to study team/hospital
- ☐ Providing participants with easy access to the research, clinical and community resources
- ☐ Assist in planning ,development and implementation of Burkitt cancer research
- ☐ Assess community impact and ensure that community concerns are considered
- ☐ Serve as voice of the community and study participants before the Study Steering Committee
- ☐ Sensitise / Prepare community for future research agenda (beyond EMBLEM building local capacity)

- ❑ May serve in any capacity approved by the Study Steering Committee to advance objectives of the study

It should be noted that members of CAB will be reimbursed their sitting allowances for attending meetings, but such reimbursement will be determined by the PI and will be within the usual local government rates and will not be so high to be considered a bribe

### **3.3 Relationship of CAB with EMBLEM team**

. CAB will avail minutes of the meetings and its resolution to the local PI and study coordinator putting emphasis on the following points; action points and responsible persons assigned to follow its resolutions clearly stated. The Local PI or Study Coordinator will report all major challenges that EMBLEM may meet in the community as a result of conduct of the study. Likewise the community will make their concerns about EMBLEM known. The chairman of CAB will assign the Liaison officer to investigate any allegation arising both from the community and the Hospitals. He will be assisted by the study coordinator. Both will file a report to the committee who, after careful deliberation will forward their decision for review and final decision by the Study Steering Committee. The study coordinator will also report all adverse events and its impacts at community level to CAB

### **3.4. Professional organizations support**

The study will also be presented to professional fora involved in research such as Makerere University School of Public Health, Gulu University, Moi University and Educational meetings for clinicians in Lacor, Kuluva, Webuye District Hospital and Bugando Medical Centre, Uganda cancer institute. Through these presentations, EMBLEM intends to gain useful critique from professional colleagues about the design and conduct of the research.

#### **3.4.1 Cultural institutions support**

Through courtesy calls, visits the study coordinator with permission from the PI will represent the study to cultural, opinion, religious leaders, and village elders. Support from these institutions will be very important for mobilizing community consent and support for the study especially where resistance is anticipated. These leaders are also important for maintaining the continued support of the community in the project. Support of politicians will not be ignored. EMBLEM community mobilizer will through visits explain to them the objectives, strategies and benefits of the study



### **3.4.2.. Other organizations support**

Support also will be solicited from organizations that are doing complementary work in the field. Joint planning, sharing of information resources will avoid conflicting and parallel activities. In addition this will minimize inter-organizational conflict.

Arrangements will be made to place the study teams in ongoing field exercises like surveys organized by these organizations in order to gain some hands on experiences in the field. Such collaborations will strengthen inter organization links and reduce conflicts of interest.

## **4.0 Community mobilization for census**

The EMBLEM census will be implemented through three interlinked phases in 12 parishes selected from northern Uganda; (i) pre-enumeration phase (ii) enumeration phase (iii) Post enumeration phase the following will form the core of the community mobilization activities for the census

### **4.1 District Leadership approach**

The study Coordinator may decide to invite district leaders from the districts where the selected villages for the survey for a sensitization meeting. During the sensitization workshop, a various EMBLEM staff may present information about the study design and operational plan.

The participants of the workshop will be determined by the objectives of the workshop, but they may be drawn from the District Health Offices, District Planning, Community Development, and District Population Offices from all the Districts where the EMBLEM project will be implemented. The workshop will be the first official step to outline the necessary arrangements for the Census. Pertinent issues regarding the overall EMBLEM survey exercise will be discussed. A consensus by the participants to support the project is expected to be obtained. It is envisaged that getting support from the necessary technical and other staff will serve as a gateway to indirectly advertise EMBLEM project.

### **4.2. Publicity and mobilization for census.**

The successes of the EMBLEM project will be to a large extent depend on the population knowing the benefits of the exercise to them. For this reason a public awareness campaigns will be conducted prior to and during the EMBLEM field work. The aim of the campaign is to maintain the cooperation of the public and therefore reduce on non response and non consent during the Census implementation by informing the

public of the usefulness of the information obtained and the confidentiality of the data provided.

For the household census, a provision is made to meet opinion leaders and the technical staff in the study villages. Before the census. The proposed people to be met in each village includes 2 members of the Local council One executive, a member of the District health Team, 2 other opinion leaders. The village team will be charged with responsibility of sensitizing the rest of the population to support the Census. This is likely to take two weeks.

#### **4.4 Households Approach for census**

After the two weeks has elapsed, EMBLEM team will convene a bigger meeting in the survey villages. During these meetings, the UBOS together with EMBLEM team agree with the village team on how best to conduct the census, fix a date, time and place for the exercise. This is the time the village team will be requested to suggest names of capable people who will not only meet the selection criteria of UBOS but should also be trusted by the community. To identify the households, UBOS and EMBLEM team will work with village elders like 'Rwot Kweri' who will act like village guides. Additional work force will includes village Health Team Members

#### **5.0 HC II for enrollment of population control**

To gain full cooperation of the HCII community for the enrollment of population controls, the HC II managers will be given clear and timely information about EMBLEM. This information is contained in BL tutorial and process of obtaining consent poster. Two weeks before the enrollment exercise, EMBLEM team will invite the HC II managers for one day sensitization meetings. To enhance response rate, there will be one meeting for each of these sub-regions of Lango, Acholi, and West Nile. The venue for these meetings will preferably be in the respective District Health Offices. The EMBLEM team will discuss with the HC II managers to chart the best way to obtain consent taking into account the local beliefs and cultures. At the end of the workshop, the HC II managers will be given copies of the BL tutorial, Consent process posters, posters to take to their respective units to be used for sensitization of the other staff who will not attend the meeting.

The following key messages will be emphasized to the HC II managers (a) Lacor and Kuluva is conducting a study on Burkitt lymphoma (b) the HC II managers should have a high suspicion index for BL (d) once suspected they should timely refer these cases to these two hospitals where diagnosis and treatment is freely available. Others posters that addresses community misconceptions about cancer in general and BL in particular

will be discussed. Other focus of discussions will be challenges that the EMBLEM team are likely to face during the enrollment of HC II population controls. The EMBLEM team will also clarify the link between the census, and HC II control enrollment. One community mobilizer will be task to mobilize the survey villages

Just before the actual enrollment, the EMBLEM team will organize a second meeting with the HC II staff. The following are expected to attend, the chairman Health Unit management Committee and his executives, HC II staff, Local Council I chairperson or his representative, one opinion leader, cultural, leader, a church leader. During these meetings, EMBLEM team together with the HC II team decide on the date, time, and persons to assist the EMBLEM team

## **6.0 Mobilizing the Health Centre II for matched controls**

EMBLEM team will keep track all of newly screened and eligible cases of BL from the hospital BL Log book, the key details of age, sex, and residence will be kept in a diary. From this information, the EMBLEM team will use the Health facility register to identify the nearest HC II these cases came from.

### **6.1. Initial stage of enrollment**

In the initial stages of matched control enrollment, all approaches to the HCII will be done through the office of the District Health Office. One week prior to the teams visit to the HC II, the study Coordinator will give a written prior notice to office of the DHO in the district where the case originate from. The Coordinator will give details such as name of HC II, purpose, day, date and time of their intended visit. In the same letter, the Coordinator will request the DHO to inform the HC II manager to prepare for this visit.

### **6.2. Informing HC IIs manager**

The DHO will inform the HC II manager request them to disseminate the information of the team's visit to all the other HC II staff in order to sensitize them of the visit. On the day of enrollment the EMBLEM team will inform the office of the DHO of their presence in the district before proceeding to the HC II for enrollment. On arrival at the HC II, the team will report their presence to the area LCI chairman for security purposes. After this the team will proceed to convene a brief meeting with all the HC II staff. They together with the HC II staff will agree on the best way to carry out the exercise. The HC II staff may be motivated with light refreshments

### **6.3 Later stage of enrollment**

All the above protocol may not be necessary at a later stage of the matched control recruitment because the information about EMBLEM will have reached all the hierarchy of the district health infrastructure. The DHO may delegate authority to the managers of Health Sub-Districts where the HC II are located. In that case the EMBLEM team will deal directly with the Health Sub-District managers. It is hoped that this will lessen the bureaucracy.

## **6.4 Hospital for enrollment of cases**

Each Hospital where BL cases will be enrolled has signed a Limited Scope of Work with NCI to provide support for planning and establishment of infrastructure needed to conduct the EMBLEM study. Through this SOW agreement, each hospital will secure an office space that can be used for the study. Another space will be provided for the deep freezer and other Laboratory equipments to be used in the study. They have also agreed on a percentage time for each of the following hospital staff; Pediatrician, Nurse Specialist, Health Educator, and Data Clerk, to assist the study Clinical Coordinator and Data Manager in planning and preparation for enrollment of cases. Under the SOW agreement, the Hospitals will coordinate logistics needed to implement the study in the hospital settings including; identification of eligible patients, obtaining consent, collecting, processing and testing samples, collecting subject data and lab results.

The Hospital community however apart from policy matters needs to be educated about EMBLEM in order to gain their full cooperation. This is the task of the EMBLEM team. The team will organize education seminars for the various cadres of staff in the hospital. Hold planning meetings with hospital administrators, Posters with messages will be displayed in all BL activity areas like X-ray and Ultra sound departments, Dental units, children ward, outpatient Department

## **7.0 Working in hospital catchment areas**

When EMBLEM activity falls in the catchment of the hospitals providing care for the BL children or other district hospital, EMBLEM team will liaise with the hospital's staff particularly, Health Educators, Health Visitors, to mobilize the community in that way the EMBLEM team will not only strengthen the collaboration with the hospital but use the good name of the hospital in the community.

### **7.1 Hospital routines**

At all times, the EMBLEM team will try as much as possible to respect the routines of the hospitals. This is to avoid interfering with the care of the patients. The study Coordinator will be informed of all y conflicts of interest between the EMBLEM team and the hospital staff. The study coordinator will be informed. After making a thorough investigation, of the origin of such conflicts, he will liaise with the unit incharges to solve such issues that may arise. Should it not be possible, then the coordinator will inform the Local PI whose decision will be final.

## **Appendix A: Letter to District Health Officer requesting for permission**

Dear Sir/Madam

### **RE: PERMISSION TO CONDUCT STUDY ACTIVITY IN THE DISTRICT**

Lacor Hospital In collaboration with the National Cancer Institute USA is conducting a study called Epidemiology of Burkitt Lymphoma in East African Minors (EMBLEM).

EMBLEM is a case control study that will enroll 1500 BL cases and 3000 age, sex, residence frequency matched controls from four regions of East Africa namely north central and north western regions of Uganda, western Kenya, and Tanzania. BL cases will be enrolled from four regional Hospitals of Lacor and Kuluva Hospitals in north central and northwestern Uganda respectively, Bugando Medical Centre in northern Tanzania and Webuye District Hospital in Western Province and New Nyanza Provincial Hospital in Nyanza Province in Kenya. Your district was chosen because endemic BL is fairly common in these areas.

The major activities of EMBLEM will involve a) Case enrollment from participating hospitals b) Enrollment of controls from HC II and community and c) conducting a census in 12 selected parishes during wet and dry seasons by Uganda Bureau of Statistics personnel.

The purpose of this letter therefore is to request you to inform the district leadership, senior health managers, health partners, other stake holders together with HC II managers in your district about the study and request them to give as much cooperation to the EMBLEM team whenever they are carrying out study activities in their area.

For more information about the study, please contact Dr. David Martin Ogwang the Local Principal Investigator or Dr. Tobias Kinyera the Clinical Study Coordinator through these contacts:

Dr. David Martin Ogwang +256772593901, E-mail: [ogwang.martin@lacorhospital.org](mailto:ogwang.martin@lacorhospital.org)

Dr. Tobias Kinyera +256 772541976 or +256 E-mail: [tobiaskinyera@gmail.com](mailto:tobiaskinyera@gmail.com)

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**DR. David Martin Ogwang**

**The Institutional Director Lacor Hospital**

## **Appendix B: A letter to the HC II managers informing them about study activity**

**Dear Sir/Madam**

### **RE: ENROLLMENT OF POPULATION CONTROLS**

Lacor Hospital In collaboration with the National Cancer Institute USA is conducting a study called Epidemiology of Burkitt Lymphoma in East African Minors (EMBLEM).

EMBLEM is a case control study that will enroll 1500 BL cases and 3000 age, sex, residence frequency matched controls from four regions of East Africa namely north central and north western regions of Uganda, western Kenya, and Tanzania. BL cases will be enrolled from four regional Hospitals of Lacor and Kuluva Hospitals in north central and northwestern Uganda respectively, Bugando Medical Centre in northern Tanzania and Webuye District Hospital in Western Province and New Nyanza Provincial Hospital in Nyanza Province in Kenya. The study areas were chosen because endemic BL is fairly common in these areas.

The EMBLEM team, through the office of the District Health Officer was granted permission to conduct enrollment of HC II population controls from your HC starting from Date-----to date-----

On behalf of the EMBLEM team, I am requesting you to pass as much information about the study and this exercise to other health centre Staff as you can and request them to work with the EMBLEM team as much as possible. You will find much of the information about the study in the posters enclosed which I am requesting you to distribute to all the staff of the health centre. Every effort has been made to make sure that the exercise does not interfere with your daily routine patients care

For more information about the study and the exercise please contact the following persons:

Dr. David Martin Ogwang +256772593901, E-mail: [ogwang.martin@lacorhospital.org](mailto:ogwang.martin@lacorhospital.org)

Dr. Tobias Kinyera +256 772541976 or +256 E-mail: [tobiaskinyera@gmail.com](mailto:tobiaskinyera@gmail.com)

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**Dr. David martin Ogwang**

**Institutional Director Lacor Hospital**

## **Appendix C: A letter to LC I Chairman of a village**

**Dear Sir/Madam**

### **RE: PERMISSION FOR A MEETING**

Lacor Hospital In collaboration with the National Cancer Institute USA is conducting a study called Epidemiology of Burkitt Lymphoma in East African Minors (EMBLEM).

EMBLEM is a case control study that will enroll 1500 BL cases and 3000 age, sex, residence frequency matched controls from four regions of East Africa namely north central and north western regions of Uganda, western Kenya, and Tanzania. BL cases will be enrolled from four regional Hospitals of Lacor and Kuluva Hospitals in north central and northwestern Uganda respectively, Bugando Medical Centre in northern Tanzania and Webuye District Hospital in Western Province and New Nyanza Provincial Hospital in Nyanza Province in Kenya. The study areas were chosen because endemic BL is fairly common in these areas.

The EMBLEM team, with the help of the Uganda Bureau Of Statistics (UBOS) has been granted permission by the District Health Officer to conduct a census in your village starting from Date.....

The purpose of this letter is to request you to organize your LC I executive members for a meeting with the EMBLEM team headed by Tobias Kinyera on Date-----Time  
The key agenda will be as follows.

1. Introductions and prayer
2. Background to the Census
3. Obtaining community consent to conduct the Census
4. Identification of resource persons to work with EMBLEM team
5. Deciding on the appropriate date of Census

I request you to mobilize key opinion, religious and cultural leaders to attend the meeting as their contribution is invaluable. For more information, please contact: Dr. Tobias Kinyera the Study Coordinator

Tobias Kinyera on: +256 772541976 E-mail [tobiaskinyera@gmail.com](mailto:tobiaskinyera@gmail.com)

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**Dr. David martin Ogwang**

**Institutional Director Lacor Hospital**



## **Appendix C: A LETTER TO HOSPITAL DIRECTOR OF A HOSPITAL**

**Dear Sir/Madam**

### **RE: PERMISSION TO OBTAIN RELEASE OF TISSUE BIOPSY**

Lacor Hospital In collaboration with the National Cancer Institute USA is conducting a study called Epidemiology of Burkitt Lymphoma in East African Minors (EMBLEM).

EMBLEM is a case control study that will enroll 1500 BL cases and 3000 age, sex, residence frequency matched controls from four regions of East Africa namely north central and north western regions of Uganda, western Kenya, and Tanzania. BL cases will be enrolled from four regional Hospitals of Lacor and Kuluva Hospitals in north central and northwestern Uganda respectively, Bugando Medical Centre in northern Tanzania and Webuye District Hospital in Western Province and New Nyanza Provincial Hospital in Nyanza Province in Kenya. The study areas were chosen because endemic BL is fairly common in these areas.

For confirmatory diagnosis of BL, a tissue biopsy is being obtained from the eligible patients enrolled in the EMBLEM study. A patient with the following particulars was referred from your hospital with a diagnosis of BL. The medical record shows that a tissues sample was taken as part of the investigations. The same patient satisfied our eligibility criteria for enrollment in the study.

Lacor Hospital has obtained consent from patient X allowing you to release the tissues sample taken for the diagnosis so that it becomes part of the study samples. We guarantee all confidentiality that the patient deserves in handling the tissue sample

For more information about the study and the exercise please contact the following persons:

Dr. David Martin Ogwang +256772593901, E-mail: [ogwang.martin@lacorhospital.org](mailto:ogwang.martin@lacorhospital.org)

Dr. Tobias Kinyera +256 772541976 or +256 E-mail: [tobiaskinyera@gmail.com](mailto:tobiaskinyera@gmail.com)

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**Dr. David martin Ogwang**

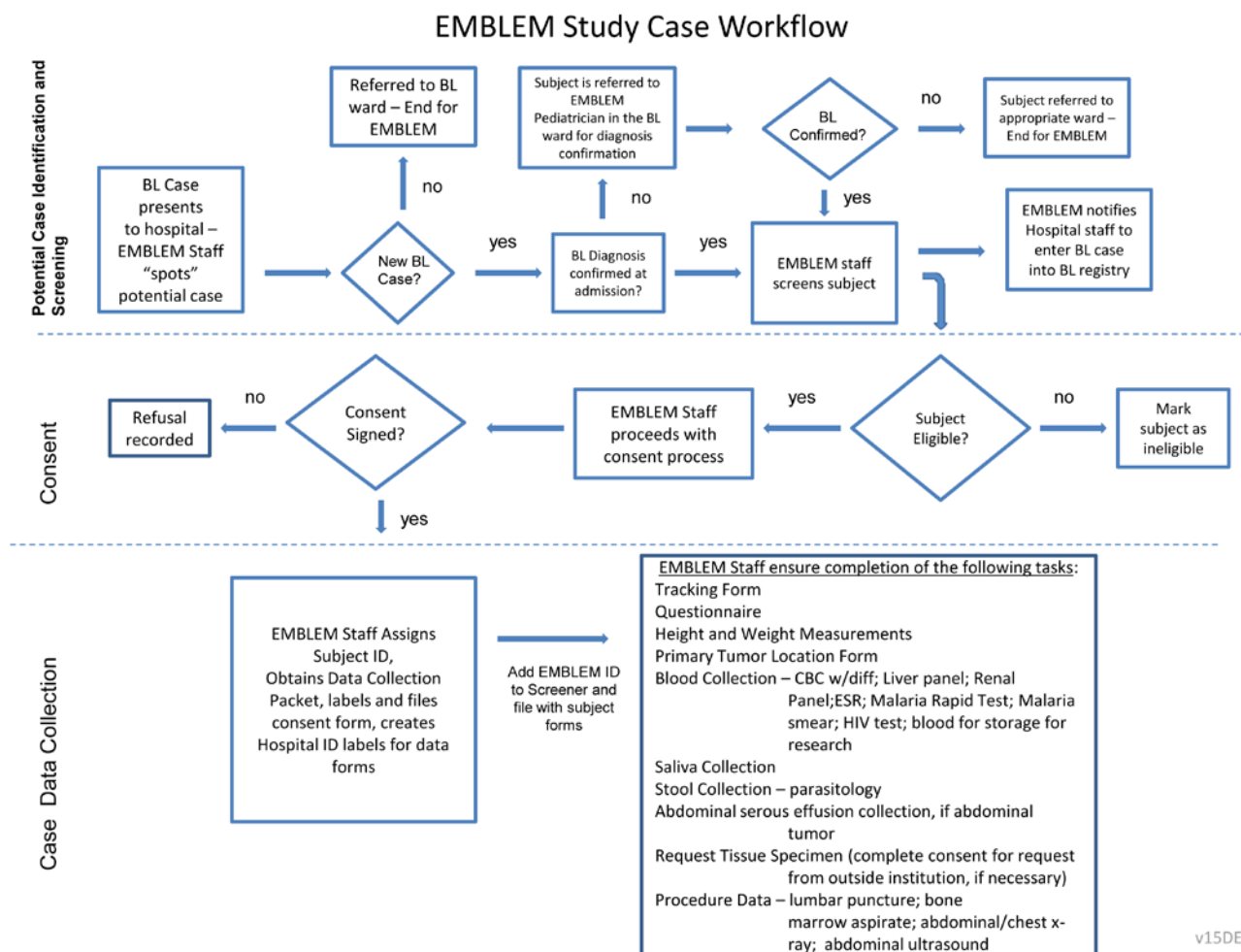
**Institutional Director Lacor Hospital**

## Chapter 3 – Enrollment of Case Subjects

### 3.1 Introduction

The primary sites for enrollment of BL cases in Uganda are St. Mary's Hospital, Lacor, in Gulu District, and Kuluva Hospital, in Arua District located in Northcentral and Northwest regions of Uganda respectively. The case catchment area is defined as the geographical area covered by the northcentral and northwest regions of Uganda according to the Uganda census report of 2002 (see list of current districts by region in Appendix 3-1). Any case residing for at least four months in any district located in these two regions will be eligible for enrollment when they present to either of the two hospitals, if they meet the other enrollment criteria. This chapter discusses the activities and data collection requirements related to BL cases for the EMBLEM Study. Figure 3-1 shows the overall workflow as subjects enter the hospital setting, they are screened for eligibility and enrolled in the study.

**FIGURE 3-1**



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### 3.1.1 Study Staff Roles in the Hospital

At the hospital, study activities will be conducted by a local EMBLEM team consisting of a staff directly hired by EMBLEM for the study and staff working for the hospital but seconded to EMBLEM through agreements between EMBLEM and the hospital. The table below shows the different cadres of staff who will be assigned to the study and their key roles/duties. Some individuals may fulfill multiple roles according to the discretion of the local EMBLEM team leader and skills of the individuals in the team.

Team Member Role	Study Responsibilities
<b>Study Coordinator</b>	The EMBLEM Team Leader's responsibilities will be to manage, monitor and supervise all study activities and staff for EMBLEM. He is the principal AFENET representative and manager on site in charge of EMBLEM. Reports to local PI, NCI, AFENET, the Local advisory Committee, and any other partnering institutions.
<b>BL Case Spotter</b> <b>BL Focal Persons</b>	Spots cases of BL cases in the outpatient departments and clinics and informs EMBLEM staff responsible for screening and enrollment.  BL focal persons identified and strategically placed at every place where BL related activities take place, namely, OPD especially where patient's history is taken, clerking, X-ray/ultrasound dept, Oral surgery, Pathology dept, laboratory, medicine and ward, children wards. Sensitized specifically on EMBLEM to facilitate case identification, referral, investigation, including processing of samples. Instructed to liaise with and supervised by EMBLEM staff. This arrangement has more coverage, division of labour spelt out. There may be more than one focal person in one unit, any category from nurse assistant to senior staff
<b>Pediatrician for BL patients</b>	Examines potential new EMBLEM cases at the hospital to confirm clinical diagnosis of the case that are referred to the wards, provide health information to the patient as needed, and requests tests required both for clinical care of the patient and as needed for EMBLEM, relays test results to study subject and family. Advises Study Coordinator on how to conduct EMBLEM's work with least interference with patient's care.

<b>BL Case Screener</b>	Screens new cases for eligibility to be enrolled by EMBLEM using the Screener form, documents screening results for both eligible and ineligible cases screened using appropriate study forms, administers informed consent to eligible cases, and assigns EMBLEM subject ID to consented cases. Schedules interview and sample draw for participants.
<b>Nurse for BL patients</b>	Administers interview questionnaire, takes height and weight measurements, and collects biological samples from participant; transfers samples to appropriate labs with corresponding test request forms. Assists pediatrician in booking for procedures like biopsy, x-ray, ultrasound and following patients results
<b>Laboratory technician(s)</b>	Collects biological samples from study participants; receives biological samples and accompanying request forms; processes biological samples and stores them according to protocol procedure; ,and record test results in the results forms and returns them to participant or to EMBLEM as per protocol data.
<b>Data manager</b>	Reports to Study Clinical coordinator. Ensures the correct study forms are available and used for all study processes; ensures study forms are completely and accurately filled out as stipulated in the protocol; designs and implements a system to perform regular form audits in the field, identify areas of ambiguity in the forms and advises on remedial action; ensures timely computerization of data collected on paper. Performs regular training of field study staff to collect accurate and complete data; provides regular reports about study accrual and targets, and quality control issues for all aspects of the study; advises and supports the local hospital BL Registries. Transfers data routinely as required; responds to data requests from NCI, Lacor PI, AFENET and other partnering institutions.
<b>Survey and Data Entry Clerks</b>	To work in the Data department to perform data review, queries, editing, and entry for EMBLEM. To work closely with interviewers, laboratory technicians, and other staff to ensure high quality of data collection.

### **3.2 Identification of Potential Cases**

Identifying potential EMBLEM cases is the first study-related activity. A BL Case Spotter will be posted to the outpatient department to monitor patients' activity in the outpatient department and clinics at the enrolling hospital. A spotter will be posted to the Pediatric ward to monitor patient activity after regular outpatient department hours. A spotter will be any staff allocated the duty of scouting by the Study Clinical Coordinator. The Spotter will either observe potential BL cases as they arrive for diagnosis and/or treatment or will request the hospital staff in the outpatient department to notify him or her that a potential case has come to the hospital and where they have been referred. The spotter will locate the patient, perform preliminary assessment, and then notify the EMBLEM staff person responsible for screening (the Screener) patients for EMBLEM study eligibility. Simultaneously, the Spotter will notify the EMBLEM Pediatrician that a new case has come and schedule clinical examination to confirm clinical diagnosis of BL. The Pediatrician will examine the patient as early as possible, including a quick assessment to approve the plan to consent and enroll the patient. A complete examination required for clinical care will be done according to hospital established schedule and procedures. In some cases, the Pediatrician may screen and consent the subject; in most cases the pediatrician will provide approval for the subject to be screened, consented, and enrolled. If the pediatrician doubts the diagnosis of BL, the child will not be enrolled until the impression of the pediatrician is reversed. Because the tasks of scouting for, and screening, BL cases must take place within intimate places of the hospital, the Study Clinical Coordinator will always allocate these roles to staff seconded to the study by the hospital. When this is not possible, EMBLEM hired staff will work as unobtrusively as possible within the hospital spaces, respecting the privilege the hospital has extended to EMBLEM to conduct this important study at the hospital.

### **3.3 Screening for Patient Eligibility**

Every patient who is screened will be given a local serial EMBLEM Screening Number. This number will consist of the first three letters of the site, serial number and year (yyyy). Children with BL must meet pre-specified criteria to be eligible for EMBLEM as case subjects. This eligibility information will be elicited and entered on Case Eligibility Screener form (See Attachment 1-X) by the BL Case Screener by asking the participant, guardian, and reviewing referral forms, or discussing with the Spotter or Pediatrician. The Case Eligibility Screener form is the first EMBLEM form that will be completed to document assessment and fulfillment or not of eligibility. Care must be taken to treat the information as privileged, so standard procedures to keep private information in the hands of authorized staff will be adhered to (see section 3.3.6).

The Case Eligibility Screener form must be completed for all subjects screened for EMBLEM regardless of whether they meet the eligibility criteria or not. If the subject does not meet the eligibility criteria, the reason for exclusion is noted on the screener form, thank the patient, and explain why the child is not eligible for EMBLEM. If the subject meets all of the eligibility requirements, the BL Case Screener will proceed with the consent process as described in section 3.4, and if the subject provides consent, the BL Case Screener will assign an EMBLEM Subject ID (see section 3.5), and label the screener form with the assigned Subject ID label, schedule or proceed with interview and sample collection. All the forms required to complete enrollment of the case are listed in section 3.6. These forms are numbered (in the footnote), for ease of reference. The forms that are completed for cases only are numbered sequentially starting from Form 01 to Form 12. Forms that are completed for both cases and controls are numbered sequentially starting from Form 31 to Form 39. All forms will be placed in an enrollment folder in preparation for data collection and will be placed in the folder after data collection. When all the forms are completed, the folder will be returned to the EMBLEM office for data review, entry and filing. The Data Manager or Survey clerks will check all folders to confirm that all forms that must be completed were completed and are placed in the correct folder.

The first form to be completed in the folder is the Screener Form, if the subject is ineligible, patient evaluation for the study will end, the form sent to the EMBLEM office for data entry into the EMBLEM database. If the subject is eligible, but does not provide consent, this fact and the reason will be marked on the Screener Form and patient evaluation for the study will end, the form sent to the EMBLEM office for data entry into the EMBLEM database. An EMBLEM subject ID will be assigned only to eligible and consented subjects. All other patients will be identified by their hospital In Patient IDs and a screening number containing a local sequential serial number assigned by EMBLEM staff.

### **3.3.1 Age Requirement**

EMBLEM cases must be at least 1 year of age and less than 15 years of age at the time of diagnosis.

### **3.3.2 Geographic Eligibility**

To be eligible, EMBLEM cases should have resided in the study region for at least 4 months prior to the onset of initial BL-related symptoms. The study region includes the Northcentral and Northwest regions as defined in the 2002 Uganda National census (see Appendix 3-1 for list of included districts).

### **3.3.3 Diagnostic Eligibility Criteria**

A diagnosis of BL will be based on a clinical picture compatible with BL and local histology or cytology report. Children will be eligible for enrollment based on a clinical diagnosis, but it is important that biopsy should be scheduled to confirm diagnosis. When biopsy is performed at the hospital, the histo-technologist will prepare two blocks, one for routine clinical diagnosis and one for review, and prepare two H&E stains for clinical diagnosis and review as per standard procedure. For children admitted after a biopsy was performed at the referring hospital, repeat biopsy will not be requested. Permission to approach the referring hospital to obtain the diagnostic specimen for review will be obtained (see Appendix 3-7) as provided for in section 3.6.2, of the study manual. If the patient refuses to give permission for the diagnostic specimen to be obtained, they will be enrolled based on the clinical diagnosis and the local report in the referral note from the referring hospital.

### **3.3.4 Definition of Prior Treatment for Burkitt Lymphoma**

Burkitt lymphoma children should be enrolled into the study before BL specific chemotherapy has been started at that hospital or the referring hospital. Specific BL treatment includes drugs such as cyclophosphamide, methotrexate, vincristine or prednisone (see Appendix 3-2 for list of Burkitt lymphoma chemotherapeutic drugs). It does not include intravenous fluids like normal saline, dextrose or drugs such as antibiotics, antimalarials or allopurinol. In case of doubt, please consult the pediatrician, or enroll the child and write this query on top of the patient's enrollment folder for later clarification.

### **3.3.5 Clinical Stability**

Children must be enrolled when in a stable clinical condition. Clinical stability will be determined by the hospital team in charge of caring for patients. If a potential case is deemed clinically unstable, the screener will discuss with the pediatrician when the patient should be re-contacted before BL specific treatment is initiated. If the paediatrician deems that the child has stabilized for the patient to be enrolled, he/she will obtain consent and obtain the clinical and study samples.

### **3.3.6 Case Recruitment Considerations**

Patient enrollment will take place in private to safeguard the privacy and confidentiality of the patient and his or her family. A private place will be designated on the ward or will be created by using a screen where the parent/guardian is comfortable to answer questions about the child's

illness and their family. The screener must be sensitive not only to the physical condition of both parent and child but also the emotional state that can impair optimum participation. The screener should be sensitive to the fact that the patient's primary interest is to get care, so the interruption to obtain study information must be minimized so that patient's care continues as quickly as possible. The Screener should be familiar with information in Appendix 3-3 (Script to Approach Potential EMBLEM Subjects) and use it as a guide when first approaching a parent/guardian about the study. The Screener should explain that the initial information is to help decide whether the patient is eligible for the EMBLEM study. Explanations about EMBLEM should be brief at this time and limited to verifying eligibility of the participant. Patients who are not eligible should be informed about the reasons for ineligibility, thanked for their time and escorted to the next point of care on the ward at the hospital. Patients who are eligible should receive detailed explanations about EMBLEM and allowed to ask questions about what they have not understood about the study before being invited to consent. The patient's caregivers must be informed that their participation in the study is completely voluntary and that if they decline to participate, their children and family members will continue to get the care they are entitled to at the hospital.

### **3.4 Informed Consent for cases**

The informed consent procedure is the formal process through which participants are informed about the EMBLEM Study, get a chance to ask and have all their questions answered, and when they agree to participate, sign the consent form to indicate exactly what they have consented to. The consent form will record the consent of the child to participate, which includes:: a) being interviewed by questionnaire; b) providing biological samples (venous blood, saliva, stool, and tumor tissue); and c) allowing for blood, saliva, and tumor tissues, if provided, to be stored indefinitely for use in future tests. In addition to the parental or guardian consent, children who are 7 years or older should be asked to assent to the study. The consent and assent are to be indicated by a signature or mark on the form and each signature or mark dated, and counter signed by a witness and interviewer. A thorough discussion of the consent form, including assent, follows.

Many patients will only speak the local vernacular. Therefore the consent forms are available in both the local vernacular and in English. The participant can sign or take a copy of the form in the language of their choice.

The Consent Form (Appendix 3-4) provides the subject with the following categories of information: Please make sure you use the version of the consent form with the most recent approval from the local Institutional Review Board as determined by the footnote Version Stamp in



the right bottom corner. The EMBLEM Consent Form for Cases is divided into two parts:

The first part of the consent (pages 1-4) provides information about the study, including the researchers conducting the study and their institutions under the following categories:

- Background and purpose of the study
- How long will your child be in this study?
- What will your child be asked to do in this study?
- What are the benefits to your child being in EMBLEM?
- What are the discomforts and possible risks to your child being in EMBLEM?
- How will your privacy be protected?
- Storage and future use of blood, saliva and tumor samples.
- The right to refuse or withdraw from the study.
- Contact information for local investigators and the chairman of the local IRB that reviewed and approved the study.

The second part of the Consent Form (pages 5-6) has the pages that must be signed by the participant to confirm their voluntary consent to participate. The participant can request a copy of the whole form or of only the first page (with names) and the last two pages (with the signatures). For all participants, the first page and the signature pages with the original signatures will be retained at the study office and filed for future reference. In each case, the version stamp of the signed pages should always be the same.

All study staff must be trained in the administration of the consent process because they may be given this role by the Study Clinical Coordinator at any time. The consent forms have been designed to be compliant with the local policies of the countries where the EMBLEM Study is being conducted as well as the basic Policy for Protection of Human Research Subjects of the Department of Health and Human Services of the United States as defined [45 CFR 46.103](#).

To administer the informed consent, the study staff doing so will ask the participant the language of choice to administer the consent form and whether the patient wishes to read or be read to. If the child's parent/guardian wishes to read, the parent should be allowed to read the consent form aloud as the staff follows along. Otherwise, the staff will read the consent form to the parent. Study staff should answer all the questions that the participant asks during this session, and if the participant needs more information that cannot be given by the staff administering the consent, the study staff will make it a point to bring this information to the participant later, or schedule for a

more senior study staff to further discuss with the parent. It is only when the parent is satisfied, that the study staff should request them to sign the consent forms.

Parents or guardians will indicate that they understood the procedures explained to them and consent to their children's participation by signing or thumb printing the consent form. This process must be witnessed by a neutral person, who is not the same person as the one administering the consent form. The neutral person should be one who speaks and understands the language that was used to administer the consent form. The study staff administering the consent form will also sign or thumb print the form. The witness will affirm by signing or thumb printing the "Witness" section on the form. All signatures will be dated today's date. Children aged 7 years or older will be requested to sign or thumb print to indicate their assent (Appendix 3-4).

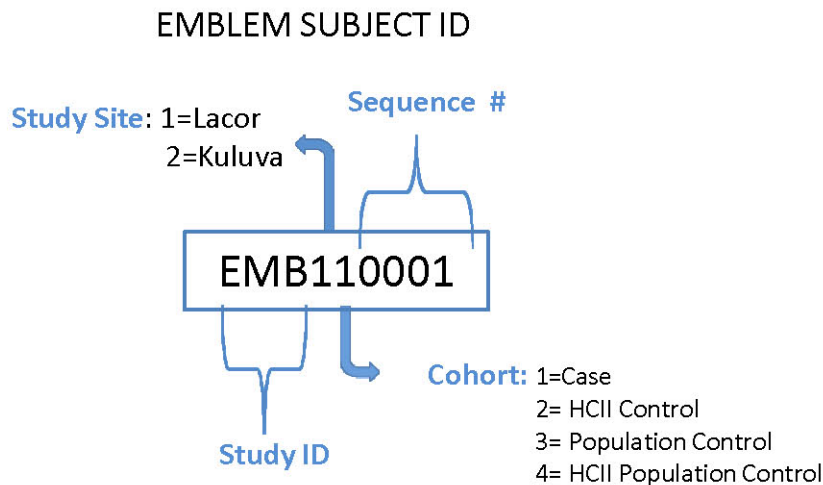
A signed/thumb printed, copy of the consent form will be offered to the participant's parent/guardian while the original signed/thumb printed copy of the consent will be retained by the EMBLEM study office. Because of the confidential information on the consent forms, the signed informed consents will be kept in a locked file at the hospital and access to the locker limited to authorized staff. The informed consents will be retained at the local sites as long as the EMBLEM Study protocol is active. Participants will be issued a Participant Enrollment ID Card (Appendix 3-5). This card will be completed with the participant's name, age, gender and date of participation. The participant will be required to present this card to study personnel at any time in the future in case they desire to discontinue participation in EMBLEM.

### **3.5 EMBLEM Subject ID Assignment**

The EMBLEM Subject ID must be assigned to all subjects who are screened, found to be eligible, and have consented to participate in EMBLEM. The process of assigning an EMBLEM Subject ID involves assigning the participant an Enrollment Folder (see below), which will have an EMBLEM Subject ID already printed on the top as well as on all the Forms in that Folder. The EMBLEM Subject ID number (See Figure 3-2) consists of a 3 letter study ID (EMB) followed by a 5 digit sequence number to reflect the enrolling hospital (1<sup>st</sup> digit), the cohort (case vs. control status) (2<sup>nd</sup> digit) and the order of enrollment into EMBLEM (last 4 digits). At the time that a case subject signs the informed consent, the next available subject ID number is assigned. The identifying information for the subject including, name, gender, age and hospital ID number are recorded in the log used for tracking subject ID assignments. This will ensure that no EMBLEM subject ID number is used more than one time and that the appropriate link between the hospital ID

and the EMBLEM subject ID is maintained.

**Figure 3-2**



The subject's Hospital ID will be used for tracking forms as they circulate among the hospital staff. In the interest of privacy, no EMBLEM Subject ID label will be affixed to forms that are not kept in the possession of EMBLEM Study staff at all times. A print utility is used to create labels printed with the subject's Hospital ID. A supply of Hospital ID labels will be printed at the time an EMBLEM Subject ID number is assigned and included in the data collection packet to facilitate proper identification of forms. Forms that circulate amongst both Hospital and Study staff, are multi-part and require both hospital and subject ID labels be affixed to the top and middle copies of the forms since these are maintained in the EMBLEM Study files. The bottom copies will be labeled with the hospital ID only and maintained for the records of the hospital staff.

### **3.6.1 The EMBLEM CASE Enrollment Folder**

The Case Enrollment Folder is designed to simplify handling and completion of all the forms used in EMBLEM. Each Enrollment Folder consists of a folder pre-numbered with EMBLEM Study Subject ID number. The Enrollment Folder will have a set of labels and associated data collection forms that will need to be completed at enrollment of that participant. All forms must be completed in ball point pen. This will ensure that responses to items on the form and any notations on the form are legible on all three copies of the multi-part forms. If a mistake is made when completing a form, a single line should be drawn through the error and the corrected response

recorded. Mistakes in completing the form should not be erased or obliterated.

The first page of the Enrollment Folder will be a Tracking Form (see Attachment 1-X), to record all of the procedures done and when each was completed. The Tracking Form has the following fields that must be completed when the Enrollment Folder is first opened: a) EMBLEM Subject ID; Date Consent Form was signed; Date of Clinical BL Diagnosis (this is the same as the date of admission); Was biopsy for the patient already done; where biopsy was performed and whether permission to obtain part of the diagnostic specimen has been obtained; and whether the patient wishes to know their HIV status.

In addition, the Tracking Form collects completion of each of the following items:

- Interview questionnaire
- height, weight, and anatomic site tumor information
- Collection of diagnostic specimen for histology
- Collection of saliva
- Collection of a venous blood sample for research
- Collection of a blood sample for HIV testing
- Collection of a blood sample CBC, differential, and ESR
- Collection of a blood sample for liver function tests
- Collection of a blood sample for renal function tests
- Collection of a blood sample for malaria tests
- Collection of a stool sample
- Chest x-ray requested
- Ultrasound request
- Bone marrow request
- Lumbar puncture request

For each of these, the response may be completed or not done and, if so, one the options for not

doing the procedure should be indicated.

The Enrollment Folder will contain the following forms and items:

1. Participant Information Sheet
2. Pathology Release Request Form
3. Participant Enrollment ID Card
4. Interview Questionnaire
5. Height and Weight Measurements and Anatomic Site Tumor Location Form
6. Lab Requisition Forms
7. Procedure Requisition Forms
8. EMBLEM Study Blood Collection and Processing Form
9. EMBLEM Study Saliva Collection and Processing Form
10. Extra EMBLEM Subject ID labels
11. Hospital ID labels will be generated at the time of EMBLEM enrollment. These will be placed in the packet when generated.

The completed Eligibility Screener form will be labeled and inserted into the Enrollment Folder for entry into the EMBLEM database.

### **3.6 EMBLEM Case Enrollment Procedures**

The study staff will explain the sequence of the study procedures to the patient, using the Study Information Sheet (Appendix 3-6), and guide the patient through the procedures in such a manner that patient's care will not be delayed to the detriment of the patient. The staff can alter the sequence of enrollment procedures as appropriate, but the following sequence is recommended: complete the Height, Weight, and Anatomic Site Form. Weights and heights are routine measurements on cancer wards, so completing this procedure will involve labeling the physical measurement form with the subject's EMBLEM Subject ID, and completing the weight and height measurement from the Patient's Hospital record. This form should be labeled and placed in the Enrollment Folder. The examining doctor will complete the bottom section. Pediatricians routinely carry out a complete medical evaluation of the patient including determining the site of the tumor, order basic investigations, request for procedure that should be done.

The questionnaire can be administered when the patient is waiting for their scheduled hospital procedures to be done. The study staff should exercise flexibility in setting the order of the

data collection activities in the hospital setting, such that sample collection can also happen before the questionnaire is administered. In some cases, where the child is accompanied by several relatives, some of these procedures may take place simultaneously.

When all forms in the Enrollment Folder have been completed, The Folder should be sent to the Hospital EMBLEM Study Office. At the Office, Data Entry Clerks will key the forms in the data entry screens and then file the folder in the designated Data Cabinet, whose access will be controlled by the Study Data Manager. The questionnaire form will be scanned (except for the cover sheet which contains personal identifying information) using Data Fax machine and sent to the NIH/Uganda office in Entebbe, and then stored away in an appropriate binder in the Case Data Cabinet at the EMBLEM Study Office. Periodically, questionnaire forms will be transferred for safe custody at the NIH/Uganda office at the Uganda Virus Research Institute.

### **3.6.1 Case Subject Data Collections**

Details of each data collection procedure are provided in the sections below. Please update the EMBLEM Study Tracking Form as each procedure is completed.

#### **3.6.1.1 Questionnaire**

The questionnaire is the main method EMBLEM is using to collect information about experiences of the cases. The questionnaire asks information about age, sex, residence and socioeconomic information, past medical history, and information about the child's family and home environment. Asking these questions consistently of all subjects will allow the researchers to reach the correct conclusions about the average experiences of children with Burkitt lymphoma. The researchers accept that patients will recollect their experiences differently; however, they wish to minimize distortions that the study personnel can add to the responses. Therefore, the following instructions are intended to give you an accurate understanding of the question, taking into account local norms and contexts that the respondents may consider when answering the question. As an interviewer, the researchers would like you to record the patients' responses and ***not to alter*** the patient's response by suggesting what ***you*** think is the correct or best answer.

The questionnaire is in English. You as an interviewer will be free to translate the questions into the participants' language. The right phrasing in the different languages will be discussed and agreed upon during the regular training sessions. Thus, the staff who will administer the questionnaire must be fluent in both English and the local vernacular or be accompanied by an individual who is fluent in both languages. The study personnel administering the questionnaire

will record the predominant language used to administer the Questionnaire.

Study personnel must become familiar with and regularly refer to Attachment 1-X for details regarding techniques and considerations when interviewing participants and Question-by-Question instructions for completing the Interview Form which are provided in Attachment 1-X. when conducting the interview. This attachment may be updated during the conduct of the study, with approval from the PI, when new situations that were not anticipated are encountered.

The Questionnaire consists of two parts: a) cover sheet, and b) the main questionnaire. The cover sheet will be marked with both the Subject ID and Hospital ID labels; the questionnaire will be marked only with the Subject ID label. The cover sheet will assure proper linkage between the Hospital ID and the Subject ID and will not be faxed for processing since there are personal identifiers also included on the cover sheet.

### **3.6.1.2 Height, Weight, and Anatomic Site Tumor Location**

It is usual, because the measurements are crucial to determining the dosage, for weight and height of children to be taken as part of their admission to a pediatric ward. The child's weight and height should be copied (measured if not previously done) and recorded on the Height, Weight, and Anatomic Site Tumor Location Form (Attachment 1-X).

The height of children who are 2 years and above ( $\geq 85\text{cm}$ ) will be measured when children are standing in a vertical position. The nurse will place the flat board with a vertical ruler. A caretaker or assistant removes any foot wear or headgear from the feet and head of the child, places the child on the flat board so that the shoulder blades, buttocks, and heels are touching the vertical surface of the ruler. The child stands with both feet flat on the board, legs slightly apart. The assistant kneeling down with the left hand on the knee of the child tells the child to stand 'straight and tall' looking straight ahead. The assistant brings a level board to the child's head and reads the child's height (the reading at the lower edge of the level) loud and clearly records it to the nearest 0.1 cm. With children who are either below 2 year ( $< 85\text{cm}$ ) or are unable to stand, the height measuring board is placed in a horizontal position on a flat surface. The above procedure is repeated. If the child is held on the scale, use the calculator provided to subtract the "holder's" weight from the combined weight of the holder plus the child. The resulting difference is the child's weight.

This form (see Attachment 1-X) is intended to elicit the first anatomic site where the participant noted tumor symptoms. This form should be completed by the pediatrician or a nurse very experienced in reviewing patients with Burkitt lymphoma. This form will be completed at the

time that the study pediatrician examines the child and orders any necessary lab tests and/or procedures.

### **3.6.1.3 Routine Clinical Laboratory Testing**

The Laboratory Requisition forms may be used to request routine clinical laboratory tests that are ordered as part of the care of the subject. These tests include HIV screening, complete blood count including differential cell count, sedimentation rate, malarial smears, malaria antigen testing, stool microscopy, liver and renal function tests. Because of lack of consistency in the laboratory request forms used at any one hospital or across hospitals, the EMBLEM study will use standardized forms to facilitate routine laboratory requests for the cases. The hospitals may choose to adopt aspects of these forms for their own routine use. This form, included in Attachment 1-X, is 3-part NCR (carbon-copy form). The top part is labeled with both subject ID and hospital ID, completed and retained in the EMBLEM Enrollment Folder to track results for cases for data entry. The middle page and last page carbon-copies will be sent to the laboratory with the sample. The laboratory personnel will complete the “receipt” part of the form and return the middle carbon copy to the EMBLEM study office. EMBLEM study personnel will affix the subject ID to the bottom of the middle carbon-copy page returned from the lab and place it in the Enrollment Folder of the subject. The laboratory will retain the bottom carbon copy of the laboratory request form for their records.

To facilitate return of clinical results to patients, the laboratory personnel will record results of each laboratory test on reporting forms (See Attachment 1-X). These result reporting forms are 2-part NCR (carbon-copy) so that results can be returned to the patient as well as to the EMBLEM Study. The laboratory will copy results that are normally reported in writing onto the form on the result reporting form. The top part of the form will be returned to the EMBLEM study office; the carbon-copy will be returned to the Patient’s file on the ward. Where the laboratory result is a machine result printout, the laboratory personnel will print two sets of results and staple one set to the top part of the result form and return both the EMBLEM Study office, and staple the second copy of the result on the carbon copy and return the results to the ward. At the EMBLEM Study office, staff will place the result in the patient Enrollment Folder and affix a subject ID label to the top of each copy of the result form. The Enrollment Folder will be in Custody of the Data Entry Department and will remain open until all the Tracking Form indicates all procedures for the patient have been completed.

Participants who wish to receive HIV results will undergo Voluntary HIV Counseling and Testing (VCT) that will prepare them to receive their HIV/AIDS results. The EMBLEM study will



use the existing HIV/AIDS Voluntary and Counseling system at the study site including reporting of HIV results, but will have on its staff certified HIV counselors in case this need is required. If needed, a subject may receive both counseling and HIV results from an EMBLEM staff, who will follow the established National HIV/AIDS Voluntary Counseling procedures. HIV results are handled in the most confidential manner and this confidential procedure will not be breached by study personnel, regardless of whether the participant elects to learn the results of the test.

The HIV pre-test counseling session will be held with the parent/guardian, and also with the child if aged 7 years or older, shortly after the patient has provided informed consent to participate in the study. It will consist of the following: (1) an explanation of what HIV infection is, how the virus can be transmitted, and the meaning of having AIDS, (2) an explanation of the measures one can take to decrease the chances of getting infected with HIV, or transmitting HIV or other STDs to loved ones, and (3) a discussion about the options for supportive care available to people who test positive for HIV, including the opportunity to be referred to doctors who specialize in treating and caring for people with HIV/AIDS. During pre-test counseling, the counselor will explain the process of accessing the HIV result and schedule an appointment to relay the child's HIV results, if they wish. The participant's desire or refusal to learn the HIV results will be recorded on the tracking form in the Enrollment Folder.

HIV serology will be performed by the hospital laboratory using the procedure in use at the time. EMBLEM staff will keep a record of the tests in use at any given time (the test kit inserts and the test algorithm will be kept on file along with the dates when those kits were introduced and when their use was discontinued). Routinely, the HIV serology for the public is performed using rapid tests (about 15 minutes each) using two different testing technologies. When results from the two tests are concordant, the result is returned to the patient as is; when the results are discordant, the sample is tested on a third test based on a different technology as a tie-breaker test. The samples from discordant samples are sent to an HIV Reference Laboratory at the Uganda Virus Research Institute for repeat testing and review of testing policy. The standard practice is to place the HIV test results in a sealed envelope with the patient's identifying information and return them to the HIV Counselor originating the request. For EMBLEM subjects, the results will be returned in a sealed envelope marked at the front with the Hospital ID number. The sealed envelope will be placed in the Enrollment Folder and the linkage with the EMBLEM Subject ID number will be performed by only one authorized staff, who will also computerize the data in a special HIV Result Data application. Thus the remaining Study staff will remain blinded to HIV status of participants.

#### **3.6.1.4 Routine Clinical Procedures**

Children admitted with cancer, including Burkitt lymphoma, undergo routine investigations to stage their cancer. These procedures include abdominal ultrasound, chest x-ray, tumor or bone marrow biopsy and lumbar puncture. The EMBLEM Study will track all procedures that are requested for each patient, and where it may speed up the process, EMBLEM Staff will complete the request forms and direct or accompany the patients to the location where the procedure will be performed. The procedure form, included in Attachment 1-X, is 3-part (carbon-copy) NCR. The top part has fields for both subject ID and hospital ID, which should be completed. The top form should be placed in the Enrollment Folder of the patient and sent to the EMBLEM study office. The middle and bottom carbon-copies are sent with the patient (or patient's sample) to the personnel performing the procedure who will complete and return the middle carbon-copy to the study office. EMBLEM study personnel will affix the subject ID to the bottom of the middle part of the form and place it in the Enrollment Folder.

Results of each procedure are recorded separately on the appropriate result reporting form (See Attachment 1-X). The results forms are 2-part carbon-copy NCR with the top part being completed and returned to the EMBLEM study office (where the EMBLEM subject ID label is affixed) and the bottom carbon-copy can be retained in the laboratory or procedure location for their records. Two extra hospital ID labels will be clipped to the requisition form so that the lab will have labels available to affix to both copies of the results form.

#### **3.6.1.5 Research Specimen Collections – Blood and Saliva**

There are two specimen types that are collected specifically for the EMBLEM study. These are the blood and the saliva. It is desired that these specimens are collected at the time that other blood specimens are being drawn for clinical tests. These specimens must be collected prior to the initiation of chemotherapy, but only after consent is obtained.

Details regarding the collection and processing of these specimens can be found in Chapter 4 – Specimen Collection, Handling and Shipping Procedures. Research Specimen Forms are included in Attachment 1-X and 1-X and should be completed when these samples are collected. The forms are 3-part carbon-copy NCR forms with the top part with fields for both subject ID and hospital ID. The top part of the completed form should be retained and placed in the Enrollment Folder of the subject. The middle carbon-copy of the form should be completed by the personnel in the processing laboratory and returned to the study office to be placed in the Enrollment Folder, while the bottom carbon copy should be retained in the processing laboratory. EMBLEM study

personnel will affix the EMBLEM subject ID to the bottom of the middle part of the on the middle carbon-copy returned from the laboratory and placed in the Enrollment Folder. The laboratory personnel will affix the BSI ID to the middle and bottom copies (ID used to track the specimen in the bio-specimen inventory system) and retain the bottom copy for their records.

### **3.6.2 Permission to Obtain Diagnostic Tissue from an Outside Institution**

Study subjects may have undergone biopsies at an outside institution before coming to an EMBLEM study site will be asked for permission to obtain diagnostic tissue from the outside institution using the form in Appendix 3-7. The Form will be completed and placed in the subject's Enrollment Folder. Periodically, EMBLEM study personnel will go to these institutions and request the diagnostic tissue specimens and/or slides be provided to EMBLEM for review to confirm the pathological BL diagnosis.

Refer to Chapter 4 –Specimen Collection, Handling and Shipping Procedures for details regarding the handling of tissues and slides once they are returned to the EMBLEM pathology laboratory.

### **3.6.3 Case Subject Withdrawal after Enrollment**

If a participant decides to withdraw from some or all of the EMBLEM procedures, they will do so by informing the EMBLEM team leaders whose names are written on the consent form. When the request to withdraw is received, it will be sent to the PI at NCI, who will write to you to confirm that the request has been implemented according to the request of the participant.

**Appendix 3-1 – Districted Included in Uganda Catchment Area**

<b>NORTH CENTRAL DISTRICTS</b>
<b>Amuru</b>
<b>Gulu</b>
<b>Kitgum</b>
<b>Pader</b>
<b>Oyam</b>
<b>Lira</b>
<b>Apac</b>
<b>Dokolo</b>
<b>Amolata</b>
<b>WEST NILE DISTRICTS</b>
<b>Adjumani</b>
<b>Arua</b>
<b>Nebbi</b>
<b>Yumbe</b>
<b>Moyo</b>
<b>Koboko</b>
<b>Maracha-Terego</b>
<b>Nyadri</b>

### **Appendix 3-2 Drugs used to specifically treat Burkitt Lymphoma\***

- 1. Vincristine**
- 2. Cyclophosphamide or cytoxan**
- 3. Methotrexate**
- 4. Methotrexate intrathecally.**
- 5. Cytarabine (or ara-C)**
- 6. Etoposide**
- 7. Ifosfamide**
- 8. MESNA**

**\* Adopted from INCTR 03-06A Treatment Protocol**

### **Appendix 3-3**

#### **Script to Approach Potential EMBLEM Subjects**

“Ms./Mr. [Name of caregiver], I understand you are taking care of [name of child]? If you have a few minutes, I would like to tell you about a research study that we are doing on Burkitt lymphoma, which is a cancer that affects a lot of children in East Africa.

If declines: OK, can I check with you later today or tomorrow?

Accepts: Good. I will check with you later.

If still declines: OK. I wish the best for you and your child.

If accepts: Good. Burkitt lymphoma, which we call BL, is a cancer that typically causes a tumor in the face or abdomen, of children but it can affect the whole body. Children who have BL must receive intensive treatment in a hospital. BL is curable. We are working with doctors at several hospitals in East Africa and in the United States to try to understand why BL occurs. Some people think that malaria is involved, but most people think that many other things are also involved. Our goal is to figure out ways to prevent or improve treatment of BL.

Our research called the EMBLEM study, is trying to recruit more than 4000 children in East Africa. I have here a form that describes what we are doing and how it would involve your child, if you agree to have him/her participate. First, I would like to determine whether your child is eligible to participate. To do this, I will complete a Screener Eligibility Form. If your child is eligible, then I will read to you aloud or allow you to read the consent form which ever your preference will be.. You will learn more details about the study from the consent form. After we have read the consent form together, I will invite you to ask me any questions about anything, which are not clear to you about the study. , I will answer most of your questions to the best of my ability. For questions that I may not be able to answer, I will give you the answer later alternatively, I will arrange for the questions to be answered by some study staff more senior than me at a later time OK?

If accepts: Good. [Read complete the Screener Eligibility Form, stopping as needed to discuss or answer questions.]

If declines: OK, can I check with you later today or tomorrow?

Accepts: Good. I will check with you later.

If still declines: OK. I wish the best for you and your child.”

**Appendix 3-4**  
**The Consent and Assent Forms**



Study Contact Tel: +1(256) 471432492/ 471432490  
P.O. Box 180 Gulu, Uganda, or  
P.O. Box 12874, Kampala, Uganda  
Study contact email: [emblem@afenet.net](mailto:emblem@afenet.net)

### **CONSENT TO BE IN A RESEARCH STUDY AS A CASE SUBJECT**

Approving IRBs: NCISSIRB-IORG#0000460; UVRI-SEC IORG# 00001274; NIMR-IORG#0002002 & MCHS -SEC- IORG#0002223

Consent Form Version Date: 11<sup>th</sup> March, 2010

Title of Study: EPIDEMIOLOGY OF BURKITT LYMPHOMA IN EAST AFRICAN CHILDREN AND MINORS (EMBLEM)

Principal Investigator: Dr. Sam M. Mbulaiteye (National Cancer Institute, USA)

Co-Principal Investigator: Dr. Martin D. Ogwang (St. Mary's Hospital, Lacor, Uganda)  
Dr. Steven Reynolds (NIH/Uganda Virus Research Institute)

Other Investigators: Drs. Kishor Bhatia, James J. Goedert, Ruth Pfeiffer, Stephen Chanock (National Cancer Institute, USA), Dr. Leona Ayers (Ohio State University, USA), Dr. Patrick Kerchan ((Kuluva Hospital, Arua), Drs. Esther Kawira, Nestory Masalu (Bugando medical Center, Tanzania), Dr. Constance Tenge, Robert Kuremu, Walter Wekesa, and Ms. Ms. Pamela Akinyi Were (Moi University, Kenya).

Name of Child: \_\_\_\_\_ Date: \_\_\_\_/\_\_\_\_/\_\_\_\_  
Day Month Year

**Background and purpose of the study:** Researchers here at [name of hospital], at other hospitals in East Africa, and at the U.S. National Cancer Institute (NCI) are working to understand a cancer called Burkitt lymphoma. Burkitt lymphoma occurs mostly in children and rather frequently in East Africa. Burkitt lymphoma typically causes swellings (called tumors in English) on the face or abdomen, but it can affect the whole body. Many researchers believe that frequent or severe malaria may increase the risk for Burkitt lymphoma, but there are probably other causes.

We are asking your child and more than 4000 other children to be in this study called EMBLEM to better understand the causes of Burkitt lymphoma. The researchers will study children with and those without Burkitt lymphoma to get a better understanding of the causes of Burkitt lymphoma by testing their blood, saliva and stool, and by asking questions about their home environment and previous illnesses. If you agree, the research team will ask you questions about your home and family. The questions will take about 20 minutes and will be used for research use only. The researchers will also obtain a sample of blood, saliva, or stool for testing. The details of what will be done, how long your child will be in the study, the benefit and discomfort to you and your child and your privacy are explained in detail below.



**How long will your child be in this study?** Your child will participate only once in this study and will not have to do anything after today. The research team will contact you if they find something in your child's blood or stool that should be treated. You may contact the leader of the research study (Principal or co-Principal Investigators) using the addresses and telephone numbers on page 4 of this consent form) at anytime, if you have any questions.

**What will your child be asked to do in this study?** If you agree for your child to take part in the study, the following will be done:

- Your child's medical records will be reviewed to determine whether he or she has Burkitt lymphoma.
  - If a diagnosis has not been confirmed by examining a piece of tissue from the swelling, a doctor at [name of hospital] will take a piece of Burkitt lymphoma to be examined to determine whether your child has or does not have Burkitt lymphoma.
  - A small piece of the tissue will be taken by the study for research.
  - The results from this examination will be returned to your doctor, who will give them to you. If Burkitt lymphoma is found, your child will be given the treatment for Burkitt lymphoma by the doctors at this hospital.
  - If your child has already been diagnosed by tissue examination at another hospital, no new tissue will be taken at this hospital to confirm Burkitt lymphoma as part of the research study. If you agree to participate in the EMBLEM study, we will ask for your permission to receive a piece of the tissue that was used to make the diagnosis at the hospital laboratory where the child was diagnosed so that our doctors can examine it and confirm the Burkitt lymphoma diagnosis. [SHOW PATHOLOGY RELEASE FORM TO THE PREVIOUSLY DIAGNOSED PARTICIPANT. THE FORM SHOULD BE COMPLETED ONLY FOR PARTICIPANTS WHO HAVE CONSENTED TO PARTICIPATE IN THE STUDY]
- You and your child will be asked questions (questionnaire interview) about previous illnesses, family, home life, and home environment by a research study nurse.
- Your child will be weighed and his or her height measured by a research study nurse.
- Your child will be asked to give a blood sample (1-2 teaspoonfuls), a saliva sample, and a stool sample to be tested as part of the research study. A research study nurse will take the blood from your child and, to avoid your child being pricked several times to get blood samples for tests required as part of treatment and for research, the nurse will take the blood samples at the same time when blood samples requested by your child's doctor.
- Your child's information (questionnaire) and samples will be labeled only with numbers, not your child's name.
- Your child's blood will be tested for anemia (low blood count), for the function of the kidneys and liver, for malaria (the parasite that causes fevers and chills), and for HIV (the virus that causes AIDS). HIV is detected frequently in adults with Burkitt lymphoma in America and Europe, but it has not been found to be very common in children with Burkitt lymphoma in Africa. Thus, the chances that your child has HIV are very small.
  - Your child's HIV test result will be given to you in person, if you want it. The other blood test results (not HIV) will be given to your child's doctor so that the treatment your child may need can be arranged quickly.

- Your child's saliva will be tested for two very common viruses (EBV and HHV8). Most people who are infected with these viruses experience no symptoms, but researchers think that these viruses may increase the chance of developing Burkitt lymphoma. The tests for EBV and HHV8 will be done after about one year and because there is no treatment for them, the results will not be returned to you.
- Your child's stool will be tested for parasites. Your child will be given treatment if parasites are detected in stool.
- Your child's blood sample, along with blood from all children in the EMBLEM study, will be frozen and tested in the future for certain changes in some genes. Genes are inherited from parents and ancestors and may influence who gets or does not get a particular disease.
  - About 1-2 years from now, the researchers will test the blood for changes in approximately 20 genes that increase or decrease risk for malaria. By comparing the frequency of genes that influence the risk for malaria in children with and without Burkitt lymphoma, the researchers will be able to show indirectly whether malaria is related to Burkitt lymphoma. Because there are no treatments for differences in genes, the results from the gene tests will not be returned to you.
  - Farther in the future, perhaps in 5 years, EMBLEM researchers will test the frozen blood for changes in all the genes (approximately 30,000 genes) to find if there are differences in genes between children with and without Burkitt lymphoma. Someday it might be possible to identify a person by differences in the 30,000 genes. So to make sure your child cannot be identified, the results for the 30,000 genes will be available only in a way (by grouping or scrambling) that your child and other children in the study cannot be identified. The results for the 30,000 genes will not be returned to you because they will not change your child's medical care.
- After the researchers complete the tests described above, the remaining specimens will be stored indefinitely by the NCI. These stored specimens may be used in future tests after obtaining permission from NCI. You or your child will not be contacted about these tests.
- You will receive an Information Sheet listing the procedures we have discussed today.

**What are the benefits to your child being in EMBLEM?** By participating in EMBLEM, your child will help doctors to learn about the causes of Burkitt lymphoma in children. This knowledge may lead to prevention or better treatments of other children with Burkitt lymphoma in the future. You and your child will benefit by learning about causes of common illnesses and by having improved blood tests for anemia (low blood count) and malaria, a test for stool parasites, and timely treatment of parasites that are detected. We do not expect to find that your child has HIV infection. If we do, you and your child will be referred for HIV counseling and treatment at clinics that provide these services at no cost. Finally, you, your child and other children in the house will receive treated mosquito bednets to use at night so that the risk of being bitten by mosquitoes at night and, therefore, of getting malaria is reduced.

**What are the discomforts and possible risks to your child being in EMBLEM?** You or your child may experience some discomfort with some questions you will be asked during the interview. Your or your child are free not to answer any questions. Your child will experience discomfort from the needle prick when taking a blood sample. We will avoid an extra needle prick by trying to take blood for the research tests at the same time as the blood that your child's doctor here at [name of hospital] has requested. You or your child may experience discomfort because of the plan to test your child's blood for HIV, the virus that causes AIDS. We are following the advice (policy) of the Uganda AIDS Commission. So you and your child have the right to know the HIV test result, and we encourage you,

to find out. You also have the right to choose not to know your child's HIV result. If the results show that your child is HIV-positive, you and your child may experience emotional distress from learning those results. You and your child will be referred to a hospital that specializes in treating children with HIV infection, where you and your child will receive additional counseling and treatment for HIV and its complications at no cost to you.

**Your privacy:** Your child's information that EMBLEM is collecting will be kept private and will be in locked files. Only people who are authorized to handle that information will see it. To make sure that you and your child cannot be identified when the research information is being compared, your child's name will be separated from the research information (questionnaire and samples). Only information (questionnaire) and samples labeled with numbers, not your child's name, will be sent to researchers. Any forms bearing your child's name will be left in the country at the hospital where the study is conducted, where they will be kept in locked files. The research results will be presented to other scientists using numbers that are not linked to you or your family's name and village. Your child's name will never be used to communicate information to other researchers.

**Storage and future use of blood, saliva and tumor samples:** Your child's samples (except the stool) will be frozen and stored indefinitely by the National Cancer Institute (NCI) in the United States. The samples will be labeled only with numbers that are not linked to your child's name. NCI will make sure that any research tests conducted in the future using your child's samples are consistent with the consent that you and your child will sign today. You and your child will not be contacted about these future research tests, and the results from those research tests will not be returned to you. You and your child will not receive any money or other benefit from any discoveries made from using the samples. You or your child can ask the local study staff to be removed from future comparisons using the procedures explained below.

**The right to refuse or withdraw from the study:** Your child's participation in EMBLEM is voluntary. Your child's treatment here at [name of hospital] will be the same whether your child participates or not. Your decision will not change your child's medical treatment. Refusal or withdrawal will not reduce any benefits to which you or your child are otherwise entitled. You or your child can refuse to participate now, or you can change your mind and withdraw from the study in the future without penalty. You or your child can withdraw from the study by informing the EMBLEM team leaders whose names are written on this consent form. When your request to withdraw is received, it will be sent to the PI at NCI, who will write to you to confirm that your information was deleted from the study files and your samples were removed and destroyed.

If you have other questions about EMBLEM, you can ask them at any time-now or in the future. You can ask Dr. Tobias Kinvera, the Project Leader for EMBLEM, Dr. Martin D. Ogwang, the head of the local research team at St. Mary's Hospital, Lacor, Gulu, Dr. Patrick Kerchan, the head of the local research team at Kuluva Hospital, Arua, or Mr. Tom Lutalo who is the chairman of the Uganda Virus Research Institute Science and Ethics Committee, the ethics committee that approved EMBLEM in Uganda. The contact information is provided below:

Contact Information: Name, Station, and Telephone number:

Dr. Tobias Kinvera, St. Mary's Hospital Lacor, Gulu Tel: +256 (772) 541976 (Mobile); Dr. Martin D. Ogwang, St. Mary's Hospital Lacor, Gulu - Tel: +256 (471) 432310 (Office) +256 (772) 593901 (Mobile); Dr. Patrick Kerchan, Kuluva Hospital, Arua; Tel: + 256 (476) 421198 (office) + 256 (772) 444135 (Mobile); Mr. Tom Lutalo, Chairman, Uganda Virus Research Institute Science and Ethics Committee, Nakiwogo Road, P.O. Box 49, Entebbe, Tel: +256 (414) 321962 (Office) +256 (712) 732129 (Mobile).

(1) **Consent of Parent or Guardian for the Child to Participate in the Study**

I have read or somebody has explained to me all of the above. I understand the following:

- What the EMBLEM study is about and how and why it is being done.
- If I decide to participate my child will undergo an interview and samples will be obtained, and that my child and I will not have to do anything after today; this is a one time participation.
- I have been told of the risks, discomforts, and possible benefits from the study.
- I have been told about the gene tests that will be done over the next 2-5 years.
- I have been explained that the storage of any remaining samples of blood, saliva, and Burkitt lymphoma tumor tissue that remain from these tests will be stored indefinitely by NCI and may be used in future tests.
- I understand that I will not be contacted about any future tests, but I can withdraw my information or samples if I wish. I am aware that my information will remain confidential
- I will receive a copy of this consent form and am aware that if I need more questions answered that the study contact phone numbers are located on the first page of this form.

I voluntarily consent for my child to participate as follows.

1. BE INTERVIEWED

\_\_\_\_\_  
Signature or Mark of Parent or Guardian

Date: \_\_\_\_/\_\_\_\_/\_\_\_\_  
Day Month Year

2. FOR SAMPLES TO BE OBTAINED

\_\_\_\_\_  
Signature or Mark of Parent or Guardian

Date: \_\_\_\_/\_\_\_\_/\_\_\_\_  
Day Month Year

3. FOR BLOOD AND SALIVA SAMPLES TO BE STORED INDEFINITELY FOR FUTURE TESTS

\_\_\_\_\_  
Signature or Mark of Parent or Guardian

Date: \_\_\_\_/\_\_\_\_/\_\_\_\_  
Day Month Year

\_\_\_\_\_  
Name of adult giving consent for the child to participate

Relationship to Child: Birth Mother \_\_\_\_\_ Other Parent or Guardian \_\_\_\_\_

THE FOLLOWING SECTION MUST BE COMPLETED BY A WITNESS WHO OBSERVED THE WHOLE PROCESS FOR PARTICIPANTS WHO CANNOT READ AND WRITE:

I was present while the benefits, risks, procedures, and storage of blood, saliva, and tumor tissue samples were read to the parent or guardian. The parent or guardian was given the opportunity to ask questions about his or her child being in the study and has agreed to have his or her child take part in this research.

Date: \_\_\_\_/\_\_\_\_/\_\_\_\_ Signature of Witness: \_\_\_\_\_  
Day Month Year

Date: \_\_\_\_/\_\_\_\_/\_\_\_\_ Signature of Interviewer: \_\_\_\_\_  
Day Month Year

**(2) Assent of Child to Participate in the Study (For Children Aged 7 or Older)**

- The researcher has explained to me about the study.
- I understand that the researchers will ask questions about me.
- I understand that they will take a little blood from me and they will ask me to give them some saliva and stool.
- They need these samples so they can test them to understand why some children get Burkitt lymphoma (like me) and others do not.
- I understand that the samples that remain from this research will be kept and used in the future.

**My parent/guardian has agreed that I should participate in the study. I also agree to the following. I voluntarily agree that I will participate as follows:**

**1. BE INTERVIEWED**

\_\_\_\_\_  
Signature or Mark of child

Date: \_\_\_\_/\_\_\_\_/\_\_\_\_\_  
Day Month Year

**2. FOR SAMPLES TO BE OBTAINED**

\_\_\_\_\_  
Signature or Mark of child

Date: \_\_\_\_/\_\_\_\_/\_\_\_\_\_  
Day Month Year

**3. FOR BLOOD AND SALIVA SAMPLES TO BE STORED INDEFINITELY FOR FUTURE TETS**

\_\_\_\_\_  
Signature or Mark of child

Date: \_\_\_\_/\_\_\_\_/\_\_\_\_\_  
Day Month Year

\_\_\_\_\_  
Signature or Mark of Child

Date: \_\_\_\_/\_\_\_\_/\_\_\_\_\_  
Day Month Year

THE FOLLOWING SECTION MUST BE COMPLETED BY A WITNESS WHO OBSERVED THE WHOLE PROCESS FOR PARTICIPANTS WHO CANNOT READ AND WRITE:

I was present while the benefits, risks, procedures, and storage of blood, saliva and tumor tissue samples were read to the participant. The participant was given the opportunity to ask questions about being in the study and has agreed to take part in this research.

Date: \_\_\_\_/\_\_\_\_/\_\_\_\_\_  
Day Month Year

Signature of Witness: \_\_\_\_\_

Date: \_\_\_\_/\_\_\_\_/\_\_\_\_\_  
Day Month Year

Signature of Interviewer: \_\_\_\_\_

### Appendix 3-5

#### EMBLEM Study Participant ID Card

<b>EMBLEM STUDY PARTICIPANT CARD</b>
--------------------------------------

This is to certify that Master/Miss \_\_\_\_\_ participated in EMBLEM Study after an informed voluntary decision of the parent/guardian. The parent/guardian of the participating child also voluntarily consented to the following parts of the study:

Patient Age: |\_\_| |\_\_| . |\_\_|      ☐ Years      ☐ Weeks

☐ Months   ☐ Days

Patient Sex:      ☐ Male      ☐ Female

Enrollment Date   |\_\_| |\_\_| / |\_\_| |\_\_| / |\_\_| |\_\_| |\_\_|

                         DD      MM      YYYY

Please keep this card for future communication about your continuing involvement in the study. In particular, present this card to the local EMBLEM investigator [Dr. Tobias Kinyera] along with any request to have the specimens withdrawn from genome-wide association (GWA) or other studies.

## Appendix 3-6

### EMBLEM Information Sheet for participants

Name of Child: \_\_\_\_\_ Date: \_\_\_\_ / \_\_\_\_ / \_\_\_\_

Day    Month    Year

#### **Check all that has been done**

1. Informed Consent Form/Assent Form:

- a. Purpose of study explained
- b. How long the child will participate in the study explained
- c. What the child will be asked to do explained
- d. Benefits for participating in EMBLEM explained
- e. Discomforts and possible risks explained
- f. Rights of privacy explained
- g. Storage and future use of blood, saliva, and tumor (cases only) samples explained
- h. The rights to refuse or withdraw explained
- i. Guardian consented to participate
  - i. Child assented to participate (only children aged 7 years or older)

2. Enrollment Form:

- a. Screener form completed to confirm eligibility
- b. Enrollment form completed to collect individual risk factor information

3. Specimens:

- a. The following specimens taken
  - i. Venous blood sample
  - ii. Saliva sample
  - iii. Tumor sample (if case was not diagnosed before; if diagnosed before, permission to retrieve sample obtained.
- b. Plan to return results promised to the patient explained
  - i. Improved complete blood count
  - ii. Malaria slide
  - iii. Stool examination for parasites, ova, and cysts
  - iv. HIV serology results
- c. Plan to freeze and store blood and saliva for many years to test
  - i. for genetic differences in 25 malaria genes
  - ii. all genes (30,000) called GWAS
  - iii. other newly developed tests relevant to BL, other cancers or malaria

4. Other investigations for cases:

- a. For cases, request forms for requested investigations completed
  - i. Ultrasound and chest x-rays
  - ii. Lumbar puncture
  - iii. Renal and liver function tests

5. Participant card and contact information for the local PI

## **Appendix 3-7**

### **Permission to Release Pathology Tissue for EMBLEM Study**



**FORM CEF3 - PERMISSION TO RELEASE PATHOLOGY TISSUE FOR  
THE EMBLEM STUDY**

With your permission, we will request that the hospital named provide a tissue sample from the biopsy or surgery that was performed on your child at that hospital. These tissues will be examined by pathologists who are participating in our study: "Epidemiology of Burkitt Lymphoma in East African Children or Minors" (EMBLEM).

Name of hospital: \_\_\_\_\_

Name of Child: \_\_\_\_\_

**Consent of Parent or Guardian for the Child for release of tissue specimens:**

The EMBLEM study has been explained to me and my questions have been answered. I understand what the study is about and how and why it is being done. I have read or somebody has read to me all details of the study.

With this form, I now request \_\_\_\_\_ [INSERT NAME OF HOSPITAL]  
to release my child's tissue specimens to the doctors who are participating in the EMBLEM study.

\_\_\_\_\_  
Name of adult giving consent for the child to participate

\_\_\_\_\_  
Signature or mark of adult providing consent Date: \_\_\_\_/\_\_\_\_/\_\_\_\_  
Day Month Year

Relationship to Child: ☐ Birth Mother  
☐ Other Parent or Guardian

**If a participant cannot read the form, a witness must sign here:**

I was present while the EMBLEM study and the use of tumor tissue samples were read to the parent or guardian. The parent or guardian was given the opportunity to ask questions about this have agreed to this in behalf of his or her child.

\_\_\_\_\_  
Signature of Witness Date: \_\_\_\_/\_\_\_\_/\_\_\_\_  
Day Month Year

Printed name of witness: \_\_\_\_\_

**Study Contact Phone numbers:** +1(256) 772403192/ +1(256) 711433112

**Study Contact addresses:** P.O. Box 180 Gulu, Uganda, or  
P.O. Box 12874, Kampala, Uganda

**Study Contact e-mail:** [emblem@afenet.net](mailto:emblem@afenet.net)

**Hospital ID**

(stick label here)

Transcribed by: \_\_\_\_\_  
Checked by: \_\_\_\_\_  
*Initials/Date*

**Subject ID**

(stick label here)

Form CEF3 V20OCT2010

# EMBLEM Study Manual

## Chapter 3: Attachment 1

### Forms Completion Instructions

#### 1.1 Introduction

The following document provides supplemental information to ensure that all of the study forms are completed consistently and accurately.

The case enrolment folder has different forms. These forms are given numbers according to the departments/points of first use/filling to ease identification, tracking, filling and filling. Empty forms are placed in respective departments where tests are to be done. Every form has a well labeled pigeon hall/tray. Table below shows all the forms, their number and point of first use/filling.

	<i>Form Type</i>	<i>Form Number</i>	<i>Form description</i>	<i>Point of first use</i>
Case Enrolment forms		CEF1	Screener forms	BL Ward
		CEF2	Consent/assent form	BL Ward
		CEF3	Tissue Release Form	BL Ward
Case report Forms		CRF1	Questionnaire	
Research investigation Forms		RIF1	Research blood processing form	BL Ward
		RIF2	Research saliva processing form	BL Ward
Hospital Investigation Forms	Physical Examination	HIPE1	Height and Weight Measurements form	BL Ward
		HIPE2	BL Tumour Location Form	BL Ward
	Procedures	PROC1	Lab requisition	BL Ward
		PROC2	Procedure requisition	BL Ward
	Chemistry	HIFC1	Liver Function Results form	Bio chemistry Lab
		HIFC2	Renal Function Test Results Forms	Bio chemistry Lab
	Haematology	HIFH1	CBC Results Form	
	Microbiology	HIFM1	Malaria Thin Parasite Smear Test results Form	Microbiology Lab
		HIFM2	Thick Malaria Parasite Smear Results Form	Microbiology Lab
		HIFM3	Malaria Rapid Test Results Form	Microbiology Lab
		HIFM4	Stool Microscopy result Form	Microbiology Lab

		HIFM5	Lumbar Puncture Results Form	Microbiology Lab
		HIFM6	HIV Serology Results Form	Microbiology Lab
	Pathology	HIFP1	Bone Marrow Aspirate Results Form	Pathology Lab
		HIFP2	BL Histology Results Form	Pathology Lab
	RADIOLOGY	HIFR1	Chest X-Ray Results Form	Radiology Lab
		HIFR2	Ultrasound Results Form	Radiology Lab
Study Linking forms	LINK	SLF1	Lab link form	Emblem processing Lab
		SLF2	Blood and Saliva Specimen Tracking Form	Emblem Processing Lab
		SLF3	Participant Tracking Form	BL Ward
		SLF4	Participant Card	BL Ward
		SLF5	Tissue Specimen Tracking Log	
		SLF6	Participant Tracking Form (Population Controls)	
		SLF7	Participant Tracking Form (HCII Controls)	
		SLF8	Participant Tracking Form (Matched Controls)	
Controls		GWS1	General Work Schedule	
		PVN1	Pre-Visit Notification	
		DWS1	Daily Work Schedule	

General instructions are followed by form-specific instructions.

## 1.2 General Instructions

All study forms must be completed in black ball-point pen. This will ensure that all responses to items on the form are legible on all parts of the multi-part forms. Additionally, ball-point pen does not smear or fade over time and is more easily photo-copied than pencil.

Complete forms as legibly as possible. Especially, if a box needs to be ticked, mark clearly in the center of the box with an **X**. The **X** or  $\checkmark$  should never cross from one box to another. Do not shade in the box or mark it with a slash or other character. Record only one answer except when given the instructions like “Mark all that apply.”

This will ensure that the appropriate response appears on all copies of the multi-part forms. If individual boxes or spaces are provided for entering numbers or characters, make sure to write one number or character in each space rather than fitting two into a single box. These numbers must be in the center of boxes and clearly written. For example:

Enter    

1	1
---	---

    Not    

11	
----	--

Be careful not to write on forms if they are stacked one on top of the other. The responses to the top form may be recorded on the second or third copy of an unrelated form in the stack below.

If a mistake is made while completing an item on the form, do not erase, overwrite or obliterate the original response. Make a single horizontal line through the original response and add the corrected response next to the original response. Initial and date the correction. For example:

*jd*  
*22-JUN-05*  
yes    no  
~~☒~~    ☒

Mark a response to all questions on the form. In most cases, there are either boxes to indicate “not done” if a particular test was not completed or an instruction on the form to write “N/A” to indicate that a response was not applicable or not available. If an item is sent to the EMBLEM study office for processing and it has no response, it may be thought that the item was inadvertently skipped rather than intentionally left blank.

Any personal identifiers required on the forms (name, address, etc....) will be retained in the EMBLEM Study Office for tracking purposes only and will not be transmitted to NCI or any other outside consultant.

Other identifying information regarding the subjects such as age or gender will only be reported in aggregate format so as to protect the confidentiality of the children.

Make sure that the appropriate labels have been affixed to each part of the multi-part forms. Copies that stay at the EMBLEM office for tracking purposes (i.e. top copy of the Lab and Procedure Request Form) need both hospital ID and the subject ID labels affixed. Copies of the forms that are circulated throughout the hospital or retained by the hospital lab for their records are labeled only with the hospital ID (i.e. the bottom copies of the lab/procedure request forms and the lab/procedure results forms). The middle copies of the lab and procedure request forms and the top copies of the results forms will initially be labeled only with the hospital ID, but will have the subject ID label affixed at the time they are returned to the EMBLEM Study office prior to entry into the computer.

Record all dates are in the DDMMYYYY format, unless otherwise specified on the form: Date/months have two digits. If the number for the day or month is less than 10, enter a zero in the first box, followed by the day and/or month. The year is entered in full (four digits). Considering dates of birth, if the birth year is known, but not the day and month then enter “99” for day and “99” for month in which the child was born. Work with the caregiver to determine the date with as much accuracy as possible by giving cues that may help to establish the date. For example, was the child born in an election year and if so, who won the election. If necessary, enter the child’s age in completed years. So, for example, if the date is January 5, 2010, it will be entered as follows:

|\_0\_|\_5\_| / |\_0\_|\_1\_| / |\_2\_|\_0\_|\_1\_|\_0\_|

DAY      MONTH      YEAR

On all the forms, don’t forget to affix the subject ID and Hospital IDs, Patient’s Name, sex and age. Transcribers/checkers need to write down their initials and the dates for when this was done. Where we have machine printouts, please attach a copy of the results

The requisition forms (procedure Requisition and Lab Requisition forms) are 3-part (carbon-copy) NCR with the top part, having fields for both subject ID and hospital ID, which should be fixed. The top copy is sent to Study office for tracking purposes, the second/ middle and third/bottom copies are sent with the patient (or patient’s sample) to the procedure /sample taking personnel who completes and returns the second carbon copy to the study office. Results of each procedure or sample processing are recorded separately on the appropriate result reporting form. The results forms are 2-part carbon-copy NCR with the top part being completed and returned to the EMBLEM study office (where the EMBLEM subject ID label is affixed) and the bottom carbon-copy can be retained in the laboratory or procedure location for their records. Two extra hospital ID labels are clipped to the requisition form so that the lab will have labels available to affix to both copies of the results form.

## 1.3 Form Specific Instructions

### 1.3.1 *Eligibility Screener Form*

This form is used to determine and to document eligibility of a child identified by the Spotter as a potential case for EMBLEM. At the end of the form, the child will be eligible if:

- a) he/she meet ALL required inclusion criteria
- b) he/she exhibit NONE of the exclusion criteria.

Screening assessment is terminated at any point when an exclusion criterion is correct for the child. Please explain to the caregiver why the child cannot be enrolled in the study and thank the caregiver and child for their time and willingness to answer the questions. If the family decides not to participate in the study, please thank the family for taking the time to talk to you and wish them well.

Place the hospital ID labels on the bottom left hand corners of both pages of the screener form.

Determine the next screening number assignment (site code/sequence number/year) and enter this number at the top of the Screener Form.

If a subject is eligible, the next EMBLEM Subject ID will be assigned at the end of the screening process and the Subject ID label affixed to the bottom right corner of both pages of the Screener Form. The completed Screener Form should be placed in the subject's Enrollment Folder. If the subject is not eligible, place the Screener Form in the Screened, but Not Eligible Subject's Binder. This binder will be in Data Section of the EMBLEM Office.

Q1. Enter the date ("Today's Date") on which the child is screened in the boxes provided. If the number for the day of the month or of the month is less than 10, enter a zero in the first box, followed by the day and/or month. The year is entered as its four digit number. For example, for January 5, 2010, enter the date as follows:

_0_ _ _5_	/	_0_ _ _1_	/	_2_ _ _0_ _ _1_ _ _0_
DAY		MONTH		YEAR

Q2. Print the child's name on the line provided. The child's name will be keyed in a database that is kept at the EMBLEM Study office to keep track of all children who were screened for the EMBLEM Study. The child's name will not be returned to NCI.

Q3. Enter the child's date of birth in the spaces provided using a DD/MM/YYYY format. If the child's full birth date is not known, please enter as much of the date as is known. For example, if the birth year is known, but not the day and month then enter "99" for day and "99" for month in which the child was born, and the year as given. Work with the caregiver to figure out the most likely birth year the child was born with as much accuracy as possible by giving cues from major political or other events that may help the caregiver suggest the most likely birth year. For example, was the child born in an election year and if so, who

won the election, or when a major road was completed. Interviewers should use any civic event to as a reminder, and when the birth year is established in this manner, the fact should be noted on the form. If necessary, enter the child's age in completed years. If date of birth is known, leave the age field blank.

Q4. Using the admission records and, if necessary, ask the paediatrician on the BL ward whether the diagnosis of Burkitt Lymphoma (BL) has been made clinically and/or confirmed histologically. If the child has not been diagnosed clinically with BL, then the child is NOT ELIGIBLE and the screener need not ask any further questions.

Q5. Refer to the earliest birthdate for which children are eligible, or calculate the child's age by subtracting the birthdate from the current year to determine if the child is 14 years old or younger. If the child is 15 years, then the child is NOT ELIGIBLE to participate in EMBLEM and this is the end of the interview. Thank the caregiver for accepting to participate in the study.

Q6. Refer to the list of districts that are in the catchment area for EMBLEM. If the child does not live in one of these districts, then the child is NOT ELIGIBLE to participate in EMBLEM and end the interview.

Q7. Ask whether the child has lived in the study region for the at least four months before onset of the first BL symptom? (Refer to the list of districts included in the northcentral and northwest regions). If the answer to this question is no, mark this entry on the form as NOT ELIGIBLE to participate in EMBLEM and thank the caregiver.

Q8. Ask the child or participant whether they have received any BL specific chemotherapy treatment. You may need to cross-check with the pediatrician to make sure the right answer to this question is obtained. If the child has been started on BL-specific treatment, mark the entry as INELIGIBLE to participate in EMBLEM and thank the patient for their time. If they have questions, please answer them or refer them to the appropriate hospital staff.

Q9. Confirm with the child's pediatrician that the child is clinically stable enough to be enrolled into EMBLEM. Record the decision of the consultation with the paediatrician on the form under 9a through 9c. If the paediatrician determines that patient is stable, complete the eligibility check list and proceed to administer the consent process. If patient is still not stable, skip to Q10 and mark the patient as "ineligible".

Q10. The child is eligible to participate in EMBLEM if the answer to Q4; Q5; Q6; Q7; and Q9 are all "yes" and the answer to Q8 is "no". If the prior questions have been correctly answered and the screening has not been discontinued by the time the screener reached Q10, then it is expected that the child is eligible to participate in EMBLEM and the consent process can be initiated.

Q11. Obtain consent from the caregiver and for any child who is 7 years and older, please ask the child to agree to the study. If the parents/guardian refuse to participate in EMBLEM for any reason mark the child as NOT ELIGIBLE to participate in EMBLEM, and complete the Screener Form.

Q12. Record your EMBLEM assigned Identification Number on the form..

Once informed consent for the child to participate has been obtained, assign an EMBLEM Subject Identification Number to the child. This number is assigned by selecting the next available Enrollment Folder. The Enrollment Folder will have pre-printed subject ID labels and all forms needed to complete enrollment of the case. You will find two sticky Subject ID labels in the Enrollment Folder that are supposed to be placed on the Screener Form. Place the EMBLEM Subject ID labels on the bottom left corners of both sheets of this form. This subject identification will be this child's UNIQUE subject identification number and will be used on all study documentation related to this child in future.

DO NOT ASSIGN Enrollment Folders, (i.e., SUBJECT ID NUMBERS) TO INELIGIBLE SUBJECTS. The Screener Forms from those subjects, labeled with the hospital ID and EMBLEM Screening Number should be sent to EMBLEM Office for Data entry and filing in the "Screened, but not eligible Subjects" Binder for tracking purposes.



Screening Number: | | | | | | | | | | | | | | | |

### EMBLEM CASE ELIGIBILITY SCREENER

COMPLETE THIS FORM FOR NEWLY DIAGNOSED BURKITT LYMPHOMA (BL) PATIENTS  $\leq$  14 YEARS OF AGE.

1. TODAY'S DATE: | | | | / | | | | / | | | | | | | |  
DAY MONTH YEAR

2. PATIENT NAME: \_\_\_\_\_

3. DATE OF BIRTH: | | | | / | | | | / | | | | | | | | OR AGE: | | | |  
DAY MONTH YEAR

4. Has a diagnosis of BL been made clinically and/or confirmed histologically?

Yes ☐ 1

No ☐ 2 [PATIENT IS NOT ELIGIBLE, **SKIP TO QUESTION 10**]

5. Is patient  $\leq$  14 YEARS OF AGE?

Yes ☐ 1

No ☐ 2 [PATIENT IS NOT ELIGIBLE, **SKIP TO QUESTION 10**]

6. Is resident address within the study catchment area?

[REFER TO LIST OF ELIGIBLE DISTRICTS]

Yes ☐ 1

No ☐ 2 [PATIENT IS NOT ELIGIBLE, **SKIP TO QUESTION 10**]

7. Did patient live in study region for at least 4 months before developing symptoms of BL?

Yes ☐ 1

No ☐ 2 [PATIENT IS NOT ELIGIBLE, **SKIP TO QUESTION 10**]

8. Has patient ever received chemotherapy treatment for BL?

Yes ☐ 1 [PATIENT IS NOT ELIGIBLE, **SKIP TO QUESTION 10**]

No ☐ 2

**Hospital ID**

(stick label here)

**Subject ID**

(stick label here)

9. Is patient stable enough to be approached for study enrollment?

Yes ☐ 1

No ☐ 2 [GO TO RE-CONTACT TABLE]

### RE-CONTACT TABLE

For patients who are **not** stable enough for enrollment initially, note date of re-contact following consultation with the physician to determine favorable changes in the patient's condition.

9a. DAY 1 POST INITIAL CONTACT: |\_\_||\_\_| / |\_\_||\_\_| / |\_\_||\_\_||\_\_||\_\_|  
DAY MONTH YEAR

Is patient stable enough to be approached for study enrollment?

Yes ☐ 1 **Skip to Q10 - RE-CONTACT APPROPRIATE**

No ☐ 2 CONSULT WITH PHYSICIAN AGAIN ON **DAY 2** POST INITIAL CONTACT TO DETERMINE IF PATIENT'S CONDITION HAS STABILIZED.

9b. DAY 2 POST INITIAL CONTACT: : |\_\_||\_\_| / |\_\_||\_\_| / |\_\_||\_\_||\_\_||\_\_|  
DAY MONTH YEAR

Is patient stable enough to be approached for study enrollment?

Yes ☐ 1 **Skip to Q10 - RE-CONTACT APPROPRIATE**

No ☐ 2 CONSULT WITH PHYSICIAN AGAIN ON **DAY 3** POST INITIAL CONTACT TO DETERMINE IF PATIENT'S CONDITION HAS STABILIZED.

9c. DAY 3 POST INITIAL CONTACT: : |\_\_||\_\_| / |\_\_||\_\_| / |\_\_||\_\_||\_\_||\_\_|  
DAY MONTH YEAR

Is patient stable enough to be approached for study enrollment?

Yes ☐ 1 **Continue to Q10 - RE-CONTACT APPROPRIATE**

No ☐ 2 [PATIENT IS STILL NOT STABLE AND IS THUS NOT ELIGIBLE]

10. PATIENT ELIGIBLE FOR STUDY: Yes ☐ 1

No ☐ 2 [SKIP TO QUESTION 12]

11. CONSENT OBTAINED:

Yes ☐ 1

No ☐ 2 [PATIENT IS NOT ELIGIBLE]

12. SCREENER'S ID: |\_\_||\_\_||\_\_||\_\_||\_\_||\_\_||\_\_||\_\_||\_\_||\_\_|

**Hospital ID**

(stick label here)

**Subject ID**

(stick label here)

### **1.3.2 Tracking Form**

The Tracking Form is used to ensure that all of the procedures required by the EMBLEM Study are completed and the appropriate data forms obtained for entry into the EMBLEM Study database. The Tracking Form, pre-labeled with the Subject ID is contained in the Enrollment Folder.

Complete the information at the top of the form at the time that the subject is enrolled in EMBLEM.

- Date consent signed – obtain the date from the signed consent form. Mark each of the boxes indicating the specific activities that the subject consented to (questionnaire, specimens, storage).
  - If the subject does not consent to the questionnaire, mark “not done” under the Interview Questionnaire Activity and indicate the reason of 4=Subject refused at consent.
  - If the subject does not consent to collection of specimens, mark “not done” under Saliva specimen collection and Blood for Research and indicate the reason of 4=Subject refused at consent.
  - If the subject does not consent to storage of specimens, but does consent to collection of specimens, a note must be made in the enrollment folder to prevent shipment of aliquots for long-term storage.
- Date of clinical BL diagnosis – consult with the hospital chart or Pediatrician to complete the date that a clinical diagnosis of BL was made. This is most-often the date of hospital admission.
- Indicate if a biopsy was performed at the time of admission. If not, find out where the biopsy was performed and obtain consent for requesting the pathology specimen from the outside institution.
- Indicate if the subject wishes to be informed of their HIV serology results.

Review the Activities in the table at the bottom of the page. Ensure that the appropriate data collection form is obtained for each of the activities completed. If an activity is not done, make sure to obtain the reason that it was not done.

## EMBLEM TRACKING FORM FOR CASE SUBJECTS

●Date Consent Signed (DDMMYYYY): \_\_\_\_/\_\_\_\_/\_\_\_\_, check appropriate tiers of consent:

Questionnaire ☐1   Specimens ☐2   Storage ☐3

●Date of Clinical BL Diagnosis (DDMMYYYY): \_\_\_\_/\_\_\_\_/\_\_\_\_

●Was a BL related biopsy performed at this hospital during this admission?

1 ☐ Yes   2 ☐ No



Where was biopsy performed? \_\_\_\_\_

Was consent obtained to request specimen?

1 ☐ Yes   Date of Specimen Consent (DDMMYYYY): \_\_\_\_/\_\_\_\_/\_\_\_\_

2 ☐ No   COMMENTS: \_\_\_\_\_

●Does subject wish to have HIV results reported?      1 ☐ Yes      2 ☐ No

**Check the appropriate box to indicate final status for each of the following activities:**

Activity	Completed 1	Not Done 2	Reason Not Done-Use the following codes to report reason that an activity was not done:	
			1= test/procedure not ordered 2=unable to obtain specimen 3=equipment out of order	4=subject refused at consent 5= subject refused after consent 6=other, specify
Interview questionnaire	<input type="checkbox"/>	<input type="checkbox"/>	____	
Height, Weight, and Anatomic Site Tumor Location	<input type="checkbox"/>	<input type="checkbox"/>	____	
Histology specimen and report obtained	<input type="checkbox"/>	<input type="checkbox"/>	____	
Saliva specimen collection	<input type="checkbox"/>	<input type="checkbox"/>	____	
Blood for research	<input type="checkbox"/>	<input type="checkbox"/>	____	
HIV testing	<input type="checkbox"/>	<input type="checkbox"/>	____	
CBC w/differential and ESR	<input type="checkbox"/>	<input type="checkbox"/>	____	
Liver function tests	<input type="checkbox"/>	<input type="checkbox"/>	____	
Renal function tests	<input type="checkbox"/>	<input type="checkbox"/>	____	
Malaria thin smear	<input type="checkbox"/>	<input type="checkbox"/>	____	
Malaria thick smear	<input type="checkbox"/>	<input type="checkbox"/>	____	
Rapid Malaria Test	<input type="checkbox"/>	<input type="checkbox"/>	____	
Stool microscopy	<input type="checkbox"/>	<input type="checkbox"/>	____	
Chest x-ray	<input type="checkbox"/>	<input type="checkbox"/>	____	
Abdominal ultrasound	<input type="checkbox"/>	<input type="checkbox"/>	____	
Bone marrow aspirate	<input type="checkbox"/>	<input type="checkbox"/>	____	

Lumbar puncture	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
-----------------	--------------------------	--------------------------	--------------------------

**Hospital ID**

(stick label here)

**Subject ID**

(stick label here)

### **1.3.3 Interview Questionnaire**

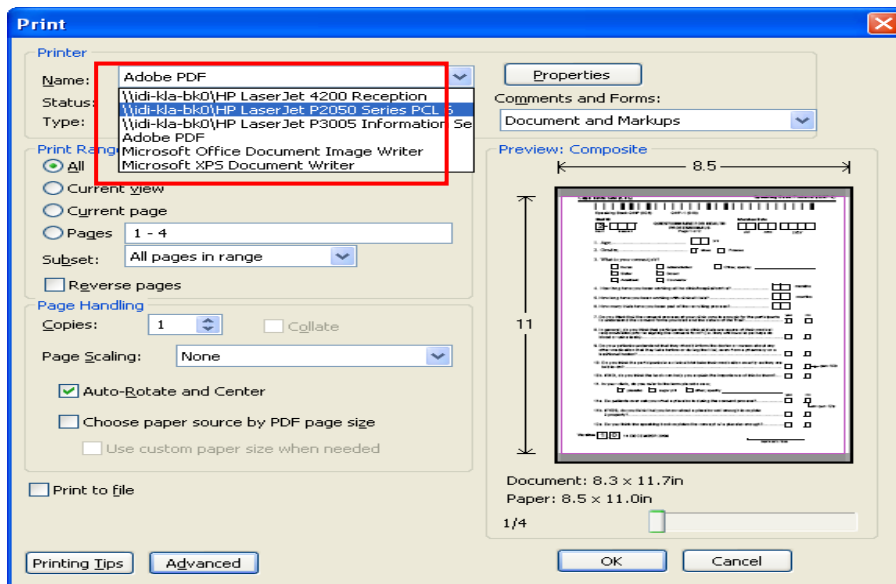
The questionnaire is one of the Forms in the Enrollment Folder. The questionnaire consists of two parts: a) a cover sheet with general information and b) the main part with the questions researchers will use to do their studies. The cover sheet is retained in the EMBLEM study office and the information is entered into the EMBLEM study database for tracking purposes. The main part of the questionnaire is formatted for optical scanning by IDI. Those pages are faxed to IDI for processing within 72 hours of data collection. The instructions for printing, completing, and faxing the scannable datafax portion of the Questionnaire are included in section 1-4a. General interview techniques are included in section 1-4b. Clarifications to responses for specific questions are included in section 1-4c.

The Questionnaire is the main method by which the patient's experiences, some of which may influence the risk for Burkitt lymphoma, will be measured. You, as an interviewer, have no control of how patients may recall their experiences. You, however, could have much influence in how patients choose the responses they give. The Interview techniques and question-by-question instructions below are designed to give you a good understanding of "what the question is" that is being posed to the participant so that you can ask the questions the same way for all participants. If you suspect the participant has misunderstood the questions, you can use the examples given in the question by question specifications to provide more clarification. *You should not, however, discourage the participant from giving an answer they genuinely believe to be correct simply because you think it is the wrong answer. The answer is only wrong when it is not given by the participant.*

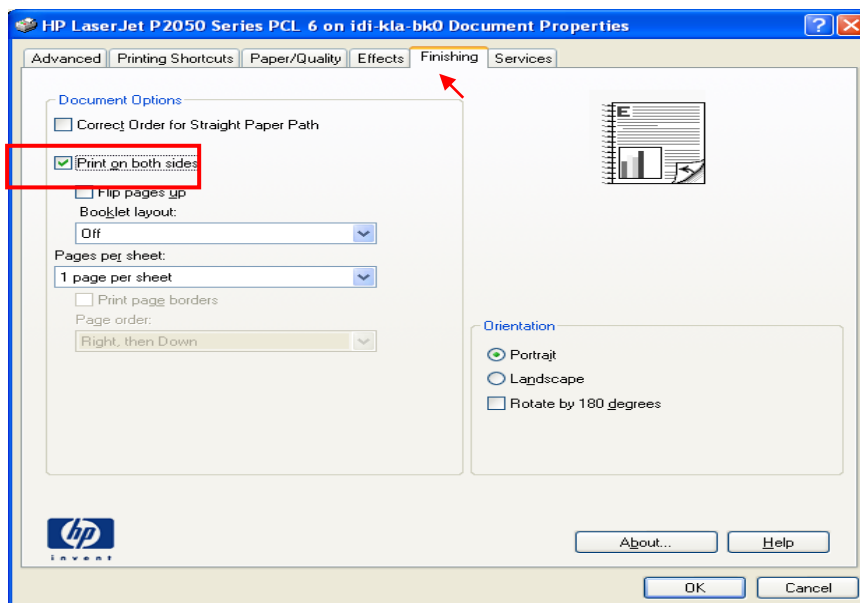
#### **1-4a Managing the Datafax portion of the Questionnaire**

##### **Printing the Datafax Questionnaire**

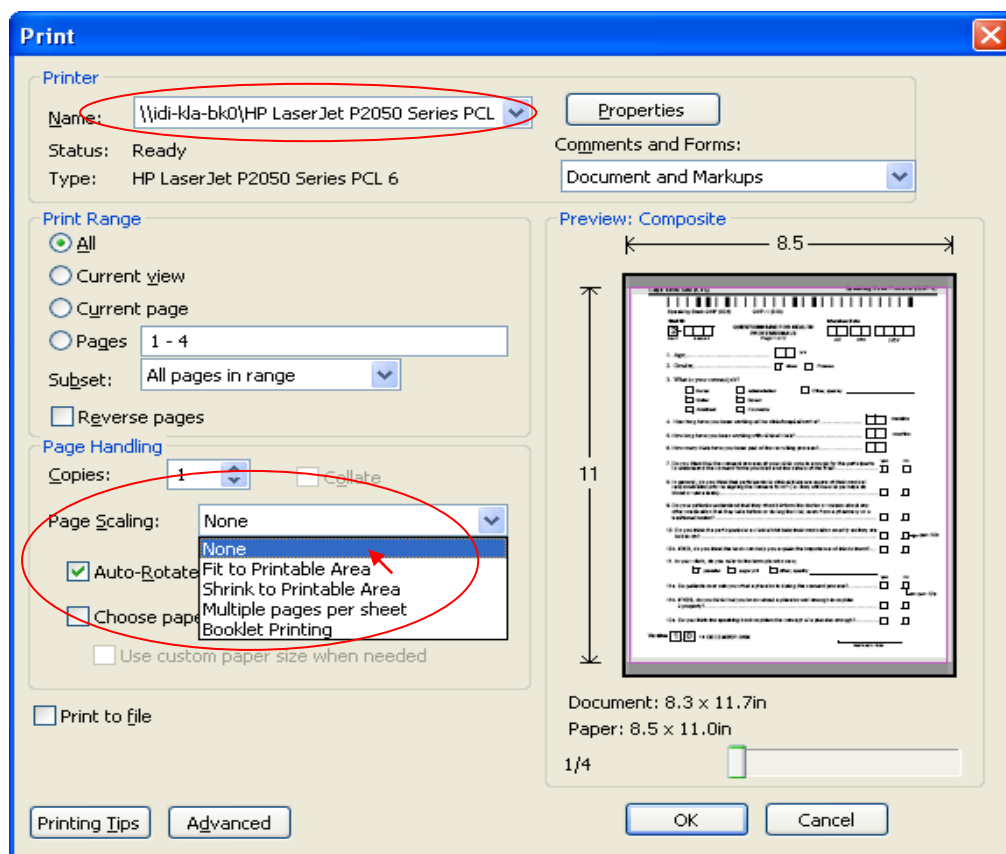
1. Open the PDF version of the Case Report Form (CRF) by double clicking it.
2. Click on File – Print and specify the printer Name your computer is attached to as illustrated below;



- Next to the Printer Name on the right, click on the 'Properties' button and this displays another dialogue box with a Menu with 6 items. Click on "Finishing" menu item and Check "Print on both sides" then OK to close the dialogue box.



- This leaves the first dialogue box open and check to ensure that the right printer Name is specified and that the page scaling is "None"



Changing the Page scaling to any other than “None” won’t give us satisfactory results with DataFax and this may call for reprinting, re-transcribing and re-faxing the completed CRFs hence avoid double work by ensuring all instructions are followed.

## Completing the Datafax Questionnaire

At the time that the questionnaire interview is administered, EMBLEM subject ID labels will be placed at the bottom of each CRF page. Prior to faxing the questionnaire, the Subject ID number will be hand-written in the designated place at top left of each CRF page. Only the hand-written ID will be read by the datafax scanner. The ID label will be used for QC purposes only.

Use only black medium ballpoint pen to complete the questionnaire (supplied by the study).

By design, DataFax does not pose any special CRF completion constraints other than what would be found in Good Clinical Practice guidelines. Above all else, forms should be completed legibly, and accurately.



All forms should be reviewed by yourself or the field data editor before being sent to the Data Management Center. The forms should be reviewed at the office by the Study Data Manager at the Data Management Center.

Faxing of forms should be done within 72 hours of completion.

Following these simple rules will eliminate many of the simple quality control issues before they happen. Every form is reviewed at Data Management Center by at least two people: the one receiving the forms and the one faxing or filing the forms. Statisticians and programmers often review forms months or even years after completion. Monitors (Field data editors) often review original CRFs in the field. The quality of the data is largely dependent on the quality of the CRFs.

- Legibly print responses or comments.
- Press firmly when recording data or writing comments. You will be provided with clip boards to write on.
- When completing number boxes, write as large as possible while still keeping all responses within the boundaries of the individual data boxes.
- Handwrite all data on all forms. Do not type data onto forms.
- Record data on the front of forms only. The back of forms may be used for other notes and quality control checks that are not required for DataFax.
- Do not use the 1/2-inch margins at the top, bottom, or sides of the forms for recording data.
- Record “Other, specify” responses on the line(s) provided. If the line(s) provided are not long enough, continue in another blank area of the form (within the page margins).
- Record only **one** answer except when given the instructions like “Mark all that apply.”
- Never mark over or punch holes through the barcodes at the top of the form. DataFax requires the barcode to identify pages.
- Never use “white-out” or other correction fluid or tape on forms.
- Because some DataFax and non-DataFax forms are source documents (documents where the research information was originally recorded), the site staff person who initially completes a page must record his/her signature and the date in the space provided in the bottom of each “module” or section.
- Before faxing forms, remove paper clips and post-it notes from form pages.

- If a data field is unclear, please use a comment line or other blank area on the form to explain it. For example, write “specimen dropped”, “refused”, or other short clarifications. Be sure to initial and date all marginal entries.

Remember that many people will review each CRF. Be as clear, legible, concise, and accurate as possible. Carefully review each form after completion for completeness and accuracy.

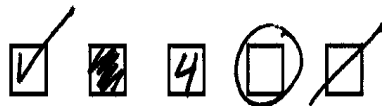
### How to Mark Response Boxes:

Many items have a box or series of boxes for recording a response. Mark the box clearly with an **X**. Do not shade in the box or mark it with a slash or other character.

#### Correct:



#### Incorrect/wrong:



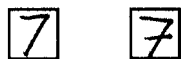
### How to Record Numbers Within Boxes:

Although DataFax has built-in capabilities to perform Intelligent Character Recognition (ICR) on the forms to augment data entry, ultimately the staff at the Data Management Center must be able to read the data on the forms.

The following instructions need to be followed when recording:

The DataFax and the Data Management Center staff can interpret a wide variety of handwriting and number styles. For example, the following are all correct:

#### Correct:



Right justify **all** numbers. For example, to record a 7 into a three-digit box, enter either a 0 or a blank in each of the first two boxes, and a 7 in the third box. Entering either leading zeros or blanks are acceptable, but you must be consistent: **For EMBLEM, the standard will be entering leading zeros.**

#### Correct:

		7
--	--	---

0	0	7
---	---	---

Write the number(s) within the boundaries of the box. Try not to touch the edges or stray outside the boundaries, but it is important to write the numbers as large as possible within the boxes

**Correct:**

3	1	0	3	0	4
<i>dd</i>		<i>mm</i>		<i>yy</i>	

**Incorrect/wrong (too small):**

3	1	0	3	0	4
<i>dd</i>		<i>mm</i>		<i>yy</i>	

**Incorrect/wrong (go outside boundaries of the box):**

3	1	0	3	0	4
<i>dd</i>		<i>mm</i>		<i>yy</i>	

**Special Considerations for Recording Dates:**

Dates on data collection forms are recorded using the format “dd mm yy”, representing a two-digit day, a two-digit month, and a two-digit year (the last two digits of the year).

If the dates are partially known, cross out the unknown date components and write “unknown” or “UK” or “Unknown” in the margin.

**Correct:**

<i>UK</i>				0	3	0	4
<del></del>		<del></del>		<i>mm</i>		<i>yy</i>	
<i>cp</i>		<i>31/03/04</i>					

### Data Corrections and Additions:

There will be times when data entered on data collection forms will need to be changed, clarified, or additional data will need to be recorded. All corrections and changes must be made to the original form that was first completed.

Data on a completed DataFax form must never be copied onto a new form unless specifically instructed by Data Management Center.

Each correction or change made to a data collection form after its initial completion must be initialed and dated (regardless of whether or not it has been faxed to the Data Management Center for the first time). Any time a data correction or addition is made, check to make sure that each addition/change is initialed and dated, then re-fax the page to the Data Management Center. If a correction or addition is made to one page of a multi-page DataFax form, only re-fax the page with the change.

***Remember:*** Make all data corrections and additions to the original form. Never transfer information from a completed form to a new form.

### Examples of data corrections or additions:

If a numeric box is completed incorrectly, draw a *single line* through the incorrect entry, write the correct answer near the box, and initial and date the correction as shown below. Remember that your responses will be read by the data management staff at the Data Management Center. As long as your corrections are clear, they will be interpreted correctly.

29 <sup>CP</sup> 31/03/04  

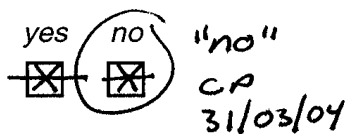
0	4	2
---	---	---

If an **X** is marked in the wrong box, draw a *single line* through the incorrectly marked box, mark the correct box, and then initial and date the correction as shown below.

jd 22-JUN-03  
yes no  

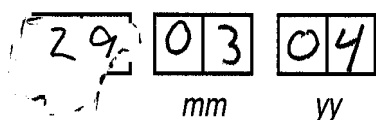
<del>X</del>	X
--------------	---

If the correct answer has previously been crossed out, circle the correct item, write an explanation in the white space near the item, and initial and date all corrections.

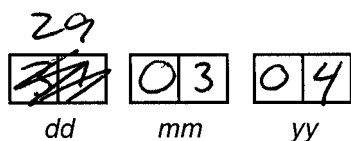


**Remember:** Each time you correct or change an entry, draw a single line through the incorrect entry so that it can still be easily read. Do not make the incorrect entry unreadable with multiple cross-outs.

**Incorrect/wrong (use of correction fluid):**



**Incorrect/wrong (not initialed and dated, previous entry obscured):**

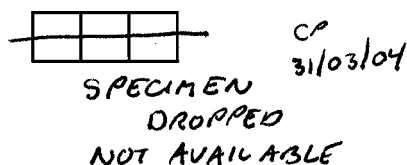


**Note:** Always initial and date each correction or addition made to the data collection forms.

### How to Handle Missing and Unknown Data

Data items that are left blank are considered missing data and will result in a QC note **unless** the item is blank as a result of skip pattern instructions. To avoid QC notes on items where the answer to an item is not known, not available, or the participant refuses to answer, draw a line through the blank boxes for the item, write “don’t know”, “refuses” or other appropriate explanation in the white space next to the box(es), and initial and date the item.

For example, when a field should have been completed, but the data is unavailable or unknown, cross the field out, write a brief explanation near the field, and initial and



date the field.

## **Review of Data Collection Forms at the Data Management Center before Faxing to DataFax**

Before faxing to DataFax, staff at the EMBLEM Data Management Center must review each page of the DataFax forms to check that it is complete and accurate, and that all writing is clear. For this review, staff responsibilities should be specified in each site's data management SOP. The following list can be used as a review checklist:

Never record any information on a DataFax form that identifies the participant, other than the Participant ID. This includes the participant's name, phone number, national identification number, or any other personal identifiers.

For each item, make sure a response has been recorded, unless the item has been skipped as instructed by skip patterns.

Make sure all handwritten responses are clearly recorded.

Make sure there are no marks on or above the DataFax barcode at the top of each DataFax page.

Check for common errors. The most avoidable QC notes involve:

- missing dates;
- missing visit codes;
- incorrect Participant IDs;
- incorrect visit codes; and/or
- missing data for items beginning a series of skip patterns.

While forms are waiting to be reviewed and faxed to DataFax, it is important that they are received and stored systematically to avoid loss of data to unauthorized persons/groups. In addition, once the the form has been faxed to DataFax, the form should be so marked and filed away with in the Faxed Forms Cupboard.

***Suggestion: Use white space on back of page for a date stamp***

Be sure to date stamp the page each time it is faxed—including re-faxes.

***Suggestion: The site staff reviewing and faxing the CRFs must write their Initials and Date at the back of each CRF in the section provided.***

Be sure to Initial and Date the CRF each time it is faxed—including re-faxes.

## **Faxing DataFax Forms to DataFax**

Make sure the adjustable paper guides are snug against the pages in the input tray. Some machines use the position of these guides to determine how large the pages are and will resize the fax, shrinking it, if the guides are not snug against the pages.

Do not overload your fax machine. The machine may pull in two pages at a time, or jam if the input tray is overloaded. There is also a limit on how many pages a fax machine can store in memory at one time. Most fax machines have a counter indicating the percentage of memory free (ranging from 100% to 0%). Be certain that there is sufficient memory when faxing.

Check the number of pages in your fax against the number your fax machine reports having read in. If it has read fewer pages than were in the input tray, it has probably pulled more than one page at a time. If this happens, abort the fax and read it in again.

Each site should maintain some form of faxing procedure and log of faxes sent. This can be quite simple, or complex, as the site feels is needed. If pages are not received, they will appear on Quality Control Reports, so no data will be lost. If in doubt, it is always better to fax a form twice than not at all.

Only DataFax forms (forms with a barcode at the top of the page) should be faxed to DataFax. Other (local, non-DataFax) forms may be completed for this study, but these are never faxed to DataFax.

The Data Management Center DataFax e-mail address is:

**[datafax@datafax1.niaid.nih.gov](mailto:datafax@datafax1.niaid.nih.gov)**

If your Internet connection is down, on an emergency basis you may fax to [cluswata@idi.co.ug](mailto:cluswata@idi.co.ug) as a backup. Please let the Data Manager, Charles Luswata know about it by calling the number below.

The Data Management Center at the IDI number is: **+256 712 679 227**

After thorough review, forms should be faxed to the Data Management Center within 3 days following the participant's visit. Keep in mind that the sooner a form is faxed, the sooner the data will enter the study database.

## **1-4b Interviewing Study Participants**

The following provides a brief overview of appropriate interviewing techniques and should be used to ensure uniformity in the quality of the data collection.

### **The Professional Interviewer**

As an interviewer, you need to maintain high professional standards when carrying out duties for the study. To perform your duties competently, you are expected to understand the reasons we are doing this study - its purpose, the type of samples we are collecting, the questionnaire, etc, so that you will be able to fully explain to the participants. You are also expected to be knowledgeable about the procedures of the study, and be prepared to answer any question a respondent might pose.

As a professional interviewer, you need to maintain a high ethical standard, collect data objectively and treat with utmost confidence and respect all the information gathered during an interview. You will have to explain to each respondent that you are completing the interview impartially and that you will keep confidential all information provided. The interview is the critical communication link between questions the researchers are seeking to understand and the causes of disease in the population where the study is being conducted. Therefore, as an interviewer, you are an important member of the research team and you ensure the integrity of that link.

When conducting the interview, the interviewer must be clearly audible when reading the questions, make sure that the respondent gives relevant and complete answers, and record all answers provided clearly and accurately on the questionnaire. The information gathered in the interview can be combined into summary that accurately reflects the experiences, thoughts, actions, and feelings of respondents only if interviewers perform their duties diligently and record the answers uniformly.

Because the data collection process is important to the success of any research study, data collection procedures have been standardized to maximize the quality of the data. Data collection procedures are standardized to minimize errors that may be introduced during data collection. We are depending on you to follow the procedures described both in this manual and in the body of the questionnaire to ensure the integrity of the data. The study investigators will serve as a resource to discuss unusual situations, review standardized procedures, or to answer questions about any aspect of the study that was not anticipated. This will ensure that the questionnaire is administered and conducted the same way by all interviewers across all sites.

Respondents are usually willing to share their private information for research purposes. Thus, your assurance to the participant that all information collected during research will be used only for research is important to make sure that participants respond



truthfully and completely. This means that your responsibilities include protecting the privacy not only the data collected on the questionnaire, but also information that you may gather through incidental observation or through casual conversation before or after the interview. Thus, you cannot divulge names, tell facts about, or reveal the opinions of anyone you interview. Information gathered during the interview can be shared only with the research team, whose members are under the same ethical and moral obligations as you are to protect the people interviewed. The information collected during the interview will be shared with other researchers in reports published, but only as percentages and totals in such a way that no individually identifiable information can be linked to any participant.

### **Privacy and Confidentiality**

As an interviewer, you may sometimes ask questions that are very private. Participants who have consented to the interview are likely to be willing to answer those questions when they are asked in private. They are willing to give information because they trust that it will be used only for research purposes. In protecting of all information gained about respondents during the interview, you will be recognizing and respecting the following rights of the participants:

- X            The right for their information to be accurately collected, recorded, and represented in the study
- X            The right to participate after giving informed consent
- X            The right to refuse or end their participation without losing their right to services at the hospitals where the study is being conducted

### **Respondents with Disabilities**

You may encounter a respondent who is disabled. Our concern is whether the respondent is physically and emotionally capable of participating in the study. You will have to make this judgment. Some people may have trouble understanding some of the questions, particularly questions that require the respondent to have a good memory of some experiences, such as the medical history questions.

If a respondent does not seem to understand the questions or is giving answers that appear to be unrelated to the questions being asked, slow down the pace of asking and give them adequate time to think about the answers. If, at any point during the interview, you suspect that the respondent does not understand the questions, or is confused or for some reason unable to give accurate answers, you should discontinue the interview with that person

and consult with the EMBLEM study coordinator to determine an alternate course of action for acquiring the information. You may encounter a respondent who cannot read. Since the questions are read aloud, illiteracy should not be a limitation that prevents participation. If the respondent is not fluent in English, make sure the question is translated accurately in the local language to ensure that the respondent clearly understands the question.

### **Standardization of Questionnaire Administration**

Standardizing the interview is crucial to guarantee that the data being collected are reliably and reproducibly. Thus, data collected from one respondent should be comparable to those collected from another respondent, regardless of date of collection or interviewer performing the interview. Listed below are general procedures that should be followed to ensure uniformity. Please review them and adhere to them when administering the interview.

- a. Ask the questions by reading verbatim the words printed on the questionnaire -

This may seem obvious, but it is the key to ensuring comparability of data gathered from the study. If you change the wording in a question, even slightly, the respondent's answer may change and, therefore, introduce distortion in the results. When translating a question into the local language for respondents who are not fluent in English, the local study team will agree on the correct translation for all the questions; that translation should be followed at all times.

- b. Read the complete question -

The respondent may interrupt you and answer before hearing the complete question or before you have finished reading the entire list of response options. When this happens, politely explain to the respondent that you wish them to hear the entire question before giving their answer, and then re-read the question from the beginning including any introductory remarks. Do not assume that a premature response is necessarily the one that the respondent will give after listening to the whole question as written in the questionnaire.

- c. In order to help you read questions with the correct emphasis, some questions may contain words and phrases that are highlighted in **bold** or are underlined. Emphasize words that appear in **bold** or that are underlined, and pause at a comma. Emphasized words are designed to make the questions easier for the respondent to understand. Also, commas are inserted after phrases to cue the interviewer that he or she is expected to pause before continuing with the remainder of the question.

- d. Do not read aloud words/phrases that appear in ALL CAPS -  
The words and sentences that are printed in ALL CAPS are either instructions to you or answer options that should not be read aloud to the respondent.

- e. Read questions slowly -  
As you become familiar with the questionnaire, you may be tempted to read through the questions more quickly. However, you must remember that this is the first time the participant is hearing the questions, and therefore you must read slowly so he or she understands everything you are asking. A pace of about 2 words per second is generally recommended.
- f. Do not suggest answers to the respondents -  
  
As you progress through the questionnaire you may come across questions that you might think you know the answer to, based upon prior information you have heard. This can lead to a tendency to suggest answers to the respondents, and should be avoided.
- g. Become comfortable with the interview material –  
  
To increase your comfort level with the material presented in the questionnaire, it is helpful to practice reading the questions before you begin interviewing. You will get better at it as you practice during training, but most interviewers find additional practice on their own to be helpful in familiarizing themselves with the material. It is best to ask a co-worker, friend, or family member to role-play with you (by making up fictitious information) so you can simulate the interview before going Alive@. In addition, carefully read the instructions provided in the Question-by-Question instructions.
- h. Discourage discussion of questions with respondent -  
Some participants may provide you with more information than you request. Participants will often view you as an interested person who would like to hear more about their concerns. It is important to gently discourage the participant from giving information that has not been asked, as it slows down the interview and may result in your recording the responses wrongly. If you find that you must listen patiently to avoid upsetting the respondent, please do so. In most cases, you can discourage this by listening attentively but not responding, or by responding with something neutral, such as AI see@ or AI understand@, and then moving on to the next question.
- i. Do not add codes –  
  
You may only choose from the codes that appear next to the response options. Use the probing strategies provided to obtain responses that can be applicable to the codes that are already provided for the question. If the participant does not know the answer after sufficient probing, provide a “DON= T KNOW” response by selecting from the answer choices or writing in the margin next to the question.
- j. Be non-judgmental –

It is essential that you interact with participants in a non-judgmental manner. Regardless of what you hear from the respondent, you must accept the information without judging. It is important that you accept all that you hear in a matter-of-fact manner so that the respondent will continue to feel comfortable providing you information.

## **Probing**

By following the procedures described in the preceding section, you will be able to improve and standardize the manner in which you ask the questions. This section discusses Aprobing@, a technique that is used to help ensure that the answers given by your respondents are as accurate and as complete as is possible. Your need to probe the respondent will occur very rarely during the administration of this questionnaire because the majority of the questions require a straightforward AYES/NO@ or ATRUE/FALSE@, and others will offer a short set of response categories with the frequently seen AOTHER@ option. Other types of questions may require probing before you are satisfied with the answer.

Probes serve two purposes: they encourage a respondent to express him/herself completely, and they help the respondent focus on the accuracy of the answer being given to a specific question. In order to know when to use a probe, you as the interviewer must be thoroughly familiar with the questionnaire and know the objective of each question; that is, you must know what is being measured and what constitutes an acceptable response. All probes must stimulate thinking and responding without suggesting possible answers. Neutral non-directive probes are the only type that may be used. Below are some examples of proper probing techniques.

### **a. Neutral Questions or Statements**

These probes encourage a respondent to further explain or elaborate upon a response. These must be stated in a neutral or non-challenging tone. Some examples of neutral probes are:

- \$ How do you mean?
- \$ What do you mean?
- \$ Tell me more about that.
- \$ Which would you say is closest to how you feel?

b. The Silent Probe

A timely pause is the easiest and often the most useful type of probe. This lets your respondent know that you are expecting or waiting for an answer or additional information.

c. Clarification

Clarification probes are to be used when you judge the respondent's answer to be unclear, inconsistent, ambiguous, or contradictory. You must take care, however, not to appear to challenge the respondent; instead, tactfully express concern over not completely understanding the nature of the response. Clarification can be obtained using statements and/or questions such as those in the examples below.

AI=m not quite sure which answer to put down. Can you tell me if that would be (*repeat answer choices*)?@

ACould you tell me a little more about that?@

d. Encouragement

This technique involves you conveying to the respondent that you understand what she had said, and you would like to hear more. You could encourage respondents to provide additional information with the use of phrases like AI see.....@, AThat=s interesting.....@, AUh-huh.....@, etc.

e. Repetition

Repetition could be either repeating the question, or repeating the response options. The former is useful when it appears that the respondent may have misunderstood the question, or deviates from the topics at hand. The latter technique of repeating the response may produce additional comments or explanation from the respondent, especially if you say it in the form of a question.

f. ADon=t Know@ Responses

When the respondent says AI don=t know@, it can mean two things.....either she/he is temporarily not sure of an answer and needs more time to think, or he/she actually does not know how to answer the question. You must be prepared to discern between the two. If the respondent simply appears to need more time to think about his/her answer, wait silently and expectantly until she/he gets her/his thoughts together.

Although ADon=t Know@ is a possible answer for all questions, always probe the respondent for his/her best guess or best estimate before

recording a ADON=T KNOW@ response. Do not leave any questions blank - either record the respondent=s answer or record a ADON=T KNOW@ for the question. In spite of probing, the respondent may insist that she/he does not know how to answer a particular question or gives the same response repeatedly. Once you have properly probed for an answer, you should accept the responses in the interest of not alienating the respondent, even if you believe she/he may be avoiding the question. Remember, there may be times when the respondent actually may not know the answer to one or more specific questions.

#### **1-4c Question by Question Specifications for the Interview Questionnaire**

The Questionnaire consists of a cover sheet followed by an introductory script to be read to the respondent prior to initiating the interview.

Complete the Cover Sheet with the following information:

**Date of Interview** – Fill in today’s date (DD/MM/YY) for the date of the interview.

**Interview ID** – Enter the ID assigned by the study.

**Subject Name, Age, and Gender** – complete the fields

**Hospital ID** – Affix the bar coded Hospital ID label in the space provided

**EMBLEM Subject ID** – Affix the bar coded Subject ID label in the space provided. Affix additional labels to the bottom left corners of each data collection form (numbered 1-19).

**Language of Questionnaire Administration** – Check the appropriate box for the language preferred by the respondent.

**Start Time of Interview, End Time of Interview** – Complete the start and end time (including am/pm designation).

**The cover sheet must be removed following questionnaire administration and filed in the Enrollment Folder for entry in the EMBLEM Office prior to faxing the questionnaire for processing by IDI.**

The full introductory script for the interview is not included in the formatted datafax instrument to be completed by the interviewer. The Interviewer must read the following introductory script text and all interview questions verbatim.

#### **GENERAL INTRODUCTORY STATEMENT ABOUT THE STUDY AND TYPES OF QUESTIONS**

**Thank you for agreeing to participate in the emblem study. I would like to ask you several things about the child who is being enrolled, the child’s family, and child’s home life.**

**The first set of questions will be about the child’s birth and home, where the child has been residing. The next set will be about the child’s parents and some of the things the child has been exposed to in the home. I will complete this interview by asking about the child’s vaccination and recent illnesses.**

**The interview has been prepared to take about 30 minutes to complete. If the questions are not clear and you do not understand them, please tell me and i will try to make them clear.**

**I do appreciate your agreeing to participate in the study. Please remember that your answers will be used for research and will remain private. We can begin whenever you are ready.**

Instructions to the interviewer are in **UPPER CASE BOLD** and are not to be read out loud. Response categories that should not be read out loud are also in UPPER CASE.

All questions must be answered and the answers marked on the formatted questionnaire instrument.

After probing, if the respondent does not know the answer, the interviewer must choose the DON'T KNOW option in the answers provided.

**Q1.** Ask the respondent to tell you their relationship to the child who is enrolled in the study.

If the respondent is NOT the biological mother, go on to Q2a, else if the biological mother, go to Q4.

If the respondent is NOT the biological father, skip to Q2a and go on to Q2b, else if the biological father, go to Q4.

If the respondent is an other relative, skip Q2a and Q2b and go on to Q3, else if not an other relative, go to Q4.

**Q2a.** Ask the respondent whether the child's biological mother is alive (see question 1).

**Q2b.** Ask the respondent whether the child's biological father is alive (see question 1).

Quite often, the information of whether the child's parents are alive will be discussed during the process of obtaining consent for the child to participate. When this information is known to you, please use it to record the right response. You only need to reconfirm with the respondent by saying...*"during our discussion, you told me that the child's biological mother passed away, please confirm that this is correct"*. As this is a sensitive topic, you are advised to practice and use appropriate expressions of sympathy and empathy when discussing deceased parents before proceeding to the next question.

**Q3.** If the answer to Q1 is "Other Relative", ask the respondent to tell you the exact relationship with the child.

**Q4.** Usually the gender of the child is obvious from observation or name, nonetheless, this question should be asked with the intention to confirm, say, [say the child's name] is a male (or female).

**Q5.** Record the child's birth date (DD/MM/YYYY). This information may have already been collected as part of the Screener Form, so it should be merely confirmed, especially if the interviewer is not the same person who screened the patient. The dates should be recorded as explained on the Screener Form.

If the day or month is unknown, enter the year of birth.



If the year of birth is unknown, enter an estimated age (YY).

**Q6.** Ask the respondent to tell you where the child was delivered. In most cases, the respondents will spontaneously tell you the place of delivery in such a way that you can find the correct option on the questionnaire. If they do not, please tell them the types of responses we have provided on the questionnaire and ask them to tell you the closest that apply to the child who was enrolled.

**Q7a.** Ask the respondent to tell you how many months the child's mother breastfed the child. For this question, we are looking for a spontaneous response from the respondent, and some respondent may give the answers in "years" like for two years. In that case, ask the respondent to give you a clearer answer by asking whether it was exactly two years, two and a half years or more. It may be easier to ask the respondent to say how many months after two years did the mother continue to breast feed the child. You then should add up the time and confirm with the respondent whether your number of months is about correct. Record that number as the answer (enter leading zeroes, if number is only 1 digit).

If the mother did not breastfeed or if she breastfed for less than one month, enter "00".

**Q7b.** If the biological mother did not breastfeed the child, ask whether the child was breastfed by another woman. Other women who could breast feed the child, including the child's grandmother, aunt, co-wife and so on. If the answer is no, skip to Q8, else go to Q7c.

**Q7c.** Ask the number of months (MM) the child was breastfed by this other woman.

**Q8.** Ask the respondent for the child's tribe. The child's tribe is usually the tribe of the child's father. A list of tribes in Uganda is given in List A, use the three digit code to record the tribe. Use code for "Other tribe" if applicable.

**Q9.** Ask the respondent how many children the biological mother of the child delivered. This question asks about live deliveries or full term still born children. These are children whom the tribe would have given a name and would count to the total number of children a woman has delivered, but it does not include early miscarriages.

**Q10.** Ask the respondent to tell you the birth order of the child enrolled. If enrollee is an only child, then the birth order is 1 and should be entered on the form as "01". The birth order should consider children who died or moved away.

**The following questions can be answered by the child's mother, father or guardian. Some questions are designed to gather information specifically about the child's mother and father.**

**When reading these questions, select the appropriate wording from within the parentheses.**

**For example, question 11 is presented as follows:**

"Please tell me when (you were/the child's mother was) born?"

**If respondent is the child's mother, question 11 would be read as follows:** "Please tell me when you were born?"

**If the respondent is not the child's mother, question 5 would be read as follows:** "Please tell me when the child's mother was born?"

**For all such questions, select the appropriate wording from within the parentheses.**

**Q11.** Ask the respondent when the child's mother was born and record the birth date as explained for the Screener Form (DD/MM/YYYY). You can use the same methods for figuring out the birth year as explained on the Screener Form.

If the day or month is unknown, enter the year of birth.

If the year of birth is unknown, enter an estimated age (YY).

**Q12a and b.** Ask the respondent the tribe of the child's parents and use the three digit codes in List A to record the response. Use code for "Other tribe" if applicable. Enter a separate code for the child's mother's tribe and a separate code for the child's father's tribe.

**Q13.** Ask the respondent the child's mother's religion. The religion of the mother is the religion the mother currently practices. If the mother changed her religion after getting married, then her new religion is the correct one. If the mother is of a different religion from the father and did not change her religion, then her original religion is the correct answer. If the respondent's answer is in the 'other', specify option write clearly the name of the religion in the space provided.

**Q14.** Ask the respondent the child's father's religion. If the respondent's answer is in the 'other', specify option, write clearly the name of the religion in the space provided.

**Q15.** Ask the respondent the district where the mother grew up as a child. If the mother lived in several districts, then the district where she lived most of the early years of childhood is the district that should be recorded. Use the three-digit code for the districts found in List B to record your answer. Use code for "Other district" if applicable.

**Q16.** Ask the respondent to tell you the district where the mother currently lives, which can be found in List B. If the mother lives in multiple districts, record the district where she lives most of the time. Use code for "Other district" if applicable. DO NOT ask this question if the mother is dead (see question 2).

**Q17.** Ask the respondent to tell you the mother's highest education level. The level of education is the highest class attended by the respondent, not the number of years spent in school. Learning in a religious school that teaches religious studies that are not part of the regular school curriculum does not count as a formal level of schooling. If the respondent's answer is in the 'other', specify option, write clearly the mother's level of education in the space provided.

**Q18.** Ask the respondent to tell you the mother's usual occupation. This question can be asked even if the child's mother is deceased. If the respondents answer is in the 'other', specify option, write clearly mother's occupation in the space provided. We have provided you a list of occupations and how to classify each (List C).

**Q19.** Ask the respondent to tell you the father's highest education level. Education is defined and classified as explained above in the question about mother's education. If the respondent's answer is in the 'other', specify option, write clearly the father's highest level of education in the space provided.

**Q20.** Ask the respondent to tell you the father's usual occupation. Like for the mother, this question can be asked even if father is deceased, and the occupation should be classified as explained above for the question about the mother's occupation. If the respondent's answer is in the 'other', specify option, write clearly the name of the religion in the space provided.

**Q21.** Ask the respondent to tell you the average monthly household income (in USHS) of residence where child normally lives. Include income of all permanent residents of the household. Questions about income are very sensitive and the answers will be approximate. Occasionally, respondents will want to give the impression that they are well off and will give you large values. Others may think that the study will give them more help if they give the impression that they are poor. You need to remind them that this question is to help researchers understand whether income influences the risk for Burkitt lymphoma, so they should give an answer that is correct for them and their family. The income should include people who stay regularly with the child, and not relatives of the child who occasionally come to visit them from the city or town.

**Q22.** Ask the respondent about the mother's monthly income (in USHS). This is the money that the mother earns directly from whatever work she does. Sometimes, women may earn during harvests, and if that money is theirs, it should be counted as her income and a monthly average estimated.

If the mother does not work or is deceased, enter all zeroes.

**Q23.** Ask the respondent to tell you whether the family's home is in a city, town, or village. We have provided you with a list of cities, municipalities, and town councils (List D). All the others will be considered villages. Refer the list of towns to help the respondent choose an answer.

**Q24.** Ask the respondent to estimate how far the child's home is from the nearest water source (a river, swamp, or lake) (in KM). To answer this question correctly, the respondent needs to know what the study refers to as a "lake", "swamp" or "river". The most obvious definitions will be known to the respondent. ***However, you should clarify to the respondent what "is not a lake, not a river, and not a swamp".*** Seasonal water collecting points like (Springs, wells, and man-made pools) where water may collect during the wet season and may increase to a large amount are not what this question is referring to. In general the rivers, swamps, and lakes will be large such that to cross them one needs a canoe, a bridge, or to swim across. Tall adults may wade through the deep water. Please refer to the list (List E) which names some of the lakes, rivers and swamps that are found in the study region.

The second information the patient needs to answer this question is how to estimate distance. Some people may be familiar with the distance as marked by milestones on the roads. Others may be aware of the distance between electricity poles. Use examples of

distances from hospital to the nearest public places like schools, town, next trading centre, churches or bus stops. All will be familiar with the distance from the town that is nearest to the hospital. The interviewer should use any of these cues to help the respondent figure out the distance from the child's home to the nearest water body (lake, river, or swamp).

**Q25.** Ask the respondent to name all types of animals that were kept inside or outside the home of the child last year. There is no need for prompting on this question. Record each type of animal that the patient spontaneously mentions. When the patient indicates that all the type animals have been mentioned, the interviewer should confirm that the other animals that are listed on the questionnaire were not kept inside or outside the child's home over the past 12 months.

The past 12 months refers to a one year period (12 months period) from the time when the respondent is being interviewed.

**Q26a.** Ask the respondent the number of adults aged 15 and over who lived in the same house as the child in the past year, i.e., 12 months from the time when the respondent is being interviewed. This number is approximate and includes adults who regularly live in the house, including students who attend boarding schools. Occasional visitors, such as students who visited during the holidays should not be counted in this number.

**Q26b.** Ask the respondent the number of children (<15 years) who lived in the same house as the child, including the child. If the child is the only child, record "01". Occasional visitors should not be counted.

**Q27.** Ask the respondent the number of separate rooms, excluding bathrooms and closets that there are in the house where the child lives. If the child lives in a hut with separate rooms, the number of rooms should be stated. If the hut is not divided into rooms, the hut should be recorded as a one-roomed house. If the household has several huts on the compound, this question refers to the hut where the child regularly sleeps.

**Q28.** Record the number of people who sleep in the same room as the child, including the child. If the child sleeps alone in a room, enter "01". Please note that "the room" could be the hut where the child just sleeps. Questions about the household circumstances of the child should be asked with great sensitivity. It is quite possible that your home circumstances are very different from those of the respondent. Avoid showing your emotional reactions to the responses (shock, disappointment, contempt, surprise) or any other such emotion when asking and recording answers to these questions. Similarly, desist from forming impressions in your mind about what you consider to be the 'typical' circumstances of a child with Burkitt lymphoma because such impressions may introduce distortions in the responses they record.

**Q29.** Ask the number of people who sleep in the same bed as the child, including the child. If the child sleeps alone in a bed, enter "01". The concept of a bed will have to be defined as sharing the same sleeping space and sometimes sharing the same covering.

**Q30.** Ask whether the house where the child sleeps has electricity. If the child sleeps in a hut without electricity, but the main house on the compound of the principal guardian of the child has electricity, then the correct answer will be “Yes”.

**Q31.** Ask the respondent the family’s usual source of drinking water. This answer will be given spontaneously in most circumstances, but when it is not, tell the respondent the types of answers that are expected and ask them to give the “usual” source.

**Q32.** Ask the respondent whether the child sleeps under a mosquito net or not at home. Ownership of the mosquito net means a net where the child sleeps under at night. If the answer is “no” or “don’t know”, skip to Q35, else go to Q33.

**Q33.** Ask the respondent how many nights the child slept under a mosquito net in the past week. The past week means the past 7 days from the day when the respondent is being interviewed. This question assumes the interview will take place on the first or second day of admission. If the child has been in the hospital for several days, ask the respondent to consider the last 7 days at home before coming to the hospital.

**Q34.** Ask the respondent whether the child slept under a mosquito net in the night before admission to the hospital.

**Q35.** Ask the respondent whether the house where the child’s sleeps has ever been sprayed for mosquitoes in the past year. If the respondent indicates “yes”, ask if the house was sprayed in the last 0-6 months or in the last 7-12 months. Record the appropriate answer on the questionnaire. Tick only one answer for this question.

**Q36.** Ask the respondent whether the family regularly sprays for mosquitoes within the house. This question refers to mosquito sprays that may be bought from shops and sprayed in the house, usually directly at the mosquito. If the family uses anti-mosquito incense, this question should be answered in affirmative. Please use the list (List F) of the common mosquito sprays or incense that are commonly used to explain to the respondent what you mean by mosquito sprays or incense.

**Q37.** Ask the respondent whether the child has ever received herbal treatments that are administered through cuts made in the skin (tea tea or nyig kal). Such medicines may be administered through cuts made on the body, such as on the breast area, abdomen, back, and wherever else the parents may complain of symptoms. Because the child is in a western-type of institution, the respondent may be uncomfortable when answering this question. Reassure the patient that we are conducting a study to find out experiences that may influence risk of Burkitt lymphoma, so we want them to give us an answer that will help us find out whether some of those experiences may influence the risk for Burkitt lymphoma.

**Q38.** Ask the respondent whether the child has ever had treatment that involved extraction of tooth buds or herbal treatments administered through cuts on gums (in Acholi, this is called Te lak). Exercise some caution here because of the faith based nature of the institution, the caregivers may conceal the fact for fear of reprisals from the hospital staff. They should

be reassured that research information will be kept private and will not be shared with the hospital.

**Q39.** Ask the respondent the number of times the child has been admitted to the hospital for any reason. Admission is when a child is kept at the hospital for more than a detention overnight to observe condition. When children are admitted to hospital, parents are usually explicitly informed that the child is being admitted, whereas when children are kept overnight, the parents are also informed so. We will take the respondents answer, given the above clarifications, as correct. Enter leading zeroes.

**Q40.** Ask whether the child has ever been admitted to any hospital or private clinic for severe malaria. To help the respondent whether an admission was because of severe malaria or not, ask them to report back the explanation given to them by the admitting officer. If malaria was cited, then the admission was due to malaria. Interviewers should be familiar with other conditions that are due to malaria, but have specific names, these include a) severe anemia requiring blood transfusion, except when sickle cell disease is involved; b) febrile convulsions. A spontaneous mention of another condition, such as measles, meningitis, or pneumonia will rule out malaria as the main reason for admission.

**Q41.** Ask whether the child has ever been treated for any serious malaria episode as an outpatient at any private clinic or hospital. (Not admitted means non-residential treatment at a clinic or hospital, but lasting more than one day). The answer is based on self-report where the respondent is relaying information that was given to him or her by the practitioner

**Q42.** Ask whether the child has been diagnosed with sickle-cell disease. If the respondent does not know anything about sickle cell disease, then it is unlikely that a child has been diagnosed with sickle cell disease. However, for respondents who answer in affirmative, please ask the symptoms that triggered the diagnosis, referring to List G with symptoms for Sickle Cell disease. If no, skip to Q44.

**Q43.** For children with a diagnosis of sickle cell disease, ask at what the age the child was diagnosed with sickle cell disease (YY/MM). Use developmental milestones to ascertain the age at diagnosis. Enter leading zeroes.

**Q44.** Ask whether the child has ever received a blood transfusion for any reason. Although not easy to see why respondent may be confused, please remember to remind the respondent that you asking about *receiving*, not *giving* blood. If no, skip to Q46.

**Q45.** If the child has received blood transfusion, record the number of times the child has received a blood transfusion. Enter leading zeroes.

**Q46.** Ask whether the child has received a measles vaccination. Respondents should be reminded that the measles vaccine is given when the child is at least 9 months as an injection in the right arm, and usually does not leave a scar, but the site of injection may have a scar in about one-third of cases. Ideally, one might want to verify with a record on the child health card, but these might be missing for many children.

If respondent answered “yes” and scar is present, check “Yes - scar-verified”.

If respondent answered “no” and scar is not present, record “Yes – scar not verified”.

If respondent’s answer does not match visual inspection, record on child health card attempt to reconcile with respondent and record the correct code.

**Q47.** Ask whether the child has received a tuberculosis vaccination. The tuberculosis vaccine is the first vaccine to be given, usually on the first day after birth and it is given as an injection in the skin on the left arm. For children not delivered in hospital, the TB vaccine is given the first time the child comes into contact with the immunization team. Please check for scar on RIGHT deltoid before recording answer.

If respondent answered “yes” and scar is present, check “Yes - scar-verified”.

If respondent answered “no” and scar is not present, “Yes – scar not verified”.

If respondent’s answer does not match visual inspection, attempt to reconcile with respondent and record the correct code.

**Q48.** Ask the respondent whether the child has any family member who was diagnosed with Burkitt Lymphoma. Family members include mother, father, sisters and brothers. You may show the respondent pictures of children with BL

**Q49.** Ask the respondent whether there are other people, excluding child’s relatives, in the child’s village who have been diagnosed with BL.

**Q50.** If the respondent reports a family history of Burkitt lymphoma in Q48, ask and record the relationship of each family member who was diagnosed with BL and the age at BL diagnosis.

**Q51.** Ask the respondent whether the child has suffered from any of the medical conditions listed within the past 6 months, and if yes, specify the number of times.

**Q52.** Indicate “yes” or “no” for each complaint and if “yes”, for “current admission” enter the duration in the space provided. Refer to the doctor for clarification of terms if needed. Add any complaints not provided on the list under the “other” category.

**MAKE SURE TO RECORD TIME INTERVIEW ENDED ON THE COVER SHEET.**

## **1-4d Reference Lists**

**List A** – Tribal Codes

**List B** – District Codes

**List C** – Classification of Occupations

**List D** – Towns, Municipalities, and Towncouncils

**List E** – Lakes, Rivers, and Swamps

**List F** – Mosquito Sprays and Incense

**List G** – Symptoms of Sickle Cell Disease



### List A – Tribal Codes

1. Bafumbira	16. Basamia	31. Okebu
2. Bakiga	17. Bagwe	32. Alur
3. Bahororo	18. Bagwere	33. Jonam
4. Banyankole	19. Bagisu	
5. Bakonjo	20. Iteso	
6. Batoro	21. Jopadhola	
7. Bambuti	22. Sebei	
8. Bamba	23. Karimojong	
9. Baganda	24. Langi	
10. Banyoro	25. Kumam	
11. Bagungu	26. Acholi	
12. Baruli	27. Madi	
13. Bakenyi	28. Metu	
14. Basoga	29. Kakwa	
15. Banyole	30. Lugbara	

### List B – District Codes

1. Adjumani	30. Kyenjojo
2. Apac	31. Lira
3. Arua	32. Luwero
4. Bugiri	33. Masaka
5. Bundibugyo	34. Masindi
6. Bushenyi	35. Mayuge
7. Busia	36. Mbale
8. Gulu	37. Mbarara
9. Hoima	38. Moroto
10. Iganga	39. Moyo
11. Jinja	40. Mpigi
12. Kabale	41. Mubende
13. Kabarole	42. Mukono
14. Kaberamaido	43. Nakapiripit
15. Kalangala	44. Nakasongola
16. Kampala	45. Nebbi
17. Kamuli	46. Ntungamo
18. Kamwenge	47. Pader
19. Kanungu	48. Pallisa
20. Kapchorwa	49. Rakai
21. Kasese	50. Rukungiri
22. Katakwi	51. Sembabule
23. Kayunga	52. Sironko
24. Kibale	53. Soroti
25. Kiboga	54. Tororo
26. Kisoro	55. Wakiso
27. Kitgum	56. Yumbe
28. Kotido	
29. Kumi	

### List C – Classifications of Occupations

Main Category	Sub-categories
Peasant/subsistence farmer	Not working; men
	agricultural/employed
	unskilled manual
Household	Not working; females
skilled/manual labourer	agricultural/self employed
	skilled manual
	Personal and Protective Service Workers
	Market-oriented Skilled Agricultural and Fishery Workers
	Subsistence Agricultural and Fishery Workers
	Extraction and Building Trades Workers
	Metal and Machinery Trades Workers
	Precision, Handicraft, Printing and Related Trades Workers
	Other Craft and Related Workers
	Wood Trades Workers
	Industrial Plant Operators
	Stationary Machine Operators and Assemblers
	Drivers and Mobile Machinery Operators
	Agricultural, Fishery and Related Laborers
	Supervisors of Elementary Occupations
	Foreman/Supervisors in Plant, Machine Operators and Assemblers
Clerical	Office Clerks
	Customer Service Clerks
	Other Clerks
Trader sales	Sales
	Services
	Salespersons, Demonstrators and Models
	Whole Sellers
	Sales and Services Elementary Occupations
	Soldiers
	Retailers
Professional	Legislators and Senior Officials
	Managers and Administrators
	Political Mobilizers
	General Managers
	Corporate Managers
	Physical, Mathematical and Engineering Science Professionals

	Life Science and Health Professionals
	Teaching Professionals
	Other Professionals
	Physical Science and Engineering Technicians
	Life Science and Health Associate Professionals Diploma and Certificate
	Teaching Associate Professionals
	Other Associate Professionals
	Experienced Non-professionals
Other	Other Laborers

**List D – Towns, Municipalities, and Towncouncils**

<b>NORTH CENTRAL TOWNS</b>
Apac
Amolata
Dokolo
Otuke
Oyam
Lira
Pader
Kitgum
Gulu
Amuru
<b>NORTH WESTERN TOWNS</b>
Adjumani
Moyo
Yumbe
Koboko
Maracha
Arua
Nebbi
NB: Major district towns and municipalities carry same names e.g Gulu District, Gulu Town, Gulu Municipality
<b>NORTH CENTRAL MUNICIPALITIES</b>
Gulu Municipality
Lira Municipality
Kitgum Municipality
<b>NORTH WESTERN MUNICIPALITIES</b>
ARUA
<b>NORTH CENTRAL TOWN COUNCILS</b>
Apac
Dokolo
Amolata
Otuke
Oyam
Pader
Amuru
<b>NORTH WESTERN TOWN COUNCILS</b>
Adjumani
Moyo
Yumbe
Koboko
Maracha
Nebbi

## List E – Lakes, Rivers, and Swamps

<b>Lakes</b>
Kyoga
Albert
<b>Rivers</b>
Northwestern
Enyau
Ala
Acha
Osu
Kochi
Acwera
Namurwodo
Nyagak
North central
Aswa
Unyama
Ayugi
Pager
Ome
<b>Swamps</b>
Albertine swamps In Apac, Lira, Oyam Disatrics
Murchision Falls swamps
NB All swamps are along River Nile

### List F – Mosquito Sprays and Incense

<b>Mosquito sprays</b>
Doom
Baygon
Total
Tox
Top
IT

### **List G – Symptoms of Sickle Cell Disease**

<b>Symptoms of Sickle Cell Disease</b>
Anaemia or palor
Pain crisis or Sickle Cell crisis
Acute chest syndrome
Splenic sequestration or pooling
Stroke
Jaundice or yellowing of skin, conjunctiva and mucosa



### **1.3.4 Height, Weight, and Anatomic Site Tumor Location Form (Form 32)**

**Q1.** The height of children who are 2 years and above ( $\geq 85\text{cm}$ ) will be measured when children are standing in a vertical position. The nurse will place the flat board with a vertical ruler. A caretaker or assistant removes any foot wear or headgear from the feet and head of the child, places the child on the flat board so that the shoulder blades, buttocks, and heels are touching the vertical surface of the ruler. The child stands with both feet flat on the board, legs slightly apart. The assistant kneeling down with the left hand on the knee of the child tells the child to stand 'straight and tall' looking straight ahead. The assistant brings a level board to the child's head and reads the child's height (the reading at the lower edge of the level) loud and clearly records it to the nearest 0.1 cm. With children who are either below 2year ( $<85\text{cm}$ ) or are unable to stand, the height measuring board is placed in a horizontal position on a flat surface. The above procedure is repeated.

**Q2.** If the child is held on the scale, use the calculator provided to subtract the "holder's" weight from the combined weight of the holder plus the child. The resulting difference is the child's weight.

**Q3.** This question is intended to elicit the first anatomic site where the participant noted tumor symptoms. This should be completed by the pediatrician or a nurse very experienced in reviewing patients with Burkitt lymphoma.

### **1.3.5 Lab requisition (Form 03)**

Pediatricians will use this form to request for the samples and tests to be done. This form tracks the process of sample requisitions, sample taking and sample processing. The required sample type and laboratory test is specified by marking/ticking against it.

- A person ordering for the samples specifies his/her initials and the **date this order is made**. The order is then passed on to the entity collecting the specimens. After drawing the specimens, he/she records his/her initials and the date and time of specimen collection. After the collecting the samples, they are passed on to the processing laboratory/unit. The person receiving the samples is also required to write his/her initials, and the date and time of receiving these specimens and then assigns them a Lab Accession Number..
- The dates are recorded in the DDMMYYYY format while time is in 24 hour clock system.
- For tracking purposes, the Lab Requisition form is 3-part (carbon-copy) NCR with the top part, having fields for both subject ID and hospital ID, which should be fixed. The top copy should be sent to Study office for tracking purposes, the second/middle and third/bottom copies are sent with the patient (or patient's sample) to the procedure

taking personnel who completes and returns the carbon copy to the study office. The third copy is either retained by the processing unit or returned to the requesting unit for hospital records.

- Note that the results from the processed samples are not recorded on the lab requisition form but on its respective results forms
- 

### **1.3.6 Procedure Requisition Form (Form 04)**

The form is used to request for procedures (ultrasound, x-rays, bone marrow aspirate, lumbar punctures etc). The required procedure is specified by marking/ticking against it.

- A person ordering for the procedure specifies his/her initials and the date and time of making this order. The order is then passed on to the processing unit where the entity receiving the request is also required record his/her initials and the date and time he/she has received the order. When the procedure is done, the person doing it will record his/her initials, and date and time when this was done.
- The dates are recorded in the DDMMYYYY format while time is in 24 hour clock system.
- Just like the lab requisition form, the procedure form is 3-part (carbon-copy) NCR with the top part, having fields for both subject ID and hospital ID, which should be fixed. The top copy should be sent to Study office for tracking purposes, the second/middle and third/bottom copies are sent with the patient (or patient's sample) to the procedure taking personnel who completes and returns the carbon copy to the study office.
- Note that the results from the processed samples are not recorded on the lab requisition form but on its respective procedure results forms

### **1.3.7 Research Blood Form (Form 33)**

This form tracks the collection of the research blood (10ml) which is aliquot into the plasma, Buffy coat and Red Blood Cells. This blood, which is collected at the same time when the blood for clinical tests is drawn, is kept in a 10 ml purple top tube.

- **Q1, Q2 and Q3** respectively require the person drawing the blood to specify the date of specimen collection, time of specimen collection and his/her initials respectively. The dates are recorded in the DDMMYYYY format while time is in 24 hour clock system.
- **Q4** requires one to specify if the specimen was collected or not, and if not collected, the reason is specified.
- Section b of this form (**Q5 –Q14**) tracks the flow of the specimens in the laboratories from the point of receipt to processing.
  - **Q5 and Q6** record the dates and time when the specimen was received, **Q7** is for recording the initials of the person receiving the specimens while **Q8** tracks the

estimated blood quantity in the bottle (in millimeters-ml). In case of any problems with the specimens, this is recorded under **Q9**.

- **Q10-12** tracking the processing. **Q10** is for recording the date of sample processing, while **Q11** is where the person doing the processing writes his/her initials. Question 12 tracks specify the quantities (in millimeters-ml) per aliquot (Plasma, Buffy Coat and Red blood Cells respectively). Four aliquots of plasma are expected number sequentially from 0001 to 0004. The fourth specimen is the residual specimen. The Buffy coat and red blood cells each has two aliquots numbered sequentially (0005 and 0006) and (0007 and 0008) respectively.
  - Under **Q13**, the person processing specifies the problems faced in processing the blood if any.
  - **Q14** and **15** respectively are for recording the date and time when the aliquots are frozen. The dates are recorded in the DDMMYYYY format while time is in 24 hour clock system
- In addition to the Subject ID and Hospital Ids, the person processing the specimens has to affix the BSI labels on this form. This label is very important as it's the link between the study subject and study aliquots/specimens.

### **1.3.8 Research Saliva Form (Form 34)**

The Research Saliva Form tracks the collection of the research saliva (4ml). Like the research blood, saliva is also collected at the same time when the blood for clinical tests is drawn. Saliva is separated into four aliquots numbered sequentially from 0009 to 0012.

- **Q1, Q2** and **Q3** respectively require the person collecting the saliva to note the date of saliva collection, time of collecting the saliva and the initials of person collecting the specimen respectively. The dates are recorded in the DDMMYYYY format while time is in 24 hour clock system.
- **Q4** requires one to specify if the saliva specimen was collected or not, and if not collected, the reason is specified.
- Section b of this form (**Q5 –Q14**) tracks the flow of the saliva specimen in the laboratories from the point of receipt to processing.
  - **Q5** and **Q6** record the dates and time when the specimen was received, **Q7** is for recording the initials of the person receiving the specimens while **Q8** tracks the estimated saliva quantity in the collection bottle (in millimeters-ml). In case of any problems with the specimens, this is recorded under **Q9** (a corresponding code for the problem is written for example if the collecting bottle broke down, then 3 is recorded).
  - **Q10-12** tracking the processing. **Q10** is for recording the date of sample processing, while **Q11** is where the person doing the processing writes his/her initials. If saliva aliquots are processed for storage, Question 12 specifies the saliva quantities (in millimeters-ml) per aliquot.

- Under Q13, the person processing specifies the problems faced in processing the blood if any.
  - Q14 and 15 respectively are for recording the date and time when the aliquots are frozen. The dates are recorded in the DDMMYYYY format while time is in 24 hour clock system
- In addition to the Subject ID and Hospital Ids, the person processing the saliva specimens has to affix the BSI labels on this form. BSI label is the link between the study subject and study aliquots/specimens.

### ***1.3.9 Specimen Tracking Form (Form 40)***

After completion of specimen processing and aliquots attained, all vial aliquots are to be transferred to a secure storage location with appropriate temperature that will best maintain specimen integrity and stability. The plasma, Buffy coat, RBC and saliva aliquots are placed in boxes (Box A or Box B) and placed in a freezer. The Specimen Tracking Form is to document how the 12 aliquots numbered sequentially (0001 – 0004 for Plasma aliquots, 0005 – 0006 for Buffy Coat aliquots, 0007 – 0008 for Red blood cells aliquots and 0009 – 0012 for Saliva aliquots) are placed into these boxes. For each aliquot, the storing person specifies the drawing date, processing date, quantity in ml, Box number and box type, row and column and comments. The dates are recorded in the DDMMYYYY format while time is in 24 hour clock system. The BSI label must be affixed on the form.

### ***1.3.10 Lab Results and Procedure Results Forms***

These forms are used to record the results of the different tests and procedures done. Efforts should be taken to attach the machine printouts (if available). The transcriber and checker have to sign on the forms when filled. The dates are recorded in the DDMMYYYY format while time is in 24 hour clock system. Check “N/A” for any result that is not available and record the results in the appropriate standard units as on the form

These result reporting forms are 2-part NCR (carbon-copy) so that results can be returned to the patient as well as to the EMBLEM Study. The laboratory will copy results that are normally reported in writing onto the form on the result reporting form. The top part of the form will be returned to the EMBLEM study office; the carbon-copy will be returned to the Patient’s file on the ward. Where the laboratory result is a machine result printout, this implies that the laboratory personnel prints two sets of results and staple one set to the top

part of the result form and return both the EMBLEM Study office, and staple the second copy of the result on the carbon copy and return the results to the ward.

The lab results and procedure results forms under reference are:-

- 1-11a CBC/ESR Results Form (Form 35)
- 1-11b Liver Function Test Results Form (Form 05)
- 1-11c Renal Function Test Results Form (Form 06)
- 1-11d Stool Microscopy Results Form (Form 36)
- 1-10e Malaria Thin Smear Results Form (Form 07)
- 1-11f Malaria Thick Smear Results Form (Form 37)
- 1-11g Malaria Rapid Test Results Form (Form 38)
- 1-11h HIV Serology Results Form (Form 39)
- 1-12 Procedure Results Forms
  - 1-12a Bone Marrow Aspirate Results Form (Form 08)
  - 1-12b Lumbar Puncture Results Form (Form 09)
  - 1-12c Chest Xray Results Form (Form 10)
  - 1-12d Ultrasound Results Form (Form 11)
  - 1-12e BL Histology Results Form (Form 12)

## Chapter 4: Enrolment of Controls

4.0 Introduction .....	2
4.1 Census population controls .....	2
4.1.1 Selection of survey parishes.....	2
4.1.2 Selection of survey villages .....	3
4.1.3 Census procedure .....	4
4.1.4 Selection of census population controls.....	5
4.1.5 Collection of samples from census population controls.....	7
4.2 HCII controls for census population controls .....	9
4.3 Matched population controls for cases .....	10
4.4 Sample and data collection and processing .....	12
4.5 Staff involved .....	18
4.6 Field requirements per site .....	19
Annexes .....	20

## 4.0 Introduction

The Epidemiology of Burkitt Lymphoma in East African Children and Minors (EMBLEM) is a case-control study of 1,500 Burkitt lymphoma (BL) cases and 3,000 age-, sex- and residence-frequency matched controls. This chapter provides detailed procedures for control selection plan for the EMBLEM Study. Three sets of controls will be enrolled: a) Matched Population Controls. These are the main controls and will be matched to the historical cases matched on age and sex and broadly on residence; and Pilot controls, who include i) Pilot Population Controls and ii) Pilot Health Center II (HCII) Controls. The Pilot Population Controls will be randomly enrolled from a few representative villages to obtain robust population-level distribution of exposures. The distribution will be used to correct for any deviations among Matched Population Controls during analysis. Pilot HC II controls will be compared to Pilot Population Controls to evaluate how children recruited at HCII units systematically differ from population controls recruited from their home in the village. This information will be used to evaluate the cost-benefit for using population controls, who are ideal, versus facility-based control enrolment, who are less ideal. The Matched Population Controls will be recruited from 100 randomly selected villages that are representative of the geographical region where BL patients diagnosed and treated at participating hospitals arise. The geographical region was defined based on an analysis of historical cases treated at Lacor and Kuluva Hospitals during 1997-2009. The age- and sex-distribution of the historical BL cases will also be used to guide the age- and sex-distribution of the Matched Population Controls.

The operational field plan for enrolling controls is described in detail below. We describe the enrolment of the Pilot Controls first (Pilot Population Controls and HCII Pilot Controls) first and the Matched Population Controls last. The ethics considerations for human subject research are described in the scientific protocol and copies of approvals to conduct this research are available in separate appendices. The success of EMBLEM depends on the target population understanding the benefits of participating in the study. For this reason public awareness campaigns will be conducted prior to and during the EMBLEM field work. Details of this community mobilization strategy are contained in Chapter 2.

## 4.1 Pilot Population Controls

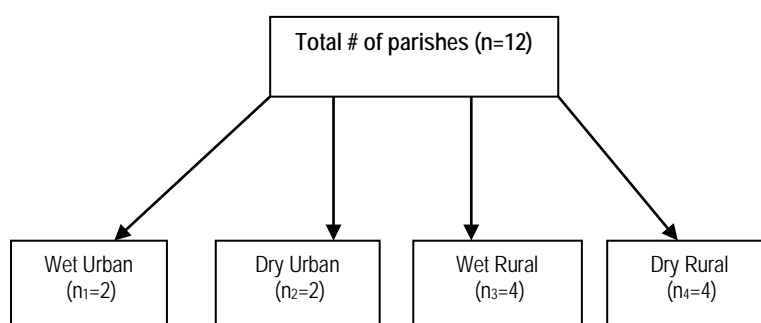
Pilot Population Controls will be enrolled from 12 of 100 randomly selected villages.

### 4.1.1 Selection of pilot parishes

The villages were randomly selected through a two stage procedure. In the first stage, 12 survey parishes were randomly selected from all parishes in the north-central and northwest regions. In the second stage one village was randomly selected from each parish. The parish sample frame was stratified on wet versus dry condition and rural versus urban

environments and then parishes randomly selected from each stratum. Wet versus dry was defined according to GIS maps with parishes defined as wet if their boundary was next to or within 0.5km of an all-season water body and dry if its boundary was further than 0.5km. It was decided that 0.5 km is a reasonable distance based on risk of malaria varies according to distance from a water body and the risk is substantially lower for individuals living >0.5 km from water compared to those living closer. Rural versus urban was defined based on population density. An assumption was made that rural areas have a sparse population, whereas urban areas have a high density population. (See appendix 2 for the selected parishes).

The study area (Northern and North western Uganda) was therefore stratified into four categories as, i.e., wet urban, dry urban, wet rural and dry rural. Because most of the population in Uganda is rural, we selected rural: urban parishes in a ratio of 2:1 and wet: dry parishes in a ratio of 1:1. The number of parishes selected from each strata is as given below:



All parishes from each strata were serially numbered and therefore uniquely identified. The required sample of parishes from each strata was therefore selected scientifically using a random number generator. Hence assuming the wet urban strata has  $N_1$  parishes then using a random generator two numbers in the range 1-  $N_1$  were generated and the two parishes with these numbers were selected.

#### 4.1.2 Selection of pilot villages

The villages within the parishes were selected by the Uganda Bureau of Statistics. The UBOS team randomly selected one village from each parish 10 times and provided this set of random selections to NCI. Random selection was accomplished by listing villages in each of the selected pilot parishes, serially numbering the village and randomly selecting one serial number from each of the parishes using a random number generator. After 10 rounds, 10 different samples of villages had been selected, numbered according to the order in which they were selected, were forwarded to NCI. Using a pre-determined random selection



for a set of 10, NCI selected one set and returned the set to UBOS as the set of villages where census would be performed to facilitate enrolment of Pilot Population and HCII Population Controls.

Following the Parish selection structure, the ratio of rural: urban villages are 2:1 (8 rural villages and 4 urban villages) and the ratio of wet: dry villages in 1:1 (6 dry and 6 wet villages). The table below summarizes the sampling by location and proximity to water. Each cell is equally divided between spring and fall season. (See appendix 2 for the selected villages).

Table 4.1.2. showing selection criteria for 12 Pilot Villages

Proximity to water	Population density		Total
	Urban	Rural	
<b>Wet</b>	1/1	2/2	6
<b>Dry</b>	1/1	2/2	6
<b>Totals</b>	4	8	12

### 4.1.3 Census of pilot villages

Households will be enumerated in each of the 12 pilot villages through conducting of a population and housing census. The census will be carried out both in the wet and dry seasons by a team from UBOS. The census activities to be undertaken will include:

- ✓ Developing instruments for gathering of data from households including field questionnaires (refer to annex 8), enumerator's manual of instructions (this is contained in a separate document) and field control forms (see appendix 10);
- ✓ Developing the appropriate guidelines for identification of field staff;
- ✓ Recruiting and training of census field staff to undertake enumeration;
- ✓ Supervision and general implementation of enumeration;
- ✓ Designing of an appropriate system for capturing of the collected census data.
- ✓ Census data capturing and cleaning.

The household census questionnaire gathers personal and household characteristics based on the objectives of the EMBLEM Study. The UBOS team drafted the initial version which was edited by staff from the EMBLEM project and later finalised by the UBOS team. The core personal variables for the questionnaire include age, sex, survival status of parents and education characteristics. In addition information will be collected on all members of the household and housing characteristics.

Field Enumeration will be carried out by well-trained interviewers. Training will take place for one week in a central location in Northern Uganda prior to starting each round of the census (either during the wet seasons or dry seasons). The training will involve classroom discussions, mock interviews and field practice.

The enumerators will canvass the whole selected LC1 and enumerate all households within the targeted villages. They will use GPS coordinates to map all households they enumerate.

They will assign a household number to each household and an individual number to each member the household.

The number of enumerators deployed to each village will be dependent on the size of the village. All the enumerators shall be recruited locally from within the LC1s in which they will be operating. Enumerators shall have the following qualities:

- ✓ Must be a resident of the LC1;
- ✓ Should preferably have a minimum education of “A level”;
- ✓ Should be a socially acceptable person;
- ✓ Aged 18-45 years;
- ✓ Those with medical background will stand an advantage;
- ✓ Must be of a sound mind.

The questionnaires will be manually edited both in the field and central office before being keyed by well-trained data entry clerks. The capture of the EMBLEM census records will be done under the CsPro (Census and Survey Processing Software) environment.

#### **4.1.4 Selection of Pilot Population Controls**

After the completion of each round of the census, the UBOS will provide a cleaned dataset and a file description to EMBLEM within 3 weeks after field work. The data set will be in CSPro (ASCII format) and the description in form of a data dictionary. All variables collected from the census will be included in the submitted data set including household and personal IDs, age, household GPS coordinates, sex etc. This format allows for data to be imported to different software including database systems, spreadsheets and statistical packages in addition to extracting/filtering a subset of the dataset based on specified conditions. From the dataset, EMBLEM with technical support from IMS will:

- ✓ extract and compile a list of all the households per village (based on the household number assignment).
- ✓ extract and compile a list of all the household members per household (based on the household member number assignment).
- ✓ extract and compile a list of eligible children for enrolment as controls per household (those within the age range of 0-15 years).
- ✓ map/trace the location of the households using the GPS coordinates.

Thirty-Five households will be randomly selected from the village using a specifically designed random selection program. All households with eligible children will be selected from the census data set, serially numbered and uniquely identified. Then Systematic random sampling method will be used to select the 35 households. The selection procedure will be as specified below:

- (i) Households from the field are numbered as 001,002,003....., N'. The data set containing individual and household characteristics is ordered in ascending order basing on household number and personal ID.

- (ii) Assuming a village is composed of N households with eligible children, without changing the order of the data set, after filtering out households with eligible children, generate N random numbers. The first household is assigned the first random number, the second the second random number etc.
- (iii) . Arrange the households in ascending order by the random number assigned and the first 35 households will form the sample. In case practically less than 35 households are able to raise 900 eligible children, say 34 then stop at household number 34 and likewise if exactly 35 households have less than 900 children, then move ahead to the number that is able to raise 900 eligible children.

**EMBLEM staff will then construct daily work schedules for each staff participating in the sero-survey. The following work schedule are envisaged:**

- ✓ **Notification schedule: this is a schedule of all households to be visited on a given day. This schedule will only include the date the schedule was prepared, the date of notification, the date of sero-survey visit to the home, the name of the district, county, sub-county, parish, and village, the number of the household, the name of the head of household and household size. The notifier will send a report to EMBLEM confirming the appointments scheduled.**
- ✓ **An individual staff sero-survey schedule. This is a schedule of households a particular staff should visit on a given day. The schedule will include the notification schedule for the day with the names of the households the staff is supposed to visit marked with a check mark. In addition, detailed roll call household schedule will be given to enable the staff to conduct a roll call. This schedule will have the basic household information as well as a listing of all individuals resident in the household, their sex, and age. This list will include a column to check and confirm the information contained in the roll call schedule, a comment field for correction, as well as several rows to accommodate new information about joiners to the household.**
- ✓ **These schedules will be generated only for the randomly selected households.**
- ✓ **The EMBLEM staff will return all the schedules to the EMBLEM office with appropriate comments for action and filing.**

All of the children from the selected households will be eligible for enrolment as Pilot Population Controls if:

- a) They are at least 1 year of age and less than 15 years of age at the time of the census survey.
- b) They have resided in the study region/survey village for at least 4 months prior to the census survey.

Each village, household and household member will be given a unique number. For ease of management, there is a three-level system for numbering household members: the first two numbers identify the village, the next three numbers identify the household and last two numbers identify the household member.

Example of household and household member numbers:

1	1	0	0	2	1	9
Village No.11		Household No.2			Member No.19	

The sero-survey is to begin three to four weeks after the census and should be completed within one month of starting. This is to minimise on the effects of both climatic changes and retention of children included in the census (due to migrations both in and out of the village).

The Population of Uganda is estimated at 31.8 million as of mid 2010 with an average household size of about 5 persons. A large proportion of this population (51.8 percent) is below 15 years of age. By age group, the piloting exercise by EMBLEM in one of the villages in Amuru district showed that 94% of the households would have at least one child eligible. Among those eligible, 51.8% were male while 48.2% females.

The number of households sampled per village will be based on the number required to obtain a sample of 900 children aged 1-14 years willing to be enrolled in the EMBLEM study. It is estimated that the sampled area has an average of 2.4 eligible children per household. The number of households per village is estimated as:

$$900 / (12 * .90^b * 2.4) = 35$$

where 900= final sample size of eligible children required for enrolment

12 = Number of Villages

.90 = estimated consent rate,

<sup>b</sup> Based on figures from Uganda 2004-2005 Sero-Behavioral Survey

#### **4.1.5 Collection of samples from Pilot Population Controls**

The collection of samples from controls will be the same as the procedures used to collect samples from cases. The main difference is that samples will be collected in the home of the participant for Pilot Population Controls and from the HCII unit for HCII Pilot Controls.

- ✓ The households selected for Pilot Population Controls will be visited to identify eligible children for enrolment into the EMBLEM study.
- ✓ The staff visiting the home will conduct a household roll call to confirm household membership and to note any changes that may have happened since the census was performed. The roll call will use household information collected during the

census and include the names of the children in the household, their ages, sex, and date of birth.

- ✓ They will then use the confirmed household list to screen eligibility of children in the household to participate in EMBLEM.
- ✓ Eligible children to answer the questionnaire and request for biological samples (blood, saliva, and stools) to be taken.

Prior to starting sero-survey in the village, EMBLEM Teams will visit the local councils to inform them of the planned activities in the village as described in chapter 3 about community mobilisation. A day before the sero-survey, an EMBLEM mobiliser will visit the households to be visited the next day and inform them about the visit from the EMBLEM team the next day.

- ✓ The number of houses to be visited will be determined and listed in a detailed diary will be constructed stipulating the households that will be visited.
- ✓ Village mobilizers will use notification cards (see appendix 10) to inform the household about the visit and confirm the house visit appointments to enrol the selected child in the household the next day.
- ✓ The EMBLEM mobilisers will be local people from the village recruited to work with the EMBLEM staff to coordinate community mobilisation.
- ✓ These mobilizers will be facilitated with a bicycle allowance to move to every household so as to pass over the information about the exercise and will be present during the house visits.

Informed consent will be obtained from the parents/guardians to enrol the children into EMBLEM. EMBLEM Study staff will explain to the participants the objectives of the study, what the child will do to participate, what the study staff will take from the child the procedures that will be done, including answering a questionnaire and giving biological samples (blood, saliva, and stool). Participants will be informed of their right to participate or not.

- ✓ Parents or guardians will provide witnessed written informed consent.
- ✓ They will receive a copy of the consent form, if they wish
- ✓ Collecting questionnaire data and taking of the specimens will only take place after the concerned parents have given witnessed written informed consent.

For children who are above 7 years, assent will also be obtained. A child will qualify for specimen taking if:

- a) He/she does not have a severe illness requiring immediate admission to hospital
- b) He/she does not have cancer

The EMBLEM staff will carry their chairs and clip boards to the house but they will be responsible for finding a place where the interview can be done in private. Confidentiality will be maintained in these tents. Staff will be trained in sample taking (saliva and blood) and questionnaire administration.

## 4.2 Pilot HCII controls

Pilot HCII controls will be enrolled from HCII units serving the 12 pilot survey villages. They will be compared to Pilot Population Controls. All children attending an HCII unit serving the pilot village during the village survey period will be registered in to compile an attendance register. Children (0-15 years) from the pilot survey village will be eligible.

- ✓ The study aims to recruit at least 30 Pilot HCII Controls from each HCII unit.
- ✓ The age- and sex-distribution of the pilot HCII controls will be similar to the age and sex distribution for BL cases seen in the whole region.
- ✓ The number of Pilot HCII Controls needed from each center within each category will be fixed based on the age- and sex-distribution of BL cases from that region (Table 4.2.).
- ✓ Pilot HCII controls will be enrolled consecutively according to eligibility criteria until the required number is obtained in the age and sex category then enrolment for that group will stop.
- ✓ The duration of stay in the HCII will depend on how long it takes to enrol the required number of children in all desired age and sex categories.

Table 4.2.: Pilot Population and Pilot HCII Controls by age and sex.

	Sex	Age group (Years)					Total
		0 - 2	3 - 5	6 - 8	9 - 11	12+	
Pilot Population Control	Male (per village)	0	3	9	0	6	19
	Female (per village)	6	3	9	9	3	31
	Total(per village)	6	6	19	9	9	50
	Over all in 12 villages	75	75	225	113	113	600
Pilot HC-II Controls	Male(per village)	0	1	3	0	2	6
	Female(per village)	2	1	3	3	1	9
	Total(per village)	2	2	6	3	3	15
	Overall in 12 HC-II	46	46	136	68	68	360

Our plans for HCII activities are guided by preliminary data collected at 4 HCII units in Arua and Gulu. Attendance at these units varied according to the day of the week, by units, population size of the communities served by the unit, accessibility, location, and availability of investigations or human resources. For example, some units were HCIII units and therefore had higher attendance than pure HCII units. In some HCII units, fewer than 20 patients attended the outpatient department during the two week period, with a daily average of 2 patients. This variability of attendance will be monitored and taken into account when

constructing HCII unit visit diaries. The table below shows the predicted estimates of the 600 controls from the survey villages and the 360 controls from HCII by age group:

To ensure smooth conduct of enrolling Pilot HCII Controls, EMBLEM staff will:

1. Schedule dates for enrolment of pilot HCII controls at HCII units serving pilot villages
2. Based on the BL age- and sex-case distribution for the region, pre-specify the number of pilot HCII controls required and their age and sex distribution. Staff will also obtain attendance data 2 weeks prior to the date of data collection to guide on how long the HCII unit should be covered.
3. During the period of enrolment, EMBLEM staff will arrive at the HCII unit at 8:00 AM and set up a work space/hub at the HCII unit. Considerations for space include an area where attendance log will be recorded, a waiting area for the identified eligible children and their guardians; secure and confidential areas for consenting, counselling and specimen collecting.
4. Staff will monitor the day's attendance log which captures the out-patient number, limited personal information (name, sex, age, and local address including village), symptoms, and a provisional diagnosis.
5. The attendance log will be computerized sequentially to generate an HCII Control Register or sampling frame (Annex 4).
6. The screener form will be applied to all children attending the HCII unit to identify children eligible for enrolment (see Appendix----).
7. Children who are eligible, based on pre-specified age- and sex-, and village criteria will immediately be invited to participate.
8. If many children from a given village attend the HCII unit, based on the report from the HCII attendance survey, we will use a random number generator to select sequence numbers from the HCII Register so that children whose registration coincides with the selected number are invited as soon as they are registered. Simple random sampling without replacement will be used in the selection.
9. Enrol the eligible children as controls as soon as they are registered. The enrolment process includes consenting/counselling, specimen collection and interviewing. Informed consent, with signature or witnessed thumb print will be obtained from the mother or guardian of the child and informed assent from children 7 years or older. If consent is not obtained, the next number will be attained (details are specified in chapter 5).

### **4.3 Matched Population Controls for cases**

The primary controls are Matched Population Controls. The controls will be enrolled from 100 control villages randomly selected to be representative of the entire study region. Two controls will be selected for each case. They will be frequency-matched to the cases on age (+/-2 year) and sex.

- ✓ Matched Population Controls will be selected randomly from Pilot Population Controls enrolled from the 12 villages.
- ✓ For the 88 control villages where village census will not be done, Matched Population Controls will be selected from households randomly selected from a village household list.
- ✓ A household list containing basic information about the composition of the household including number, age, and sex of the children in the household (refer to annex 7 for the variables in the household list) will be constructed by village local council members.
- ✓ The number of children to be selected from a given village will depend on the number of cases expected from the region represented by that village and their age- and sex-distribution. This number will be predetermined for the village.
- ✓ To harmonize and simplify enrolment, children in the household who are selected as Matched Population Controls will be taken to the local HCII unit for enrolment. The EMBLEM study will facilitate their transport to the HC II unit and back.

Similar to the 12 villages, the 88 control villages will be grouped into 4 strata of wet-urban, wet-rural, dry-urban and dry-rural and randomly selected from study districts directly proportional to the historical cases seen at the hospitals. Thus, 28 villages will be selected from urban areas and 60 from rural areas and equally divided into the wet and dry strata. The table below summarizes the sampling by location and proximity to water. Each cell is equally divided between wet and dry season. The selection of villages will follow a two stage procedure, with parish being randomly selected in the first stage and village being randomly selected in the second stage.

Table 4.3 showing selection criteria for 88 control Villages

Proximity to water	Population density		Total
	Urban	Rural	
<b>Wet</b>	7/7	15/15	44
<b>Dry</b>	7/7	15/15	44
	28	60	88

Based on the historical BL case activity both at Lacor and Kuluva hospitals during 1997-2009, and the number cases by age- and sex from each district, we can estimate the number Matched Population Controls required from each district in the region (see annex 6). These controls will be distributed in the control villages according to the number of cases in the district and where they originated in the sub-district regions.



As explained above, prior to the visiting the village to contact the households, the village leadership will be informed about the study (see chapter 3 for details on community mobilisation). Two local people per village will be recruited to work with the EMBLEM staff to coordinate community mobilisation. The mobilizers will be identified from the local council one (LC-I) or other influential local people and they will be trained to collect high-quality household data. This training will involve classroom discussions, mock interviews and field practice. These mobilizers will be facilitated to move to every household so as to pass over the information about the exercise and will be present during the house visits.

To be successful, the mobilizers will have the following qualities:

- ✓ Must be a resident of the LC1;
- ✓ Should preferably have a minimum education of “A level”;
- ✓ Should be a socially acceptable person;
- ✓ Must be 18-45 years of age;
- ✓ Must be of a sound mind.

The day before the household is visited, the village mobilizer will visit the household and inform them about the planned visit and give them a notification card (see appendix 10) to schedule house visit appointments. On the day of the home visit, the EMBLEM team will visit the house and take a roll call of the children in the household and invite the eligible children to go to the HCII unit to be enrolled. The roll call will confirm household composition and record the household sampling frame, including the names of the children in the household, their ages, sex, and date of birth.

Informed consent will be obtained from the parents/guardians before the children are taken to the HCII unit to be enrolled and a questionnaire and blood specimen taken. The EMBLEM staff will explain to the participants the objectives of the study, the procedures that will be done, including answering a questionnaire and giving biological samples (blood, saliva, and stool), and their right to participate or not.

- ✓ They will then request the parents or guardians to provide witnessed written informed consent.
- ✓ The consent form states the participants' rights, including that any the information they provide and the test results will be kept confidential.
- ✓ Taking of the specimen and administering the Questionnaire will only take place after the concerned parents have given witnessed written informed consent.

Field supervision will be done to ensure that data is accurately collected. The questionnaire will be the same as that used for cases using datafax.

## **4.4 Sample and data collection and processing**

### **Labelling of Participants specimens/ Test Results:**

The correct and safe procedures for specimen collection from subjects are covered in Chapter 4. Briefly, controls will provide 3 specimens: venous blood, saliva, and stool. The

venous blood includes a research sample and a clinical specimen. The research sample will be returned to the processing laboratory for processing, aliquoting, and storage, while the clinical sample will be used for testing for malaria parasites in the field. The saliva specimen is a research sample, while the stool specimen is a clinical sample.

- ✓ Appropriate forms must be completed for each sample type. Complete the upper section of form as described in Chapter 4.
- ✓ For both blood specimens and saliva specimens, the Research Specimen form is a 3-part carbon-copy NCR form.
- ✓ The forms should be completed using black ball-point pen.
- ✓ The original (top form) is to be retained in the Enrollment Folder of the participant; the first carbon copy and the second carbon copy are both sent to the processing laboratory. The first copy is returned to EMBLEM office after the laboratory has finished processing the research specimen. The second copy is retained in the processing laboratory for their files.
- ✓ The forms for the processing laboratory and for the EMBLEM office may be separated immediately after sample collection or at the end of the field workday.
- ✓ To complete a Research Specimen Form, affix a survey identification numbers to each of the three pages of the form in the lower left area and EMBLEM Subject ID label to the original (top) page only in the lower right area of the page.
- ✓ EMBLEM unique subject identification numbers and numbers for samples will be generated specific for EMBLEM. Names will not be included on specimen labels. Only labels with EMBLEM unique identification number will be affixed to each sample. A master list that provides a link between the specimen and survey identification numbers will be encrypted and password protected in electronic media.

Therefore all specimen collection devices will be labelled with a bar-coded identification label, a copy of which was also pasted on the study participant's Questionnaire and on any laboratory forms for the participant. Sticky labels will therefore be produced for this purpose.

The following specimens are collected from each control participant enrolled:

Table 4.4				
Specimen Collection and Application				
Specimen Type	Volume/amount	Control	Clinical Test	NCI Storage
Whole blood (EDTA)	10 ml	Yes	None	Buffy Coat, Plasma, Red Cells
Saliva	4 ml	Yes	None	w/STM
Whole blood (EDTA)	2 ml	Yes	Malaria RDT Malaria thick and thin smears	Digital image of result
Whole blood (EDTA)	3 ml	Yes	CBC w/diff ESR	No
Stool	1 gm	Yes	Wet preparation for ova, cysts, and larvae	Digital image of result

**Venous blood:** The research blood specimen is collected in a 10 ml purple-top EDTA tube (two 6 ml purple tops may be substituted if necessary). Additionally, a 2 ml purple top EDTA tube will be collected for malaria testing and a 3 ml purple top EDTA tube will be collected for CBC/differential and ESR (Refer to chapter 5 for details on blood collection procedures).

- ✓ **Saliva specimen:** The saliva specimen is not intended to be sterile, so the cups used for saliva collection will be clean but not sterile. Saliva specimens should be collected at least 30 minutes after a child has eaten solid food or 10 minutes after liquid food. The child can collect saliva over a period of about 10 minutes in order to obtain the required 4 mls. Saliva cups will be labelled with the unique participant ID numbers (Refer to chapter 5 for details on saliva collection procedures).
- ✓ **Stool sample:** will be collected to examine for intestinal parasites, which may confound the malaria and EBV associations

In addition to assess the nutritional status of the children, anthropometric measurements (weight and height) will also be undertaken.

The following data are collected from each control participant enrolled:

- ✓ **The questionnaire:** This is the main tool EMBLEM is using to collect information about experiences of the study participants. The datafax formatted questionnaire will be used. The questionnaire asks information about age, sex, residence and socioeconomic information, past medical history, and information about the child's family and home environment. Asking these questions consistently of all subjects will allow the researchers to reach the correct conclusions about BL, and malaria. Interviewers are required to record the responses as given by the respondents (Refer to Chapter 3 for details on question-by question instructions of the questionnaire).

The sub-section below provides a detailed description of the arrangement for specimen collection for the different sets of study controls.

The collection of specimens and administration of the questionnaire for the HCII controls for census population controls and the matched population controls will be performed by the EMBLEM staff. The number of staff will depend on the number of controls that are estimated to be enrolled from each location (see section 2 for more details on staff needed for control enrolment).

Note:

- ✓ The same staff taking samples will administer the questionnaires. ***The staff with superior skills for taking blood samples will guide and supervise all blood sampling activities in the field.*** All staff should know how to administer informed consent and administer the questionnaire. Knowing and understanding the local languages will be a great advantage.
- ✓ One of the field staff will always be designated as the field supervisor. This staff will be responsible for monitoring compliance with these SOPs. At no point should two persons be interviewed and bled simultaneously using the same small work area. This is intended to minimise the mix-up of samples. A document will be provided to track the samples taken, time of taking and delivery to the field storage centre.

#### ***a) Organisation of specimen collection centre at HCII Units***

Matched Population Controls and Pilot HCII controls will be enrolled at HCII units; this is in contrast to Pilot Population Controls who will be enrolled at their home. The EMBLEM team will liaise with the HCII unit staff or management that serves the village to obtain space for EMBLEM enrolment activities. The space will include a general meeting/reception area for the participants, counselling and consenting area, and specimen collecting area (preferably, this space should be sheltered to make the participants comfortable).

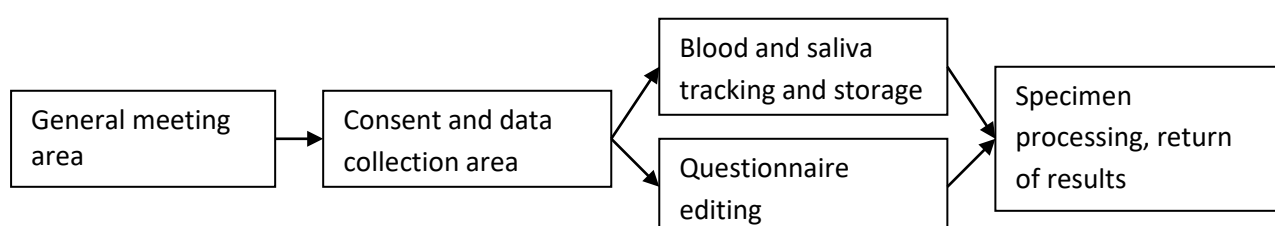
If no permanent structure is available for study enrolment, EMBLEM staff will organize a mobile encampment equipped with tents. One tent will be used as the general meeting area where the parents and their children are received and welcomed by the EMBLEM staff. Other tent will be used for consenting and specimen collection. In the main tent, the parents/children will be taken through the consent process in a group and given an opportunity to ask for clarifications. The EMBLEM staff in the tent will be responsible for

ushering the guardians to the other more-private tents where further individual consenting/assenting, counselling and specimen taking will be done. Below is a detailed description of the required space at each enrolling centre:-

- i. One general meeting/reception area and serving as a mobile clinic. This mobile clinic will cater for emergencies or other minor sickness reported by the community.
- ii. Tents set up for specimen collections and storage and for questionnaire editing and safe keeping

When enrolment occurs at the HC-II, the EMBLEM staff will make arrangements with the HC-II staff to provide the necessary emergency needs.

The diagram below presents the simple flow of participants/specimens



#### ***b) Safe keeping and Transporting specimens in the field***

The specimens collected in the field will be kept in racks in cold boxes. The cold boxes will have fresh cold freezer packs. ***The temperature of these cold boxes will be monitored using electronic temperature loggers to ensure samples are kept at a cool temperature during their stay in the field as well as transport back to the processing laboratory.*** When putting samples in the cold boxes, EMBLEM staff will verify that the information on the tubes is correct, including the EMBLEM sample IDs on the blood tubes and saliva cups, the estimated volumes drawn, by checking against the information on the sample-link forms and they will record the time the samples were placed in the cold boxes.

EMBLEM samples should be transported to the EMBLEM Laboratory within 8 hours. A vehicle in good working condition will be available to transport the specimen. The last time for collecting a sample will be determined by when the vehicle has to leave the field to take the specimens to the laboratory. The last time when samples should be received at the laboratory cannot be later than 7:00PM on any given day.

#### ***c) Editing Questionnaires in the Field***

Questionnaires will be edited in the field. Editing will be done by team members swapping questionnaires and performing quick edits. The designated team supervisor will ensure this is done so that any queries can be done immediately before the respondents leave.

#### ***d) Sample processing***

Once the samples have been delivered to the EMBLEM Laboratory, they will be recorded and aliquotted. Blood will be aliquotted into plasma, Buffy coat and Red blood cells. Each

aliquot tube each with a unique BSI number will be stored (Refer to chapter 6 for details on aliquoting and storage procedures).

***e) Returning of results to enrolled controls***

All results from clinical tests (for malaria, stool parasites, and HIV) will be returned to the controls. The clinical results will be given to their clinician at the local HCII unit and in the case of Pilot Population Controls, the results will be returned to participants by qualified EMBLEM staff.

## 4.5 Staffing Plan Control Enrolment

The table below shows the required staff for the census and control enrolment

	<b>Staff</b>	<b>Roles and requirements</b>
Census	Enumerators	Canvas the whole selected LC1 to enumerate and map (GPS coordinates) all households within the targeted village. Each village will have an enumerator
	Statisticians	Designing census methodology, train census staff, design data capturing and check programs, supervising field work and data entry, data cleaning. To be done by two statisticians from UBOS
	Geo information officer	Participate in training Census Enumerators in GIS reading and supervising the census exercise. This will be an officer from UBOS
	Programmer	Working with statisticians to develop data entry and check programs. To be done by two statisticians from UBOS
	Editors	Reviewing questionnaires before entry. Editing to be done both in the field and in office. ---- editors will be recruited.
	Data Entrants	Enter the questionnaire into the designed entry packages. UBOS will recruit two staff
	community mobilizers	Mobilising the local communities. Two will be recruited per village
Pilot Population Controls	Team Leader	Overseeing the activities at the hub
	Specimen takers and interviewers	Taking the specimens and administering the questionnaire. They will be trained in sample taking, questionnaire administration, counselling and taking participants into the consenting process. They will pass over the specimens and the questionnaire to the sample storage personnel and editors respectively immediately after completing with the subject. At least six per hub
	questionnaire editors	Editing questionnaire data. At least three per hub
	Sample storage	Recording and storing drawn sample specimens and arranging for their transportation to processing centre. One person per hub
	Drivers	Transporting specimens at the processing centres. Two vehicles to be used at each hub
	Clinical officer and assistant	To manage the mobile clinic providing clinical services at the hub. In case a hub is at a HC-II, we may work within the existing structures. One clinical officer and assistant at each hub
Pilot HCII controls and Matched population controls	Team Leader	Overseeing the activities at HCII and in the village
	Specimen takers and interviewers	Taking the specimens and administering the questionnaire. They will be trained in sample taking, questionnaire administration, counselling and taking participants into the consenting process. They will pass over the specimens and the questionnaire to the sample storage personnel and editors respectively immediately after completing with the subject. The number will be determined when the diary is constructed for the village
	questionnaire editors	Editing the questionnaire. At least three per hub
	Sample storage	Recording and storing drawn sample specimens and arranging for their transportation to processing centre.
	Drivers	transporting specimens at the processing centres
	Lab Scientist, Clinical officer and phlebotomist	To manage the mobile clinic providing clinical services at the hub. In case a hub is at a HCII, we may work within the existing structures

## 4.6 Field requirements per site

The field supplies for specimen collection are listed in the Specimen Collection and Processing Chapter. They include:

- ✓ **Needles**
- ✓ Saliva collection bottles
- ✓ Blood collection bottles
- ✓ Storage cold boxes
- ✓ Cars
- ✓ Aprons

Housing facilities

- ✓ Tents

Field first aid facilities

- ✓ Mobile clinic



## Annexes

### Annex 1: A List of Pilot Parishes and Pilot Villages nested within district and sub-district hierarchies

S/NUMBER	DISTRICT	COUNTY	SUBCOUNTY	PARISH	VILLAGE
1	GULU	GULU MUNICIPALITY	LAYIBI	KIROMBE	ALOKOLUM
2	GULU	GULU MUNICIPALITY	LAYIBI	KIROMBE	KIROMBE CUSTOM
3	GULU	GULU MUNICIPALITY	LAYIBI	KIROMBE	PABBO
4	LIRA	ERUTE	BARR	OLILO	ACANDYANG
5	LIRA	ERUTE	BARR	OLILO	ADUKU
6	LIRA	ERUTE	BARR	OLILO	AGERIWANG
7	LIRA	ERUTE	BARR	OLILO	ANGUNYDWIL
8	LIRA	ERUTE	BARR	OLILO	ANGWENYI
9	LIRA	ERUTE	BARR	OLILO	APIIKONGO
10	LIRA	ERUTE	BARR	OLILO	ARWOTNYAP
11	LIRA	ERUTE	BARR	OLILO	ATONGOKO
12	LIRA	ERUTE	BARR	OLILO	AWEAYELA
13	LIRA	ERUTE	BARR	OLILO	AWUMI
14	LIRA	ERUTE	BARR	OLILO	AYINYI
15	LIRA	ERUTE	BARR	OLILO	ODYENYERE
16	LIRA	ERUTE	BARR	OLILO	OMENY
17	LIRA	ERUTE	BARR	OLILO	OMYELONORO
18	LIRA	ERUTE	BARR	OLILO	WOROMITE
19	ARUA	AYIVU	DADAMU	ODULUBA	CONGO
20	ARUA	AYIVU	DADAMU	ODULUBA	DADAWA
21	ARUA	AYIVU	DADAMU	ODULUBA	MVARA CENTRAL
22	ARUA	AYIVU	DADAMU	ODULUBA	NDRIBA
23	ARUA	AYIVU	DADAMU	ODULUBA	ODULUBA
24	ARUA	AYIVU	DADAMU	ODULUBA	SUDAN
25	ARUA	VURRA	ARIVU	OMOO	ACIBA
26	ARUA	VURRA	ARIVU	OMOO	ALUKURU
27	ARUA	VURRA	ARIVU	OMOO	ANYAVU
28	ARUA	VURRA	ARIVU	OMOO	AWIKA
29	ARUA	VURRA	ARIVU	OMOO	AYAKA
30	ARUA	VURRA	ARIVU	OMOO	EGAMI
31	ARUA	VURRA	ARIVU	OMOO	EJAKO
32	ARUA	VURRA	ARIVU	OMOO	ELIVO
33	ARUA	VURRA	ARIVU	OMOO	ESOKO
34	ARUA	VURRA	ARIVU	OMOO	ORAPI
35	ARUA	VURRA	ARIVU	OMOO	TISI
36	KITGUM	CHUA	LABONGO-LAYAMO	PAGEN	GWENG AJUT
37	KITGUM	CHUA	LABONGO-LAYAMO	PAGEN	LALWAL
38	KITGUM	CHUA	LABONGO-LAYAMO	PAGEN	LELAMU
39	KITGUM	CHUA	LABONGO-LAYAMO	PAGEN	PAGEN CENTRAL
40	KITGUM	CHUA	LABONGO-LAYAMO	PAGEN	PAGEN EAST
41	KITGUM	CHUA	LABONGO-LAYAMO	PAGEN	PAGEN WEST
42	KOBOKO	KOBOKO	DRANYA	NYANGAZIA	BARIFA
43	KOBOKO	KOBOKO	DRANYA	NYANGAZIA	DRANYA

S/NUMBER	DISTRICT	COUNTY	SUBCOUNTY	PARISH	VILLAGE
44	KOBOKO	KOBOKO	DRANYA	NYANGAZIA	JAMURE
45	KOBOKO	KOBOKO	DRANYA	NYANGAZIA	JUNGI
46	KOBOKO	KOBOKO	DRANYA	NYANGAZIA	MUCHUNGA
47	KOBOKO	KOBOKO	DRANYA	NYANGAZIA	NGURUNGURU
48	KOBOKO	KOBOKO	DRANYA	NYANGAZIA	NGURUPI
49	KOBOKO	KOBOKO	DRANYA	NYANGAZIA	NYANGAZIA
50	DOKOLO	DOKOLO	ADOK	BARDYANG	ADWILA
51	DOKOLO	DOKOLO	ADOK	BARDYANG	AGENGI
52	DOKOLO	DOKOLO	ADOK	BARDYANG	AKAIDEBE
53	DOKOLO	DOKOLO	ADOK	BARDYANG	ALWALA
54	DOKOLO	DOKOLO	ADOK	BARDYANG	AMIAKONY
55	DOKOLO	DOKOLO	ADOK	BARDYANG	APUTI
56	DOKOLO	DOKOLO	ADOK	BARDYANG	TE ACII
57	OYAM	OYAM	ABER	WIRAO	ACANKWETTE
58	OYAM	OYAM	ABER	WIRAO	ALOBOLAC
59	OYAM	OYAM	ABER	WIRAO	ALOBOLAC B
60	OYAM	OYAM	ABER	WIRAO	APEWOTNEKI
61	OYAM	OYAM	ABER	WIRAO	APEWOTNEKI B
62	OYAM	OYAM	ABER	WIRAO	AYOM APWONO
63	OYAM	OYAM	ABER	WIRAO	BARCAL 'A'
64	OYAM	OYAM	ABER	WIRAO	BARLONGO 'A'
65	OYAM	OYAM	ABER	WIRAO	BARLONGO 'B'
66	OYAM	OYAM	ABER	WIRAO	DAGO ITOLO
67	OYAM	OYAM	ABER	WIRAO	DAM AMALO
68	OYAM	OYAM	ABER	WIRAO	DAM APINY
69	OYAM	OYAM	ABER	WIRAO	DAM IMALO B
70	OYAM	OYAM	ABER	WIRAO	LWALA
71	OYAM	OYAM	ABER	WIRAO	OYOE
72	OYAM	OYAM	ABER	WIRAO	WANGDWE
73	OYAM	OYAM	ABER	WIRAO	WIRAO
74	AMOLATAR	KIOGA	AGWINGIRI	AMOLATAR	AMOLATAR ACON A
75	AMOLATAR	KIOGA	AGWINGIRI	AMOLATAR	AMOLATAR ACON B
76	AMOLATAR	KIOGA	AGWINGIRI	AMOLATAR	OCAMOLUM A
77	AMOLATAR	KIOGA	AGWINGIRI	AMOLATAR	OCAMOLUM B
78	ARUA	AYIVU	PAJULU	NYARACU	BURRA
79	ARUA	AYIVU	PAJULU	NYARACU	GILLA
80	ARUA	AYIVU	PAJULU	NYARACU	LARUBA
81	LAMWO	LAMWO	PALOGA	PAWAJA	ATANGO
82	LAMWO	LAMWO	PALOGA	PAWAJA	KALIRO
83	LAMWO	LAMWO	PALOGA	PAWAJA	KANGOLE NORTH
84	LAMWO	LAMWO	PALOGA	PAWAJA	KANGOLE SOUTH
85	LAMWO	LAMWO	PALOGA	PAWAJA	LOTOGO
86	LAMWO	LAMWO	PALOGA	PAWAJA	MARAKACH A
87	LAMWO	LAMWO	PALOGA	PAWAJA	MARAKACH B
88	LAMWO	LAMWO	PALOGA	PAWAJA	ONGALO
89	YUMBE	ARINGA	ARIWA	RIGBONGA	AYIVU
90	YUMBE	ARINGA	ARIWA	RIGBONGA	BASUNGA
91	YUMBE	ARINGA	ARIWA	RIGBONGA	EWANYATI
92	YUMBE	ARINGA	ARIWA	RIGBONGA	KANGUDE
93	YUMBE	ARINGA	ARIWA	RIGBONGA	KIRANGA
94	YUMBE	ARINGA	ARIWA	RIGBONGA	LUGBARICAKU
95	YUMBE	ARINGA	ARIWA	RIGBONGA	OCHINGA
96	YUMBE	ARINGA	ARIWA	RIGBONGA	TIFA

## Annex 2: Census Schedule for Census of Pilot Survey Villages, by Season

	Sept 2010				Oct 2010				Nov 2010				Dec 2010					January 2011				February 2011				March 2011				April 2011				May 2011				June 2011					July 2011				August 2011			
	WEEK				WEEK				WEEK				WEEK					WEEK				WEEK				WEEK				WEEK				WEEK					WEEK				WEEK							
Activity	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	5	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	5	1	2	3	4	1	2	3	4				
Grand Total																																																		
EMBLEM -CENSUS COMPONENT																																																		
01 Develop and finalize Activity Plan and Budget		X	X																																															
02 Meet District Leaders	X																																																	
03 Design and finalize instruments		X	X																																															
04 Procurement of Stationery and materials				X	X	X	X	X	X	X	X	X																																						
05 Train and Pretest the instruments				X	X																																													
06 Finalize the field instruments					X																																													
07 Preliminary Visits/Mobilization/Staff idefn									X	X																																								
09 Identification of Field Logistics																		X	X									X	X																					
10 Train field staff																					X																													
11 Carry out field work for the Census																																																		
12 Capture of the EMBLEM Census data																																																		
13 Data Editing																																																		
14 Send clean data to EMBLEM project																																																		

### Summary, Enumeration

#### 1. Enumeration during Wet Season

May 2011 (Kangube Village (Yumbe district), Ndriba Village(Arua district) and Orapi Village (Arua district)

August 2011 (kirombe Custom Village (Gulu district), Woromite Village (Lira district) and Lotogo Village(Lamwo district)

#### 2. Enumeration during dry Season

February 2011 (Amolatar Acon B (Amolatar district), DamaImalo B (Oyam district) and Te ACII (Dokolo district)

April 2011 (Burra Village (Arua district) , Jungi Village (Koboko district), Lalwal Village (Kitgum district)

### Annex 3: Screener Form for Matched Population Controls

**COMPLETE THIS FORM TO ASSESS ELIGIBILITY OF CONTROLS RANDOMLY SELECTED FOR POSSIBLE INCLUSION IN THE CASE-CONTROL STUDY.**

TODAY'S DATE:     |\_|\_|/|\_|\_|/|\_|\_|\_|\_|\_|

DAY     MONTH     YEAR

SCREENER'S ID:     |\_|\_|\_|\_|\_|\_|\_|\_|\_|\_|\_|\_|\_|\_|\_|

**Characteristics of CASE to whom the control is being matched:**

Parish of residence:

|\_|\_|\_|\_|\_|\_|\_|\_|\_|\_|\_|\_|\_|\_|\_|\_|\_|\_|\_|\_|\_|

GENDER:           |\_|\_|MALE|\_|\_|FEMALE

AGE:               |\_|\_|\_|\_| Years

1. PATIENT'S OUT-PATIENT NUMBER:

|\_|\_|\_|\_|\_|\_|\_|\_|\_|\_|\_|\_|\_|\_|\_|\_|\_|\_|\_|\_|\_|

NUMBER

YEAR

2. DATE OF BIRTH:   |\_|\_|\_|\_|\_|/|\_|\_|\_|\_|\_|/|\_|\_|\_|\_|\_|\_|\_|\_|

DAY

MONTH

YEAR

3. GENDER:   |\_|\_|MALE     |\_|\_|FEMALE

4. Does the child live in the geographic area from where the case originated (**same parish**)?

Yes.....01

No.....02     [PATIENT IS NOT ELIGIBLE, **DISCONTINUE**]

5. Has the child lived in this parish for at least 4 months?

Yes.....01

No.....02     [PATIENT IS NOT ELIGIBLE, **DISCONTINUE**]

6. Is the child the same gender as the case?

Yes.....01

No.....02     [PATIENT IS NOT ELIGIBLE, **DISCONTINUE**]

7. Is the child in the same age group as the case (**maximum of two years younger or older**)?

Yes.....01

No.....02 [PATIENT IS NOT ELIGIBLE, **DISCONTINUE**]

8. Is patient  $\leq$  15 YEARS OF AGE?

Yes.....01

No.....02 [PATIENT IS NOT ELIGIBLE, **DISCONTINUE**]

9. Does the child have a severe illness requiring immediate admission to hospital? (For example, acute respiratory infection, diarrhea with dehydration, snake bites or fractures.)

Yes.....01

No.....02 [PATIENT IS NOT ELIGIBLE, **DISCONTINUE**]

10. Does the child have cancer (**any** type)?

Yes.....01

No.....02 [PATIENT IS NOT ELIGIBLE, **DISCONTINUE**]

Does the child have mild clinical malaria (fever 37.5°C-37.5°C and a thick blood smear positive for malaria?)

Yes.....01

No.....02 [PATIENT IS NOT ELIGIBLE, **DISCONTINUE**]

11. PATIENT ELIGIBLE FOR STUDY:

Yes.....01

No.....02 [APPROACH TO CONSENT]

12. CONSENT OBTAINED:

Yes.....01

No.....02 [PATIENT IS NOT ELIGIBLE, **DISCONTINUE**]

13. DATE CONSENT OBTAINED

|\_|\_|/|\_|\_|/|\_|\_|  
DAY MONTH YEAR

SUBJECT ID (Place sticky label with Subject ID):

--

**Annex 4: Proposed Screener Form for Pilot HCII Controls**

**COMPLETE THIS FORM FOR ALL CHILDREN PRESENTING TO THE HC-II FOR CARE ON THE DAY OF THE SCREENING.**

TODAY'S DATE: | | | | / | | | | / | | | | | |

DAY MONTH YEAR

SCREENER'S ID: | | | | | | | | | |

**SURVEY VILLAGE/Local**

council: | | | | | | | | | | | | | | | | | |

1. PATIENT'S OUT-PATIENT NUMBER:

| | | | | | | | | | / | | | | | |  
NUMBER YEAR

2. DATE OF BIRTH: | | | | / | | | | / | | | | | |  
DAY MONTH YEAR

3. Does the child live in the survey village?

Yes.....01

No.....02 [PATIENT IS NOT ELIGIBLE, **DISCONTINUE**]

4. Is patient  $\leq$  15 YEARS OF AGE?

Yes.....01

No.....02 [PATIENT IS NOT ELIGIBLE, **DISCONTINUE**]

5. Does the child have a severe illness requiring immediate admission to hospital? (For example, acute respiratory infection, diarrhea with dehydration, snake bites or fractures.)

Yes.....01

No.....02 [PATIENT IS NOT ELIGIBLE, **DISCONTINUE**]

6. Does the child have cancer (**any** type)?

Yes.....01

[PATIENT IS NOT ELIGIBLE, **DISCONTINUE**]

No.....02

PATIENT ELIGIBLE FOR STUDY:

Yes.....01

[APPROACH TO CONSENT]

No.....02

7. CONSENT OBTAINED:

Yes.....01

No.....02 [PATIENT IS NOT ELIGIBLE, **DISCONTINUE**]

8. DATE CONSENT OBTAINED

| | | | / | | | | / | | | | | |  
DAY MONTH YEAR

SUBJECT ID (Place sticky label with Subject ID):

## Annex 5: HCII Registry Form

### EMBLEM REGISTER: HC-II SURVEY CONTROLS

COMPLETE THIS FORM FOR EACH CHILD ATTENDING THE HC-II ON THE DAY OF THE SCREENING

TODAY'S DATE: |\_\_| |\_\_| / |\_\_| |\_\_| / |\_\_| |\_\_| |\_\_| |\_\_|

DAY MONTH YEAR

NAME/LOCATION OF HC-II: \_\_\_\_\_ HC-II CODE: |\_\_| |\_\_| |\_\_| |\_\_|

1. PATIENT'S OUT-PATIENT NUMBER: |\_\_| |\_\_| |\_\_| |\_\_| |\_\_| / |\_\_| |\_\_| |\_\_| |\_\_|

NUMBER YEAR

2. PATIENT SURNAME: \_\_\_\_\_ FIRST NAME: \_\_\_\_\_

3. PATIENT'S DATE OF BIRTH: |\_\_| |\_\_| / |\_\_| |\_\_| / |\_\_| |\_\_| |\_\_| |\_\_| OR AGE: |\_\_| |\_\_|

DAY MONTH YEAR

4. PATIENT'S SEX: |\_\_| MALE |\_\_| FEMALE

5. PATIENT'S TRIBE: \_\_\_\_\_

6. MOTHER'S SURNAME: \_\_\_\_\_ FIRST NAME: \_\_\_\_\_

7. FATHER'S SURNAME: \_\_\_\_\_ FIRST NAME: \_\_\_\_\_

8. GUARDIAN'S SURNAME: \_\_\_\_\_ FIRST NAME: \_\_\_\_\_

9. MAIN CAREGIVER: |\_\_| MOTHER |\_\_| FATHER |\_\_| GUARDIAN

10. RESIDENCE OF THE FAMILY (where child usually lives):
LCI/Village:  __   __   __   __   __   __   __   __   __   __   __   __   __   __   __   __
LCII/Parish:  __   __   __   __   __   __   __   __   __   __   __   __   __   __   __   __
LC III/Sub-county:  __   __   __   __   __   __   __   __   __   __   __   __   __   __   __   __
LC IV/County:  __   __   __   __   __   __   __   __   __   __   __   __   __   __   __   __
LC V/District:  __   __   __   __   __   __   __   __   __   __   __   __   __   __   __   __
Country:  __   __   __   __   __   __   __   __   __   __   __   __   __   __   __   __
TELEPHONE:  __   __   __   __   __   __   __   __   __   __   __   __   __   __   __   __
Description of address:

11. HAS THE CHILD BEEN LIVING IN THE AREA ABOVE FOR **AT LEAST IN THE LAST FOUR MONTHS?**

|\_\_| YES |\_\_| NO

12. HAS CHILD BEEN TESTED FOR MALARIA TODAY? ☐ Yes ☐ No

13. IF THE ANSWER TO No. 12 IS YES, THEN TICK APPROPRIATE BOX BELOW

☐☐



	+	<input type="checkbox"/>	++	<input type="checkbox"/>	+++	<input type="checkbox"/>	++++	<input type="checkbox"/>	Negative	<input type="checkbox"/>	Not done
Scanty			Positive		Positive		Positive				
	Positive										

14. BODY TEMPERATURE \_\_\_\_\_ °C

15. HAS STOOL MICROSCOPY BEEN DONE? YES ☐ NO ☐

16. IF THE ANSWER TO No. 15 IS YES, INDICATE THE STOOL RESULT \_\_\_\_\_

17. LIST CURRENT DIAGNOSES:

a. \_\_\_\_\_

b. \_\_\_\_\_

18. Distance from Home to HC \_\_\_\_\_ (Kms)

NAME OF PERSON COMPLETING ENTRY TO REGISTRY: \_\_\_\_\_

STAFF ID OF PERSON COMPLETING ENTRY TO REGISTRY |\_\_|\_\_|\_\_|\_\_|\_\_|\_\_|

**Annex 6: Table showing Enrolment Schedule of Matched Population Controls by age and District**

District		Predicted Cases by age group						
District		0 - 2 Years	3 - 5 Years	6 - 8 Years	9 - 11 Years	12+ Years	Total	matched controls
Lacor Hospital								
ADJUMANI	Male	1	2	6	1	1	10	20
	Female	0	1	2	1	0	4	8
	Total	1	3	8	2	1	14	28
AMOLATA	Male	1	3	4	1	0	9	18
	Female	0	3	3	2	0	8	16
	Total	1	7	7	3	0	17	34
AMURU	Male	0	15	7	6	1	29	58
	Female	0	5	4	2	0	11	22
	Total	0	20	11	8	1	40	80
APAC	Male	0	12	20	5	3	41	82
	Female	0	11	7	5	1	24	49
	Total	0	23	27	11	5	65	131
DOKOLO	Male	0	4	3	1	0	8	16
	Female	0	4	1	0	0	5	9
	Total	0	8	4	1	0	13	25
GULU	Male	1	20	15	8	6	50	100
	Female	1	5	17	4	7	35	70
	Total	3	26	32	12	13	85	170
KITGUM	Male	0	5	3	1	3	11	22
	Female	0	2	1	1	1	4	8
	Total	0	7	3	2	3	15	30
LIRA	Male	1	24	19	9	3	55	111
	Female	0	13	19	6	6	44	88
	Total	1	37	38	15	9	100	199
MOYO	Male	0	1	0	1	0	2	4
	Female	0	0	1	0	1	1	3
	Total	0	1	1	1	1	3	7
NEBBI	Male	0	1	1	0	0	2	4
	Female	0	1	0	0	0	1	1
	Total	0	1	1	0	0	3	5
OYAM	Male	0	18	14	4	2	38	75
	Female	0	5	9	5	3	22	44
	Total	0	22	23	9	5	59	119
PADER	Male	0	1	1	3	0	4	8
	Female	0	0	1	1	0	2	4
	Total	0	1	2	3	0	6	12
Total predicted cases		5	156	156	66	38	420	840
Total predicted matched controls		9	311	313	132	75	840	
Kuluva Hospital								
ADJUMANI	Male	1	0	0	0	0	1	1
	Female	0	1	1	0	0	2	4
	Total	1	1	1	0	0	3	6
ARUA	Male	0	0	1	0	0	1	1
	Female	2	15	20	10	10	57	114
	Total	6	19	23	15	10	73	146
KOBOKO	Male	8	33	44	24	21	131	261
	Female	0	3	5	1	1	10	21
	Total	0	2	9	3	3	17	35
MARACHA/TEREGO/MARACHA-TEREGO	Male	0	5	14	5	4	28	56
	Female	1	7	6	1	2	17	33
	Total	1	8	10	4	3	26	53
MOYO	Male	1	15	15	6	6	43	86
	Female	0	0	1	0	0	1	1
	Total	0	1	0	0	2	3	6

District		Predicted Cases by age group						matched controls
		0 - 2 Years	3 - 5 Years	6 - 8 Years	9 - 11 Years	12+ Years	Total	
NEBBI	Male	0	1	1	0	2	3	7
	Female	0	6	1	2	1	10	21
	Total	1	6	6	1	2	17	33
NYADRI	Male	1	13	8	3	3	27	54
	Female	0	0	1	0	0	1	1
	Total	0	0	1	0	0	1	1
YUMBE	Male	0	1	6	0	1	8	15
	Female	1	3	6	1	1	12	24
	Total	1	5	11	1	1	19	39
<b>Total predicted cases</b>		12	72	94	40	37	255	510
<b>Total predicted matched controls</b>		24	145	189	79	74	510	

## Annex 7: Household Schedule



### Epidemiology of Burkitt Lymphoma in the East African children and Minors (EMBLEM)

#### LISTING QUESTIONNAIRE

SECTION 1: IDENTIFICATION PARTICULARS			
1. DISTRICT:			
2. COUNTY			
3. SUBCOUNTY			
4. PARISH			
5. LC1			
6. LOCATION OF THE VILLAGE			

THIS LISTING PROCESS IS BEING THE EMBLEM PROJECT IN SAMPLED LC1s IN THE NORTHERN AND NORTH EASTERN UGANDA AS PART OF THE BROAD EMBLEM RESEARCH PROJECT.

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St. Mary's Hospital, Lacor, Gulu,  
Juba Road,  
P.O. Box 180 Gulu, Uganda, or  
Tel: +1(256) 772403192/ 711433112  
E-mail: emblem@afenet.net

African Field Epidemiology Network  
Plot 4B, Mabua Road, Kololo  
P.O. Box 12874, Kampala, Uganda  
Website: www.afenet.net

**SECTION 2A: LISTING SUMMARY: (To be completed after listing )**

Serial number	Item	Total		
(1)	Total number of households listed			

**SECTION 2B: STAFF DETAILS AND LISTING TIME**

STARTING LOCATION FOR LISTING: \_\_\_\_\_

1. NAME OF LISTER:

DD

MM

--	--

YYYY

2. DATE OF LISTING

--	--	--

3. NAME OF SUPERVISOR

DD

MM

--	--

YYYY

4. DATE OF CHECKING

--	--	--

5. STARTING TIME

HRS

--	--	--	--

6. ENDING TIME

HRS

--	--	--	--

6. RESPONSE CODE:

**Codes for Item 6:**

Completed 1

Partially done.....  
(Reasons for partial listing should be explained in the remarks)

--

7. REMARKS

8. SIGNATURES

\_\_\_\_\_  
LISTER\_\_\_\_\_  
SUPERVISOR

### SECTION 3: LIST OF HOUSEHOLDS AND THEIR SELECTION

House Serial Number	Household Serial Number	Name of the household head	Please give me the names of the children aged 0-14 years that <b>usually</b> live here.	How old is [NAME]?
INDICATE INFORMATION ON LOCATING HOUSEHOLD AND HOUSEHOLD HEAD, OTHER NOTES:				
House Serial Number	Household Serial Number	Name of the household head	Please give me the names of the children aged 0-14 years that <b>usually</b> live here.	How old is [NAME]?
INDICATE INFORMATION ON LOCATING HOUSEHOLD AND HOUSEHOLD HEAD, OTHER NOTES:				
House Serial Number	Household Serial Number	Name of the household head	Please give me the names of the children aged 0-14 years that <b>usually</b> live here.	How old is [NAME]?
INDICATE INFORMATION ON LOCATING HOUSEHOLD AND HOUSEHOLD HEAD, OTHER NOTES:				
House Serial Number	Household Serial Number	Name of the household head	Please give me the names of the children aged 0-14 years that <b>usually</b> live here.	How old is [NAME]?
INDICATE INFORMATION ON LOCATING HOUSEHOLD AND HOUSEHOLD HEAD, OTHER NOTES:				

[illegible]

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## Annex 8: The EMBLEM Census Questionnaire



### EMBLEM NORTHERN UGANDA SAMPLE CENSUS, 2010/11 (QUESTIONNAIRE BOOKLET)

B.1 [BOOK NUMBER]

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#### SECTION ONE: IDENTIFICATION PARTICULARS

DISTRICT :				
COUNTY :				
SUBCOUNTY:				
PARISH:				

Household Number		







HOUSEHOLD NUMBER:			
-------------------	--	--	--

# EMBLEM NORTHERN UGANDA SAMPLE CENSUS, 2010/11

SECTION 2: HOUSEHOLD SCHEDULE										PERSONS AGED 5 YEARS AND ABOVE	AGED 10 YEARS AND ABOVE	
PERSON ID	FULL NAME	RESIDENTIAL STATUS	SEX	RELATIONSHIP	DURATION OF RESIDENCE	AGE	ETHNIC GROUP OR	MOTHER'S SURVIVAL	FATHER'S SURVIVAL	SCHOOL ATTENDANCE	EDUCATION ATTAINMENT	MARITAL STATUS
	Give me full names of the household head and of all those persons who stay in this Household including Guests in the household <i>OKELLO SIMON</i>	What is the residential status of (NAME)?  <b>(for codes 5 – 7 end interview at column 2.7)</b>	Is (NAME) male or female?  1=Male 2=Female	What is (NAME's) relationship to the head of Household?  <b>(Refer to the code list)</b>	How long has (NAME) lived in this Village continuously during the last 12 months?	What is (NAME'S) age in completed years?  <b>(If less than one write 00)</b>	What is (NAME's) tribe?  <b>If non-Ugandan, write Country code of citizenship)</b>	Is (NAME'S) biological  mother  Still alive?  1=Yes 2=No 8=Don't Know	Is (NAME'S) biological  Father  still alive?  1=Yes 2=No 8=Don't Know	Is (NAME) currently attending School, left school or never been to school?  <b>(If code 3 &gt;&gt;2.13)</b>	What is the highest Grade/Class of formal Education (NAME) Completed?  <b>(Refer to the code list)</b>	What is (NAME's) current marital status?  1= Married monogamously 2= Married polygamous 3= Divorced/ Separated 4= Widow/ Widower 5= Never married
2.1	2.2	2.3	2.4	2.5	2.6	2.7	2.8	2.9	2.10	2.11	2.12	2.13

01												
02												
03												
04												
05												
06												
07												
08												
09												
10												

**EMBLEM NORTHERN UGANDA SAMPLE CENSUS, 2010/11**

	<b>SECTION 2: HOUSEHOLD SCHEDULE</b>			
<b>P E</b>	<b>LESS THAN 18 YEARS</b>	<b>BASIC NEEDS (ALL PERSONS)</b>	<b>0-17 YEARS</b>	<b>ILLNESS SUFFERED</b>

R S O N I D	MOSQUITO NET	INSECT SIDE TREATED	OWNERSHIP OF A BLANKET	OWNERSHIP OF SHOES	OWNERSHIP OF CLOTHING	HIGHEST LEVEL OF FATHER'S EDUCATION	HIGHEST LEVEL OF MOTHER'S EDUCATION	SUFFERING FROM ILLNESSES	DAYS SUFFERED IN LAST 30 DAYS	SYMPTOMS OF ILLNESS	ANYONE CONSULTED?	REASON FOR NOT CONSULTING	FACILITY WENT FOR CONSULTATION	DISTANCE TO TREATMENT PLACE	DAYS STOPPED
	Did (NAME) sleep under a mosquito net last Night?	Was this an Insect side Treated Net (ITN)?	Does (NAME) own a Blanket?  1=Yes  2=No  3= DK	Does (NAME) own at least one pair of shoes?  1=Yes  2=No  3=DK	Does (NAME) own at least two sets of clothing?  1=Yes  2=No  3=DK	What is the highest level of father's education?  <b>(Refer to the code list)</b>	What is the highest level of Mother's education?	During the past 30 days, did you/(NAME) suffer from any Illness?  1= Yes  2= No	For how many days did you/(NAME) suffer due to illness during the past 30 days?	Can you describe the symptoms that you primarily suffered from the <b>major</b> illness or injury during the past 30 days?  <b>(Record up to 3 symptom codes)</b>  <b>(See codes below)</b>	Was anyone consulted (e.g. a doctor, nurse, pharmacist or traditional healer) for the major illness during the past 30 days?    1= Yes (>>2.26)  2= No	Why was no one consulted for the major illness?  <b>(Refer to the code list)</b>	Where did you go for the first consultation during the past 30 days?  <b>(Refer to the code list)</b>	What is the Distance to the place where this treatment was sought for in km?	For how many days in total did you/(NAME) have to stop going to school or doing normal duties during the last 30 days because of this illness?

2.1	2.14	2.15	2.16	2.17	2.18	2.19	2.20	2.21	2.22	2.23 (a)	2.23 (b)	2.23 (c)	2.24	2.25	2.26	2.27	2.28
01																	
02																	
03																	
04																	
05																	
06																	
07																	
08																	
09																	
10																	

### SECTION 3: HOUSEHOLD AND HOUSING CONDITIONS

3.0(a) GPS EASTINGS							3.0(b) GPS NORTHINGS		
---------------------	--	--	--	--	--	--	----------------------	--	--

DWELLING UNIT			CONSTRUCTION MATERIALS			DISTANCE TO THE NEAREST FACILITY	
What type of dwelling is it?	What is the occupancy tenure of dwelling unit?	How many Rooms are usually used for Sleeping	What type of Material was <b>MAINLY</b> used for the construction of the roof?	What type of Material was <b>MAINLY</b> used for the construction of the Wall?	What type of Material was <b>MAINLY</b> used for the construction of the Floor?	What is the distance from home to the nearest <b>PUBLIC</b> health facility in KMs?	What is the distance from home to the nearest <b>PRIVATE</b> health facility in KMs?
3.1	3.2	3.3	3.4	3.5	3.6	3.7	3.8
Detached House (Single or Multi-Storey) 10	Owner Occupied 10		Iron sheets 1	Concrete/Stones 10	Concrete 10	Less than 1km 1	Less than 1km 1
Semi-Detached House 11	Free-Public 11		Tiles 2	Cement Blocks 11	Bricks 11	1km-<3km 2	1km-<3km 2
Flat in a block of flats 12	Free-Private 12		Asbestos 3	Burnt/Stabilized blocks	Stone 12	3km-<5kms 3	3km-<5kms 3
Room or Rooms of a main House 13	Subsidized-Public 13		Concrete 4	Unburnt Bricks with Cement 13	Cement Screed 13	5km-<10kms 4	5km-<10kms 4
Servants Quarters 14	Subsidized-Private 14		Tins 5	Unburnt bricks with mud 14	Rammed earth 14	>=10Kms 5	>=10Kms 5
Tenement (Musigo) 15	Rented-Public 15		Thatch 6	Wood 15	Wood 15		
Hut 16	Rented-Private 16		Other (Specify) 7	Mud and Pole 16	Tiles 16		
Garage 17	Other-Specify 17			Tin/Iron sheets 17	Other (Specify) 17		
Godown/Basement 18				Other (Specify) 18			
Store 19							
Other (Specify) 20							
<div><div></div><div></div></div>	<div><div></div><div></div></div>	<div><div></div><div></div></div>	<div><div></div></div>	<div><div></div><div></div></div>	<div><div></div><div></div></div>	<div><div></div></div>	<div><div></div></div>

### SECTION 3: HOUSEHOLD AND HOUSING CONDITIONS

WATER		TOILET FACILITY		SOLID DISPOSAL		BATHROOM USED		KITCHEN		SOAP AND SUGAR		SOURCE OF LIVELIHOOD		
What is the household's main source of drinking water?	What is the distance to this source of Water?	What type of toilet facility does this household MAINLY use?	What is the household's MAIN method of solid disposal?	What type of Bathroom does this household MAINLY use?	What Type of Kitchen does this household MAINLY use?	Does every member of this household ....? (1=Yes, 2=No)		What is the Household's MAIN source of Livelihood?						
3.13	3.14	3.15	3.16	3.17	3.18	3.19		3.20						
Tap/Piped water	1	On premises	1	Covered pit latrine (Private)	1	Skip Bin	1	Inside	1	Inside, Separate Room	1	(a) Use soap to bathe?	Subsistence Agriculture	1
Borehole/H and pump	2	< ½ km	2	Covered pit latrine (Shared)	1	Pit	2	Outside (Built)	2	Inside, No separate Room	2	(b) Take sugar at least once a day	Commercial farming	1
Protected well/Spring	3	½ km - < 1 km	3	VIP latrine (Private)	1	Heap	3	Outside (Makeshift)	3	Outside (Built)	3		Employment Income	1
Gravity flow scheme	4	1 km - 5 kms	4	VIP latrine (Shared)	1	Garden	4	None	4	Outside (Makeshift)	4		Business Enterprise	1
Rain water	5	More than 5 kms	5	Uncovered Pit Latrine	1	Burning	5			Outside (Open space)	5		Cottage Industry	1
Open water sources (lakes,	6			Flush Toilet (Private)	1	Waste Vendor	6			Other (Specify)	6		Property Income	1
Water truck/Water	7			Flush Toilet (Shared)	1	Other (Specify)	7						Family/Friends/Relatives	1
Other (Specify)	8			Bush	1								Institutional or Programme support	1
				Other (Specify)	1								Other (Specify)	1
					8									8

**Annex 9: Table showing Enrolment Schedule of Pilot HCII Controls by age and District**

District	HC-II	Sex	0 - 2 Years	%	3 - 5 Years	%	6 – 8 Years	%	9 - 11 Years	%	12+ Years	%	Total	%
Amuru	Amuru	Male	147	37.2	58	14.7	6	1.5	2	0.5	0	0.0	213	53.9
		Female	127	32.2	47	11.9	6	1.5	1	0.3	1	0.3	182	46.1
		Total	274	69.4	105	26.6	12	3.0	3	0.8	1	0.3	395	100.0
	Pabbo	Male	139	35.7	60	15.4	5	1.3	2	0.5	2	0.5	208	53.5
		Female	133	34.2	36	9.3	8	2.1	1	0.3	3	0.8	181	46.5
		Total	272	69.9	96	24.7	13	3.3	3	0.8	5	1.3	389	100.0
	Overall	Male	286	36	118	15	11	1	4	1	2	0	421	54
		Female	260	33	83	11	14	2	2	0	4	1	363	46
		Total	546	70	201	26	25	3	6	1	6	1	784	100
Arua	Anyiribu	Male	2	2.7	13	17.6	9	12.2	7	9.5	3	4.1	34	45.9
		Female	2	2.7	12	16.2	14	18.9	6	8.1	6	8.1	40	54.1
		Total	4	5.4	25	33.8	23	31.1	13	17.6	9	12.2	74	100.0
	Anyavu	Male	0	0	1	6.25	3	18.75	0	0	2	12.5	6	37.5
		Female	2	12.5	1	6.25	3	18.75	3	18.75	1	6.25	10	62.5
		Total	2	12.5	2	12.5	6	37.5	3	18.75	3	18.75	16	100
	Overall	Male	2	2.2	14	15.6	12	13.3	7	7.8	5	5.6	40	44.4
		Female	4	4.4	13	14.4	17	18.9	9	10.0	7	7.8	50	55.6
		Total	6	6.7	27	30.0	29	32.2	16	17.8	12	13.3	90	100.0



**Annex 10: Child notification card**

1. Name of the child
2. Residence Village:
3. Age in Completed years:
4. Parents/Guardian's Name:
5. Venue of Specimen taking:

6. Date of Specimen taking: \_\_\_\_\_

*This card serves to notify the Child whose name appears above and the Parent/Guardian also specified above to report on the above mentioned venue during the date specified for the EMBLEM study. More details can be sought from the following EMBLEM staff.*

1. Dr. Martin Ogwang, Principal Investigator, EMBLEM project mobile – 0772593901
2. Dr. Tobias Kinyera, Coordinator EMBLEM project, mobile telephone number - 0772541976

Signed \_\_\_\_\_

Dr. Tobias Kinyera (*Project Coordinator*)

**Annex 11: EMBLEM census materials control forms**



**EMBLEM NORTHERN UGANDA SAMPLE CENSUS, 2010/11**

**MATERIAL DISTRIBUTION FORM A**

<b>Serial Number</b>	<b>Item</b>	<b>Number Received</b>	<b>Number Returned</b>	<b>Comments</b>
01	Boots			
02	Umbrellas			
03	Bag			
04	Pens			
05	Tshirts			
06	Control Forms			

07	Enumerators' Manual			
08	Progress Report Form			
09	Clip Board			
10	GPS			

**Enumerator:**

**Signature :** \_\_\_\_\_

**Name :** \_\_\_\_\_

**Date :** \_\_\_\_\_

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**Issuing officer:**

**Signature :** \_\_\_\_\_

**Name :** \_\_\_\_\_

**Date :** \_\_\_\_\_



**EMBLEM NORTHERN UGANDA SAMPLE CENSUS, 2010/11**

**QUESTIONNAIRE DISTRIBUTION FORM-FORM B**

Serial Number	Received		Returned		Comments
	Questionnaire Number	Number of Household forms within	Questionnaire Number	Household forms within	
01					
02					
03					
04					
05					
06					
07					
08					
09					
10					
11					

**Enumerator:**

**Signature :** \_\_\_\_\_  
**Name :** \_\_\_\_\_  
**Date :** \_\_\_\_\_

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**Issuing officer:**

**Signature :** \_\_\_\_\_  
**Name :** \_\_\_\_\_  
**Date :** \_\_\_\_\_

## Chapter 5: EMBLEM Specimen Collection Procedures

### 5.1 Introduction

A vital component of the EMBLEM study is the collection, handling, preservation and processing of biological specimens collected from enrolled subjects (e.g. cases and controls). Specimen collection only happens when participants have completed the following steps: a) spotted (cases); b) been screened and determined to be eligible to participate in EMBLEM; and c) have consented to participate in EMBLEM. Participants will generally have also completed an interview by questionnaire before specimen collection, but if not, the questionnaire should be administered as soon as is practical following specimen collection. The enrolled participant will have an Enrollment Folder containing his or her unique EMBLEM Subject ID label set, his or her Hospital ID label set (cases only), a Tracking Form that indicates the procedures that have been completed (see Chapter 3), completed forms for the completed procedures, and forms for the procedures that still need to be completed, such as the laboratory request and processing forms for the specimens that are to be collected.

This chapter describes the operational and technical procedures that all EMBLEM staff must follow when collecting, transporting and processing of biospecimens at all study site locations (e.g. village, health center and hospital). All study personnel are advised to carefully read, understand and adhere to the instructions detailed in this chapter. They are also advised to have this manual close by for reference to settle questions about specimen collection, transport, processing and storage. This manual describes the procedures to be followed as well as the supplies that will be provided by the study to support each procedure. Following the collection procedures will ensure that biospecimen quality will be preserved so it will reflect true biological differences across study subjects, rather than differences (also referred to as “noise” by researchers) in collection or processing procedures.

Table 5.1 lists the types of biospecimens that will be collected from each study participant and the purpose for collecting the specimen. The specimens fall into two groups: a) research specimens and b) hospital specimens. Research specimens will be collected and sent to the EMBLEM processing laboratory for separation into components (plasma, buffy coat, RBC and saliva aliquots) and storage until shipped to NCI for testing. The hospital specimens are collected to perform tests that will be done on site, either at the local hospital laboratory or at a temporary laboratory established by the study in the field. In general, these specimens will not be processed into components for storage, and they will not be shipped to NCI, although digital images will be recorded of certain tests for transmission to NCI (malaria smears, rapid diagnostic test for malaria, and stool microscopy). An exception is tissue, which may be historical or collected at the time of the hospital visit. Tissue specimens will serve both research and hospital purposes and the research tissue will be shipped to NCI.

The participants in EMBLEM are classified as either cases (those who have Burkitt lymphoma) or controls (those who do not have Burkitt lymphoma). The techniques used to collect similar specimen types do not differ between cases or controls. However, the types of hospital specimens requested will be more for cases simply

because cases are very sick and require more testing in order to guide their care. Study staff can use a simple rule to remember whether a child before them is a case or control. If the child is being enrolled in the hospital, then the child is likely a case; if the child is being enrolled at a Health Center II or in the village, then the child is a control. Additionally, the EMBLEM Subject ID number assigned to each participant is specific to their cohort. The second digit in the ID represents the subject's cohort: 1=case, 2= HCII control, 3=population control, and 4= HCII population control. With these distinctions in mind, Table 5.1 can be used to collect the right set of specimens from a child. (Table note: whole blood for research and hospital tests will be drawn at the same time. The only difference is that research whole blood specimen will be processed and shipped to NCI and the hospital blood specimens will be used locally for hospital tests).

Appendices 6-1 through 6-3 provide additional information on specimen collection, labeling and processing in a graphical format.

<b>Table 5.1</b>					
<b>Specimen Collection and Application</b>					
<b>Specimen Type</b>	<b>Volume/amount</b>	<b>Case</b>	<b>Control</b>	<b>Lab Test</b>	<b>NCI Storage</b>
<b>SPECIMENS FOR RESEARCH</b>					
Whole blood (EDTA)	10 ml	Yes	Yes	None	Buffy Coat, Plasma, Red Cells
Saliva	4 ml	Yes	Yes	None	w/STM
<b>SPECIMENS FOR BOTH RESEARCH AND HOSPITAL USE</b>					
Tissue (biopsy/surgical) May be historical only.	1 gm	Yes	No	H&E slide	Paraffin block
<b>SPECIMENS FOR HOSPITAL USE</b>					
Whole blood (EDTA)	2 ml	Yes	Yes	Malaria RDT Malaria thick and thin smears	Digital image of result
Whole blood (EDTA)	3 ml	Yes	Yes	CBC w/diff ESR	No
Whole blood (SST)	2.5 ml	Yes	No	Renal panel Liver panel	No
Stool	1 gm	Yes	Yes	Wet preparation for ova, cysts, and larvae	Digital image of result



## **5.2 Collecting Specimens following Safe Practice**

Because body fluids are potential sources of dangerous infections, this study manual describes procedures that are designed to ensure the collection of specimens is safe for the participants, the study staff, and the community. You are expected to adhere to the principles of safe practice recommended here. The Study Clinical Coordinator will ensure that study teams have supplies for safe collection, safe disposal of sharp and non-sharp waste, and a system for reporting any injuries that occur during the study for whatever reason. The manual describes the supplies required for each procedure, the process of collecting the specimen, safe storage of waste in the clinic, laboratory, or the field, and safe disposal of waste back at the Study office. The adherence to these procedures will be monitored and enforced by a Safety Officer, who will be any worker so designated and trained by the Study Coordinator. The Study Coordinator will keep a log of any injuries reported to have occurred during the study and initiate corrective responses to minimize and mitigate consequences resulting from such injuries.

### **General principles**

Collection of all types of specimens involves the following five basic steps:

- a) Complete laboratory forms for the specimen for the participant and prepare labels for the specimen
- b) Set up the specimen collection tray with equipment needed to collect the specimen from the participant, including extra materials in case of equipment failure
- c) Collect the specimen, attach labels, and place specimens in the collection rack
- d) Dispose of the sharp and non-sharp waste according to safe practice
- e) Place the specimen in the transport container for transport to the testing/processing laboratory.

In addition to the basic collection procedure described above, the following safe practices will be expected when collecting biological specimens.

- Supplies for specimen collection should be prepared the day before, and their completeness and appropriateness should be checked in the morning before leaving the EMBLEM office.
- Wear the appropriate personal protection items (e.g., gloves, lab coats, and aprons). These will be provided by the study.
- Use adapter built-in needle protector and self-retracting needles to collect blood. If you use non self-retracting needles, you will destroy the needle by bending it using a forceps and then dispose it in a sharp waste bin without recapping.
- Discard waste from specimen collection according to safe practice: “sharps” into sharps waste biohazard containers and “non-sharp waste” in approved containers.
- Except where otherwise noted (e.g., excess stool specimen), carry all waste produced as part of the study back to the study office for safe disposal.

- Report any injury or exposure to body fluids to the Safety Officer and seek medical attention recommended for the injury or exposure to biologic specimens.

### **5.3 Research Blood Specimen Collection**

#### **5.3.1 Venous Blood Sample Collection Supplies**

Sufficient supplies are provided to safely collect venous blood specimens from the participant. The samples are collected for two purposes: research OR hospital purposes. The labeling and handling of the research vs. hospital specimens is different due to patient privacy considerations, labeling requirements and patient care needs. Two sets of labels will be printed and enclosed in the participants Enrollment Folder; the EMBLEM Subject ID labels and the Hospital ID labels (cases only).

The research blood specimen is collected in a 10 ml purple-top EDTA tube ( or two 6 ml purple tops may be substituted if necessary). The research blood tube will be labeled with the Hospital ID label.

The hospital specimens will be collected in smaller volumes with anticoagulant (2 ml and 3 ml purple top EDTA tubes) and in a 2.5 ml SST tube for serum testing (cases only). The hospital specimens will be labeled with Hospital ID labels (cases only).

The following supplies must be assembled for the venous blood collection

#### **a) Tubes:**

##### **Research specimen**

1 - 10 mL or 2 6mL K<sub>2</sub>EDTA BD tube(s) - (Purple top – 366643)

##### **Hospital specimens**

1 - 2.5 mL SST Greiner tube - (Red/Yellow top – 454028)

1 - 2 mL K<sub>2</sub>EDTA Greiner tube - (Purple top – 454024)

1 - 3 mL K<sub>2</sub>EDTA Greiner tube - (Purple top – 454020)

#### **b) Non-reusable supplies:**

1 - Blood Collection Set w/tube holder and butterfly needle (21 ga BD 368652 or 23ga BD 368653) or butterfly canulae

1 - Blood collection start pack (e.g. alcohol swab, 2x2 gauze, bandaid)

4 - Specimen transport bags

1- Disposable towels

#### **c) Forms/IDs**

3 - Laboratory Requisition forms (one for each hospital specimen tube)

1 - EMBLEM Research Blood Form (for the research blood specimen)

1 - Set EMBLEM Subject ID labels

1 - Set Hospital ID labels

**d) Reusable supplies**

Tourniquet

Tube racks

Specimen collection tray

Plastic apron

Ballpoint pens (black or blue ink)

Diamond pencils (to label slides and RDT tests)

Scissors for creating smaller label subsets to send to the labs

Paperclips to clip the label subsets to the Requisition and Research Blood Forms

**5.3.2 Blood Collection Procedures**

The following sequence is recommended when collecting venous blood specimens unless clinical circumstances warrant otherwise: research specimens followed by hospital specimens. Once a vein has been secured, the specimens can be collected sequentially from the same vein until all the specimen tubes have been filled as recommended. Reassure the participant that this approach will ensure the child receives only one needle prick for all collections and that blood from the vein will not be completely drained. The filled tubes should be placed in the specimen rack until collection is completed. Gently invert each tube two or three times immediately after collection to adequately mix the blood and anticoagulant. **Do not** transfer blood from one tube into another tube. If the tube loses its vacuum, it should be discarded in the sharps bin and another (replacement) tube used to collect a blood specimen.

**5.3.2.1 Pre-Blood Collection Procedures**

- Check the participants consent form to confirm that they and their guardian consented for blood draw (the consent form should be in the Enrollment Folder)
- Explain the collection procedures to the parent and child using the charts provided.
- Prepare the Blood Collection Tray by laying out the tubes, swabs, tourniquets, and racks.
- Confirm that the necessary laboratory forms are in the Enrollment Folder along with the two sets of labels that will go on the forms and on the tubes have been completed. The top of the EMBLEM Laboratory Form with fields for personal identifying information may be completed at this time.

**5.3.2.2 Safe Practice for Blood Collection**

- The participant should be seated comfortably; young children should sit in their parent's lap.
- Select a vein for venipuncture, e.g., in the antecubital area or on the back of the hand, , and apply tourniquet lightly (i.e., it should not be too tight) just above that area.

- When the vein is clearly visible or palpable (can be felt by touch), clean the area with an alcohol swab while holding the child's forearm or hand firmly to reduce jerking movement. The parent or another study staff may be asked to help you to hold the child firmly to reduce jerking movement when drawing blood.
- Unsheathe the butterfly needle and puncture the skin near where the vein is prominent so you pierce the vein slanting at an angle of about 45°. This angle will reduce the chances of tearing the vein or going through both walls of the vein. You will know that you have entered the vein when you observe blood flow into the tubing of the butterfly set. Butterfly needles help to reduce pre-analytical errors by providing better quality specimens in the field, reducing the turnaround time and minimizing the risk of exposure of the health care worker to blood-borne pathogens. Secure the butterfly wings of the needle around the hand or forearm of the child with band aid, ask the parent or an assistant to hold the hand of the child firmly to minimize movements that may cause the needle to move out of the vein.
- Remove the tourniquet as soon as you are sure the needle is in the vein to avoid blood leaking into the tissue.
- Holding the back end of the collection needle in one of your hands, pick up the blood collection tubes with your other hand and puncture the stopper diaphragm of the tube. You will know you are still in the vein when blood flows into the tube under suction of the vacuum in the tube.
- Fill the tube as recommended.
  - If blood doesn't flow into the tube, it may be that the needle opening is blocked by the tissue of the vein. Readjust the needle for better positioning.
- When the tube has filled to its stated volume and blood flow ceases, immediately remove the tube from the holder and place in the holder. Immediately after the collection of each EDTA tube, invert the tube 2-3 times to mix anticoagulant. Place the blood-filled tube upright in a rack. If additional tubes are to be collected, insert the next tube according to the order of draw and push onto the back end of the collection needle - repeat this procedure until all tubes have been collected.

### **5.3.2.3 Post -Blood Collection Procedures**

- Before you remove the needle, apply pressure with your thumb over the venipuncture site with a sterile gauze pad and then withdraw the needle in one smooth movement.
- The butterfly needle should be disposed of in a puncture proof sharps container with a valve mechanism to prevent sharps from falling out.
  - Non-sharp waste should be disposed into a biohazard trash bag.

- Ask the participant or the parent to continue applying firm pressure over the venipuncture site with sterile gauze for 3-5 minutes, and when no blood is oozing from the site, apply an adhesive or gauze bandage over the venipuncture site.
- Label the laboratory forms and the specimens with the correct matching specimen labels
  1. Research specimen: Affix a Hospital ID label on the research specimen vial and on each page of the Research Blood Form. Affix an EMBLEM Subject ID label to the original (top) page only of the Research Blood Form.
  2. Hospital specimens: Affix Hospital ID labels on all hospital specimen vials and on each page of each of the three Lab Requisition Forms. Affix an EMBLEM Subject ID label to the original (top) page only of each of the Lab Requisition Forms.
- Double-check that the forms that will accompany each specimen you have collected are completed in full.
- After verifying the accuracy of all forms, detach the original (top) page of each of the forms and place these pages in the Enrollment Folder.
- Clip a section containing four (4) Hospital ID labels to the EMBLEM laboratory form for blood. Place the research specimen, the Research Blood Form and the attached labels into a research specimen transport bag.
- Clip a section containing four (4) Hospital ID labels to each of the three Lab Requisition forms. Place each hospital specimen along with its Lab Requisition form and attached labels into its own research specimen transport bag.
- Indicate on the Lab-Link form that the blood specimens were collected.
- Transfer the blood specimens into the cold box and deliver the specimens to their respective processing stations.

## 5.4 Saliva Specimen Collection

In rare cases, a participant may be willing to provide the blood specimen but not saliva; they should be enrolled in the study but approached at additional time points to attempt to collect the saliva specimen. Saliva collection may take place earlier or later in the enrollment process if needed to minimize any discomfort from consecutive collections and to increase compliance.

The saliva specimen is not intended to be sterile, so the cups used for saliva collection will be clean but not sterile. A new collection cup should be used for each subject. Collection cups should be discarded after processing and not re-used. As for all other specimen collection procedures, the staff will wear gloves and aprons so that they can handle saliva specimens safely. Saliva specimens should be collected at least 30 minutes after a child has eaten solid food or 10 minutes after liquid food. The child can collect saliva over a period of about 10 minutes until you have about 4 mls, as described below.

Saliva is being collected for research purposes only. Thus, this specimen will be sent to the laboratory for processing and storage. Your main task is to explain to the participant how to collect the specimen, give the participant a collection cup, allow enough time to collect about 4 mls of saliva (up to the level marked on a demonstration cup), and then collect the specimen from the patient. After collection, the specimen cup and lid will be labeled with Hospital ID labels which will be in the Enrollment Folder.

The following supplies must be assembled for the saliva collection procedure:

- 1 - Specimen collection cup with lid
- 1 - Specimen transport bag
- 1 - Research Saliva Form
- 1 - Set EMBLEM Subject ID labels
- 1 - Set Hospital ID labels
- 4 ml - STM
- 1 - Gauze
- 1 - Water bottle, for use as needed
- 1 - Demonstration specimen collection cup pre-marked at the level of the 4 ml target collection
- 1 - Ballpoint pens (black or blue ink)
- 1 - Scissors for creating smaller label subset to send to the lab
- Paperclips to clip the label subset to the Research Saliva Form

#### **5.4.1 Pre – Saliva Collection Procedures**

- Check the participant forms to confirm that they gave consent for saliva to be collected.
- Fill out the top part of the Research Saliva Form.
- Explain the collection procedures to the parent and child using the charts provided. The saliva will be collected using the “pool and spit” method of saliva collection.
- Prepare the saliva collection tray with the all the required supplies.
- Mark the saliva collection cup with a line to demonstrate the target fill volume.

#### **5.4.2 Saliva Collection Procedures**

- Request the parent to hold the child in their lap.
- Remove the lid from the saliva collection cup and hand the cup to the child or the parent, depending upon the child’s age and ability to understand instructions.
- The study nurse should explain the procedure to the parent and the child and provide them with appropriate instructions to carry out the procedure.

- collect saliva using the “pool and spit” method
- Once the required ~4 ml saliva specimen has been collected to the line drawn on the cup during the pre-collection procedures, add an approximately equal volume of STM to the saliva in the collection cup.
- Replace the lid on the collection cup. Wipe down any moisture that may have contaminated the outside of the cup and lid with gauze dipped in antiseptic. After ensuring that the cup lid is secure, invert the several times to mix the saliva with the STM solution.

#### **5.4.3 Post – Saliva Collection Procedures**

- Complete the Research Saliva Form. Affix the Hospital ID labels to all pages of the form and affix an EMBLEM Subject ID label to the original (top) page of the form.
- Affix Hospital ID labels to both the body of the cup and its lid.
- Re-verify all label IDs affixed to the collection cups, lid and the Research Saliva Form.
- Detach the original (top) page of the Research Saliva Form and place it in the Enrollment Folder.
- Clip a subset of four (4) Hospital ID labels to the remaining pages of the Research Saliva Form.
- Indicate on the Lab-Link form that the saliva specimen was collected.
- Place the saliva specimen, the Research Saliva Form and the attached label subset in the transport ziplock biohazard bag and place the specimen in the cold box with pre-frozen gel packs. Transport to the EMBLEM processing lab.

### **5.5 Stool Collection Procedures**

The stool specimen is not intended to be sterile, so the containers used for stool collection will be clean but not sterile. As for all other specimen collection procedures, the staff should wear gloves and aprons when collecting and handling specimens. Containers to collect stool specimens will be given to the participants to collect the specimen when the child defecates. To facilitate stool collection, participants will be given a clean towel, plastic sheet to place below the apron, toilet paper, and a plastic bag to dispose the rest of the stool that is not used. Stool is normally thrown into a pit. If the facility where stool collection is being done has flush toilets, participants should be reminded not to throw the towels and plastic bags used to collect stool into the flush toilet because they will block the flush toilet.

The following materials are assembled for the stool collection kit:

- 1 stool collection container with lid
- 1 specimen transport bag
- 1 set Hospital ID labels for hospital clinical testing (in the Enrollment Folder)
- 1 plastic sheet
- Toilet paper

(1 disposal diaper for very young children)  
(1 sheet Saran (plastic) wrap for very young children)  
1 wooden spatula or spoon  
1 disposable linen towel  
1 large plastic bag for stool disposal  
Hand sanitizer  
Ballpoint pens (black or blue ink)  
Scissors for creating smaller label subsets to send to the lab  
Paperclips to clip the label subset to the Lab Requisition Form

#### **5.5.1 Pre – Stool Collection Procedures**

- Confirm that the consent form has been signed.
- Explain the collection procedures to the parent and child.
- Demonstrate to the patient how the stool specimen should be collected and provide the participant with gloves and toilet paper.
- If you have not completed the top of the stool laboratory request form, do so and confirm that the Hospital ID labels are affixed to all pages of the form. Affix an EMBLEM Subject ID label to the original (top) page of the form. (The Hospital ID labels for the specimen will be affixed to the specimen collection container after the specimen has been collected.)

#### **5.5.2 Stool Collection Procedures**

- Children who are old enough (socially conscious) will require privacy of the toilet or an enclosed place; young children may not require complete privacy.
- Place saran (clear plastic) wrap on the ground to collect the specimen.
- For children who are not toilet trained, a CLEAN disposable diaper can be put on the child inside-out or lined with saran (clear plastic) wrap. Specimens should not be scraped from the absorbent surface of the diaper.
- Using a wooden spatula or spoon, the parent will collect about 1 gm of stool (this is about the amount that fills the spatula). Place this in a clean dry container and securely screw on the lid.
- The participant/parent should wrap the excess stool in the linen and plastic sheet used to help collect the stool, gloves (if used) and any other contaminated materials. Wrap all of these in a plastic bag and dispose in a pit latrine.
- Hand washing and hand sanitizer should be applied at the end of the procedure.

#### **5.5.3 Post - Stool Collection Procedures**



- Check the exterior of the container to make sure it is not contaminated. If contaminated, please clean it with gauze soaked in antiseptic.
- Affix Hospital ID labels to the body of the container and to its lid.
- Re-confirm the labels on the specimen container vs. the Lab Requisition form.
- Detach the original (top) page of the Lab Requisition form and place it in the Enrollment Folder.
- Clip a subset of four (4) Hospital ID labels to the remaining pages of the Lab Requisition form.
- Place the stool specimen, the Lab Requisition form and the attached label subset in the specimen transport bag.
- Indicate on the Lab-Link form that the stool specimen was collected.
- Place the specimen bag in the cold box and transport to the lab.

## **5.6 Tumor Specimen Collection Procedures**

A review of formalin-fixed paraffin embedded tissues in Uganda showed that inconsistent and suboptimal procedures for tissue fixation review reduce the accuracy of tissue diagnosis, thus reducing its value for both clinical and epidemiological applications. Specifically, the study suggested a clinical diagnosis accuracy of about 70%, but it could be as low as 42% and, although outside review pathology can be used to improve diagnostic accuracy, poor tissue quality, labeling errors, and loss of tissue blocks precluded outside review pathology diagnosis for nearly one-third of cases. This section of the manual is intended to improve and standardize local pathology processing of diagnostic tissues. Collection of diagnostic tissue will be done as routine procedures. However, EMBLEM staff is advised to closely interact, influence, and support surgeons, clinicians, and histopathology technicians in order to ensure consistent adherence to recommended procedures. An EMBLEM affiliated pathologist will be on hand to provide technical guidance that is outside the scope of this manual. He or she will provide feedback on the quality of diagnostic H&E slides and suggest improvements. Diagnostic formalin-fixed fixed paraffin-embedded blocks will be obtained for the study for detailed characterization of Burkitt lymphoma. The EMBLEM study will facilitate processes for collecting diagnostic tissue from children suspected to have Burkitt lymphoma, including provision of some supplies and equipment.

### **5.6.1 Pre – Tissue Collection Procedures**

- The techniques of taking biopsies from patients with Burkitt lymphoma are considered minor surgical procedures and are usually completed within a few minutes, depending on the tumor site.

- Always remember to ensure that the patient has a scheduled appointment (time and date) and that the spotter or another staff is at hand to escort the patient to the procedure room and back to the ward.
- Prepare a tissue specimen tissue [a bottle with 10% neutral buffered formalin (see Appendix 6.1 for details of preparation), tru-cut needle, gauze, needles, cotton, swabs, lignocaine, Hospital specimen ID, red sticky label for EMBLEM].

### **5.6.2 Tissue Collection Procedures**

- Specimens intended for formalin fixation should be processed after the completion of other fresh tissue procedures, such as snap freezing, and submersion in RNA stabilizing reagent.
- The clinician should take a tissue specimen (biopsy) of at least 1 cm by 1 cm by 1cm.
- Rinse specimens in saline. Using sterile instruments, remove blood clots and adipose tissue from the tissue specimens.
- The specimen should be placed in the bottle with formalin in the ratio of 10:1 (formalin: tissue size). Plastic bottles of 50-100 ml capacity with tops that can be easily opened should be used.
- NB. The tissue should be put in a bottle whose mouth is wide enough for easy removal after fixation. This is because when tissue fixes it increases in volume and becomes firm.
- Before placing in the specimen, please label the container with the patients name, age and study/ hospital identification number
- In the event that the patient has a large tumor, two biopsy samples should be taken of not less than 1cm by 1cm by 1cm each. Each biopsy should be placed in its own specimen bottle. NB. Very large biopsies may be difficult to take because Burkitt lymphoma bleeds easily.

### **5.6.3 Post - Tissue Collection Procedures**

- The staff accompanying the patient should return with the tissue specimen in the formalin bottle so it is cross checked for properly labeling and sent to the histology laboratory with the appropriately filled in data collection and histology request forms with all the pertinent demographic, clinical history and examination findings details.
- Complete a subject-specimen link form.
- In addition to the Hospital specimen ID labels, a red sticky label should be attached to the bottle to indicate to the technician that the specimen is from a patient suspected to have the Burkitt lymphoma, so they can process two formalin-fixed paraffin-embedded tissue blocks and two sets of H&E and Giemsa slides (one for diagnosis and one for review) from the biopsy material.

- The specimen must be taken to the histopathology laboratory as soon as possible and handed over to the histotechnician. A receipt should be received from the histotechnician and returned to the EMBLEM office to document delivery of the specimen.

## **5.7 Completion of Research Specimen and Hospital Laboratory Requisition Forms**

The following section explains how the laboratory request and laboratory-link forms must be completed and provides specifications of where they must be taken after they have been completed. The request forms are of two types, according to the specimens that will be collected: Research Laboratory forms for blood and saliva and hospital Laboratory Requisition forms. The forms are completed in two steps. The first step is completed by the person collecting the specimen. When this step is completed, the forms can be sent to the laboratory with the associated sample. The second part is completed in the laboratory by the person who receives and processes the specimen in the laboratory. Refer to Appendix 6.6 through 6.8 for additional information.

### **5.7.1 Research Specimen Forms for Blood and Saliva**

#### **Step 1: completed by the phlebotomist or person collecting samples.**

Research Specimen Forms must accompany specimens collected for research. There will be two types of forms: one for blood and the other for saliva. Research specimens will be processed by EMBLEM staff and stored in the freezer until transported to NCI for testing. For both blood specimens and saliva specimens, the Research Specimen form is a 3-part carbon-copy NCR form. The forms should be completed using black ball-point pen. Be sure to write firmly so that the writing is clear on all three copies. If a mistake is made when completing the form, you can cross the erroneous entry with a single horizontal stroke, write the correct information immediately above the erroneous entry, and initial and date your correction. If the mistake is extensive, please use a new form.

The original (top form) is to be retained in the Enrollment Folder of the participant; the first carbon copy and the second carbon copy are both sent to the processing laboratory. The first copy is returned to EMBLEM office after the laboratory has finished processing the research specimen. The second copy is retained in the processing laboratory for their files.

To complete a Research Specimen Form, affix a Hospital ID label to each of the three pages of the form in the lower left area, and an EMBLEM Subject ID label to the original (top) page only in the lower right area of the page. Complete the upper section of form by entering the following information in the appropriate spaces: participant's name; age; gender; date and time the specimen was collected; the ID Number of the staff member who collected the specimen; and if the form was verified in the field, the ID

number of the staff member who verified the information. If there were problems associated with specimen collection, these should be included as a comment on this form and reflected in the comments section of the EMBLEM Tracking Form. If an injury happened during specimen collection, an incident report should be completed and submitted to the Team Safety Officer.

Upon the completion of the upper section of the form, the top (original) page of the form is then removed and placed in the Enrollment Folder of the participant. The specimens are sent to the processing laboratory with the first and second carbon copies.

**Step 2: completed by the laboratory technician.**

When the form and samples are received in the laboratory, they should be checked for completeness, legibility, and that the appropriate copies have been sent. The receiving laboratory technician should verify that each form matches to a sample.

Upon completion of the specimen processing tasks and completion of the remainder of the form, the first copy is returned to the EMBLEM office, where an EMBLEM Subject ID label is affixed to the lower right area of the page. The second copy of this form is retained by the lab for their files.

### **5.7.2 Hospital Laboratory Requisition Forms**

**Step 1: completed by the phlebotomist or person collecting samples.**

Lab Requisition Forms will accompany specimens collected specifically for hospital testing. Hospital specimens will be tested in the local laboratory. They will not be stored in the freezer and will not be sent to NCI. The Lab Requisition Form is a 3-part carbon-copy NCR form. The form should be completed using black ball-point pen. If a mistake is made when completing the form, you can cross the erroneous entry with a single horizontal stroke, write the correct information immediately above the erroneous entry, and write your initial and date by the correction. If the mistake is extensive, please use a new form.

The original (top form) is to be retained in the Enrollment Folder of the participant; the first carbon copy and the second carbon copy are both sent to the testing laboratory. The first copy is returned to EMBLEM office after the laboratory has entered the Laboratory Accession Number as a receipt of the specimen, and the second copy is retained in the testing laboratory for their EMBLEM or appropriate file.

A separate Lab Requisition form should accompany each specimen type, although multiple tests on that specimen may be requested on a single form. Affix a Hospital ID label to all 3 pages of the form in the space provided on the lower left of the form, and the patient's EMBLEM Subject ID label to lower right area of the original (top) copy of the form (only). Perform the initialization of the form by entering the following information in the appropriate spaces: patient's name; age; gender; checkmark in the top section to indicate the type of specimen being sent to the laboratory; the requester for the test (this will be

the Team Leader or the local PI); and ID number for the staff member who collected the specimen. Check the box(es) corresponding to the test(s) being requested.

The top copy of the form is then detached and retained in the participant's Enrollment Folder; the first and second copies are sent with the specimen to the testing laboratory.

### **Step 2: completed by the laboratory technician.**

When the form and samples are received in the laboratory, they should be checked for completeness, legibility, and that the appropriate copies have been sent. The receiving laboratory technician should verify that each form matches to a sample.

At the testing lab, the technician will complete the form by entering the date and time that the specimen was received, and entering the Lab Accession number on the line provided. Any comments about the quality of the specimen should be written in the comment area on the form. The technician then will return the first carbon copy of the form to the EMBLEM office and retain the second copy of the form for laboratory records. When the first carbon copy of the Lab Requisition form is received back at the EMBLEM office, the EMBLEM Subject ID label for that patient is affixed to the lower right area of the form.

### **5.7.3 Lab-Link Form**

The Lab-Link Form is a local form which is used for general monitoring and quality control purposes. This form is completed for each sample obtained from a participant. Prior to the collection of the hospital and research specimens, the Hospital ID label and EMBLEM ID labels are affixed to the form and the date of collection is entered onto the form. As each specimen is collected, the box corresponding to that sample collection is checked. This form is returned to the EMBLEM office at the end of the day. When the research specimen BSI ID labels are returned from the laboratory after specimen processing, one of each is added to this Lab-Link form to complete the record of specimen identifiers.

## **5.8 Transfer of Specimens from site of collection to processing or Testing Laboratories**

1. Prepare samples for delivery by packing them in cold boxes filled with ice packs and ensure the inclusion of lab request form EMBLEM requisition forms for each sample.
2. Be sure to put these forms in a folder and separate the folder from the cold boxes.
3. Fill and complete the transport register and let the driver sign for the samples he/she is transporting.
4. Samples should reach the EMBLEM study lab within 12 hours of collection.
5. If efforts are made to deliver samples immediate to the EMBLEM Lab and it is not possible, refrigerate the specimen between 2 °C to 8 °C at the field mobile lab unit and wait for delivery as soon as possible. A note on file should be made regarding such samples.
6. Deliver the samples to the lab as soon as possible after collection in a cold box and ensure to include a lab request form for each sample. If there is a delay anticipated, communicate to the laboratory in advance. Acceptable specimens should be well labeled and without leaks.

## **Chapter 6: EMBLEM Specimen Processing, Storage and Transport Procedures**

### **6.1 Hospital Lab Results forms**

The hospital Lab Results forms are described in Attachment 1 of this manual. These are forms used to report clinical results for specimens tested in the hospital laboratory. Because many hospitals in Africa do not routinely use standardized reporting forms, EMBLEM is introducing standardized test result reporting forms to improve consistency of data collection across different reporting technicians or different reporting periods. The EMBLEM study would like to capture as much data as is usually produced on any given test at any time, so the forms are complete for the type of results that may be produced for a given test. EMBLEM has provided result forms to cover CBC/Differential/ESR, the liver function panel, the renal function panel, stool microscopy, malaria thin smear, malaria thick smear, malaria RDT, and HIV serology tests. It is to be expected that some technologies used to perform any given test will generate fewer results than others, so it may not always be possible to complete the entire form for all tests listed on the form. The form, however, should be completed, indicating the results for those assays that are available and indicating that results are not available for others because the technology used does not generate those results. These forms will be placed in the testing laboratories so they are available for use to report results for EMBLEM purposes, and may be used for the hospital purposes, as desired by the hospital.

The hospital Lab Result Forms for EMBLEM participants are 2-part carbon-copy NCR forms. The hospital specimens will be accompanied by a Lab Requisition form as described above, which will arrive at the lab with Hospital ID labels to be used for results reporting and other tracking purposes. Upon completion of the requested test(s), the laboratory technician will select the appropriate Hospital Test Results form(s) to report their results. The technician will affix a Hospital ID label to the lower left of each of each page of the hospital Lab Results form and enter the patient name/age/gender information at the top of hospital Lab Results form. Upon completion of the form, the laboratory will send the original (top) copy of the hospital Lab Results form to the EMBLEM Study Office and the bottom copy to the patient's ward (or HC-II unit office for control participants). Upon receipt of the hospital Lab Results form with the patient's results at the EMBLEM office, EMBLEM study staff will affix the EMBLEM Subject ID label to the original (top) copy in the lower right area of the form and place the form in the Enrollment Folder. (If both parts of the form are returned to EMBLEM, EMBLEM staff will label only the original (top) copy and make sure that the bottom copy is immediately given to the patient's clinician for interpretation and action.)

### **6.2 Processing, Aliquoting, and Storage Procedures for Research Specimens**

This section provides detailed information about the specific processing procedures being requested by EMBLEM. Detailed descriptions of laboratory practice are outside the scope of this manual. EMBLEM, however, will be pleased to facilitate training and improvement of standards of laboratory practice in the processing

laboratory. All specimens are expected to be processed on the same day they were collected, ideally within 1 hour for excellent results, but allowable up to a maximum of within 3 hours of collection.

In general, all biological specimens should be considered as infectious and should be handled while wearing appropriate protective wear. Research specimens will be delivered to the laboratory in cold boxes. The staff delivering them will remove the specimens in their racks from the cold boxes and place them in receiving racks in the laboratory. The staff will also leave the appropriate request forms for each specimen. The staff delivering the specimens will confirm that the number of specimens delivered match the number of forms left behind. The staff will write his/her name, the number of specimens, and forms in the EMBLEM Specimen Delivery book that will always be available at the laboratory.

The receiving technician will confirm that specimens were delivered safely and are intact, and will confirm the number of specimens and forms. The blood specimen for research and the saliva specimen for research will each be accompanied by the remaining first and second copies of the Research Blood and Research Saliva Forms, respectively (the original top copy will have been retained by the EMBLEM office prior to specimen transport to the processing lab). All receipts will be entered in the laboratory accession books before they are processed. The laboratory technician will be responsible for affixing the bar-coded master BSI ID labels to each of the pages of the Research Blood and Saliva Forms and to the Specimen Tracking Log in the places provided as described in the steps below. This key step will provide the link from the research subject to the labels that will go onto the stored research materials. Bar-coded master BSI ID vial labels will also be affixed to the 10 ml whole blood collection tube and to the saliva specimen cup and lid upon receipt at the processing lab, and the final BSI ID vial labels will be affixed, in accordance with their sequence numbering scheme, to the specimen aliquots upon their creation.

Prior to performing any processing of research specimens, the next available BSI label set must be selected and the designated master labels for forms and collection containers affixed to their designated places as described below. The master BSI labels should be returned to the EMBLEM office to affix to Lab-Link and other tracking forms. When all labeling tasks are completed, remaining labels should be discarded.

The saliva specimens will be simply aliquoted and stored; the blood specimens will be spun and aliquoted into plasma, peripheral blood mononuclear cells (PBMCs), and red cells. All types of specimen materials will be stored in freezers kept at  $-75^{\circ}\text{C}$  ( $\pm 3^{\circ}\text{C}$ ) until transfer to the Ugandan Viral Research Institute (UVRI) in Entebbe. These procedures are described in greater details below and are represented in Appendix 6.6:

### **6.2.1 Processing the 10 ml ACD Whole blood Tube for Research Aliquots**

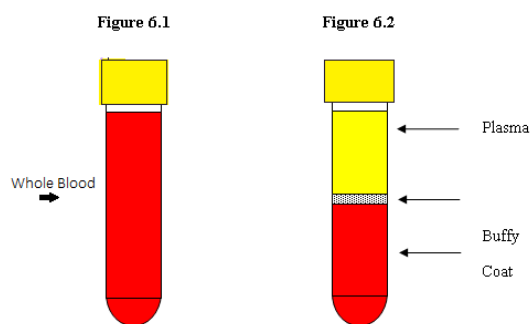
The 10 ml whole blood ACD tube should be processed for plasma, PBMCs and RBCs as soon as possible, preferably within 1-3 hours after collection to maintain the viability of live cells, specimen integrity and stability.



### 6.2.1.1 Centrifuge the 10 ml ACD with research blood sample.

Affix a master BSI ID label to the ACD tube prior to processing.

- Centrifuge the sample in the 10ml ACD tube for 10 minutes at 2000 rpm at acceleration of 9 and deceleration of 9.
- The whole blood (Figure 6.1) will separate into three distinct layers (see Figure 6.2); 1) an upper layer (plasma), 2) a lower layer (red blood cells [RBC]), and 3) a thin interface layer between the plasma and red cells that contains the Peripheral blood mononuclear cells; PBMCs (leukocytes and platelets).



**WARNING:** Excessive centrifuge speed (over 1300g) may cause tube breakage and exposure to blood and possible injury. Use the following formula to calculate your centrifuge speed for a given g force:  $x \text{ g} / \text{RCF} = 0.00001118 \times \text{radius of rotation (cm)} \times \text{rpm}^2$

### 6.2.1.2 Label the Cryovials for Receipt of the Blood Component Aliquots

- The cryovials may be prepared for the various blood component aliquots while the blood in the 10 ml ACD tube is being centrifuged.
- A total of 10 (ten) 2 ml cryovials should be labeled in preparation for the aliquot process.
- Affix the bar-coded specimen cryolabels from the selected BSI ID label stock for the PLASMA, PBMCs and RBC components to the cryovials. Cryolabels should be affixed on the non-conical surface of the vials in a horizontal orientation (that is, around the circumference of the vials). The

clear label tail should overlap the printed white portion of the label stock to ensure good adherence once frozen.

- Place the prepared, labeled cryovials in a rack pending the aliquot process. Order the vials so that the plasma vials are together and in numeric order, then the PBMC vials, and then the RBC vials.

#### **6.2.1.3 Withdraw and Aliquot Plasma Specimens**

- Before aliquoting any plasma, verify that the base BSI Sample ID number from the master BSI ID label on the ACD tube and the aliquot vials are the same (the aliquot labels also contain a sequence number suffix).
- To obtain undiluted plasma, carefully un cap the 10ml ACD tube without disrupting the various blood components by grasping the tube firmly with one hand and un-winding the cap anticlock wise. (For added stability, place arm on solid surface).
- Use a sterile pipette (Since we are dealing with live cells try as much as possible to ensure sterility of your procedure) to slowly aspirate the plasma from the 10 ml tube (Figure 6.3) according to the following aliquoting scheme. Aliquot down to approximately 0.5 centimeters above the buffy coat interface (see Figure 6.3). Be careful **NOT** to touch, mix or disturb the buffy coat (white pinkish) interface or packed red blood cells.
  1. First aliquot (sequence # 0001, Box A): 0.5 ml
  2. Second aliquot (sequence # 0002, Box B): 0.5 ml
  3. Third aliquot (sequence # 0003, Box A): 1.5 ml
  4. Fourth aliquot (sequence # 0004, Box B): residual specimen
- Cap and tighten the cryovials and hold at + 4C until placed in freezer storage at -75<sup>0</sup>C. Optimally these vials will be held for less than 24 hours at +4C before transfer to freezer storage.
- Resuspend blood to replace the plasma removed in the above steps with Phosphate buffered Saline(PBS);For example,if you removed 2 ml of plasma, then add back 2ml of PBS.
- To the blood&PBS mixture in the 10ml conical tube, add an equal volume of 1X PBS to dilute the blood and mix thoroughly in a 50ml conical tube.
- Carefully layer your blood &PBS mixture on to Ficoll in a separate 50 ml conical tube.Volume of Ficoll depends on how much blood one has.For 10-15ml of blood, use 5ml of Ficoll.
- Cap the tube and centrifuge at 2000rpm for 20 minutes at room temperature with acceleration at 9 and deceleration at 1.

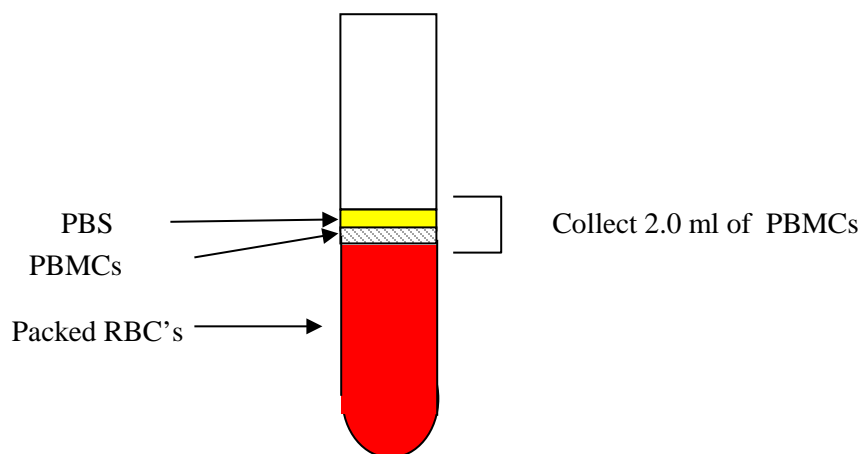
#### **6.2.1.4 Withdraw and Aliquot PBMCs Specimens**

- The PBMCs layer is sometimes difficult to see and collect.

- It can be viscous and appear as a thin milky white or pinkish colored layer just above the packed red cell layer (Figure 6.3). Some contamination of the PBMCs with the Ficoll layer is to be expected if not carefully aspirated.
- Specimens that have an extremely high WBC count will have a thicker and more defined buffy coat layer that will be easier to collect with minimal RBC contamination.
- Before collecting or dispensing PBMCs, verify that the base BSI Sample ID number from the master BSI ID label on the ACD tube and the cryovials are the same (the aliquot labels also contain a sequence number suffix).
- To collect PBMCs (the thin layer between PBS & Ficoll), first, slowly & carefully aspirate off using a sterile plastic Pasteur pipette the PBS and then pipette off the PBMCs, using a slow circular motion. Transfer to a 15ml conical centrifuge tube.
- Add 1X PBS to wash cells and centrifuge at 1200-1500 rpm for 5 minutes.
- After 5 minutes, volume up to wash the cells in 1X PBS. Centrifuge again for 5 minutes.
- Add 2mls of 1X PBS, Aliquot the cell suspension into four cryotubes (0.5ml each). Keep two cryotubes of sample on ice for NKG2D and intracellular  $Mg^{2+}$  Basal staining respectively.
- Aliquot 0.5 ml of PBMCs volume into four (4) labeled 2.0 ml cryovials as follows.
  1. First aliquot (sequence # 0011, Box A): 0.5 ml for storage in Liquid Nitrogen
  2. Second aliquot (sequence # 0012, Box B): 0.5 ml for storage in Liquid Nitrogen
  3. Third aliquot (sequence # 0013, Box A): 0.5ml for NKG2D staining
  4. Fourth aliquot (sequence # 0014, Box B): 0.5 ml for  $Mg^{2+}$  Basal Staining

Cap and tighten the cryovials and keep the first two aliquots in the Mr Frosty with 10% DMSO in freezer storage at  $-75^{\circ}\text{C}$ . Transfer the samples to liquid nitrogen.

**Figure 6.3**



**6.2.1.5 Withdraw and Aliquot Red Blood Cells (RBCs) [THIS STEP IS IN STEP 4 of PAUL's CARTOON. THIS MEANS THAT YOU HARVEST RBCs AFTER PBMCs and YOU WILL BE DOING THIS FROM a 50 mL CONICAL TUBE. RE-WRITE].**

Having taken off the PBMCs from the 50ml conical the remaining part is Ficoll and Red blood Cells.

- Aspirate off the Ficoll in the 50ml conical tube, leaving you with red cells.
- Wash the red cells in 1X PBS once and aliquot 2.0 ml of RBCs into two (2) labeled 2.0 ml cryovials as follows.
  1. First aliquot (sequence # 0005, Box A): 2.0 ml
  2. Second aliquot (sequence # 0006, Box B): 2.0 ml
- Record the volumes of aliquots and dates of processing on the respective study forms.

**6.2.2 Aliquot Saliva Specimen**

- Affix master BSI ID labels to both the saliva cup and the saliva cup lid.
- A total of 4 (four) 2.0 cryovials should have been prepared for the aliquot process by affixing the specimen bar-coded BSI ID cryolabels to the vials. Cryolabels should be affixed horizontally, around the vial and the clear label tail should overlay onto the printed portion of the label.
- Prior to aliquoting, ensure that the master BSI ID labels on the saliva cup/lid and on the cryovials match.
- Aliquot the saliva into the four (4) labeled 2.0 ml cryovials as follows.
  1. First aliquot (sequence # 0007, Box A): 2.0 ml
  2. Second aliquot (sequence # 0008, Box B): 2.0 ml
  3. Third aliquot (sequence # 0009, Box A): 2.0 ml
  4. Fourth aliquot (sequence # 0010, Box B): 2.0 ml
- Cap and tighten the cryovials and hold at + 4C until placed in freezer storage at -75°C. Optimally these vials will be held for less than 24 hours at +4C before transfer to freezer storage.

**6.2.3 Tissue specimen processing, review and storage**

All tissue specimens should be processed according to a study approved processing schedule within minutes after collection to preserve tissue for morphology, microarray and gene expression analysis and genomic biomarkers. For some of the patients, tissue will arrive as Research Tissue Specimen and Clinical Tissue Specimen. The Research Tissue Specimen should be processed according to the SOPs provided by EMBLEM. Clinical Tissue Specimens should be processed according to procedures used by the hospital, except when the hospital chooses to use EMBLEM SOPs. When tissue arrives as one block, it will be divided into two blocks and one used for research and the other used for clinical diagnosis.

Divide tumor and normal tissue specimens into at least two pieces, but the number can be higher if the pieces are large or multiple procedures for fixation and processing are being used, e.g., normal fixation and ultrasound-assisted fixation preservation methods and block amounts according to approved study procedures. Specimen sections destined for formalin fixation and paraffin embedding should be processed after specimens to be cryopreserved.

- The technician should allow the specimen to fix for at least 4 hours, or longer for larger specimens.
- Surgical blades, cassettes, baskets and sponge mops (to avoid cross contamination) should be prepared. The tissue dissection area should be located near a tap with running water. The specimen bottle labeling and demographic details should be checked to ensure that they match with those on the request form. The tissue should then be sectioned to a maximum of 3mms thickness. Two sections should be placed in two cassettes as required. At least one cassette will be used to store specimens for hospital records, and at least one cassette will be used to store specimens for research use. Consideration should be given to methods to distinguish hospital vs. research cassettes, such as the use of colored cassettes for research specimens or the use of colored permanent markers to flag research specimen cassettes. Sectioning should continue until there are a reasonable number of filled cassettes that do not crowd the basket for tissue processing (see Appendix 6.2).
- Information on sectioning may be found in Appendix 6.3.
- Information on H&E and Giemsa staining may be found in Appendix 6.4 and Appendix 6.5, respectively.

Slides for hospital diagnostic purposes will be read according to usual practice, which may include on-site readings by trained histopathologists (e.g., at Lacor Hospital) or transfer of these slides to a central laboratory if local facilities are not available. Slides and/or image files will also be transferred periodically for central research review to the EMBLEM core histopathology facilities.

EMBLEM cassettes will be stored at the EMBLEM processing laboratory in sealed appropriate cassette racks (to prevent damage by vermin) in a secure air-conditioned room to prevent the paraffin from melting. The hospital cassettes will be stored by the hospital according to their usual arrangement in separate cassette racks.

#### **6.2.4 Bar-coded BSI specimen labeling system [Paul needs to review and confirm that this will work before I give this to IMS]**

The EMBLEM research specimens will be labeled with bar-coded cryolabels which will be

preprinted and provided by the NCI. Two sets of labels will be provided; one set for the research blood specimen/saliva specimen, and one set for the tissue specimens. The coding system within the provided labels is called the BSI labeling system (BSI ID) and it is crucial to the tracking of the specimens from the time of initial processing through to final storage and use at the NCI. To ensure the success of EMBLEM, the following information should be thoroughly understood and the procedures must be followed precisely.

It is important to understand the basic principles of the BSI ID system. The structure of the BSI specimen coding system is designed to allow the NCI Biorepository the ability to not only track specimens as they are originally received but also to allow tracking of any aliquots or additional processing once specimens have been inventoried there. The BSI ID has the following general structure:

AAA 123456 0000

In this example, the AAA 123456 portion of the label provides the Sample ID of the specimen draw that is unique to the patient and the specimen collection itself, while the 0000 portion of the example is called the sequence number and provides additional information on the specific vial that is created from that specimen collection.

For the EMBLEM study, the pre-printed bar-coded cryolabels will identify the basic specimen type (BLS for the blood and saliva specimens and BLT for tissue specimens), provide the unique Sample ID for the specimen draw, and provide a special sequence number that will identify the specimen (plasma, buffy coat, RBC, saliva or tissue) and the aliquot type (first, second, etc.).

As mentioned above, two sets of BSI IDs will be used for EMBLEM. These BSI IDs have been set aside exclusively for EMBLEM study use. These two sets of BSI IDs will be provided on separate label rolls and so may be used independently by different specimen processors at different times. The label roll for the research blood/saliva specimens can be used for both cases and controls; the label roll for tissue only applies to cases because tissue is not collected from controls. Impeccable record-keeping must be maintained by EMBLEM staff to ensure that the BSI IDs assigned to the subject's various specimens are linked to that subject's EMBLEM Subject ID, and so the forms have been designed to accommodate all sets of ID numbers. This linkage will be used to identify the specimens in the future.

The label sets will be printed on rolls in a 10-up format. The label sets will be perforated so that they can be separated from the roll and used as needed for specimens from the subject to which they have been assigned. Never use leftover labels for any other purpose or for specimens from other individuals. When all laboratory labeling tasks have been completed for an individual subject, remaining BSI labels should be transferred to the EMBLEM office where they will be discarded after forms labeling tasks have been completed.

The label set for the blood/saliva specimens is illustrated in Table 6.1.

Table 6.1 Example of BSI Label Set for Research Blood/Saliva Specimens

These are master labels for specimen processing, forms and emergency use		These are final aliquot labels
START GROUP	☞ BLS000001	☞ BLS000001 0003 PLASMA #3 BOX A
☞ BLS000001	☞ BLS000001	☞ BLS000001 0004 PLASMA #4 BOX B
☞ BLS000001	☞ BLS000001	☞ BLS000001 0005 BUFFCOAT#1 BOX A
☞ BLS000001	☞ BLS000001	☞ BLS000001 0006 BUFFCOAT#2 BOX B
☞ BLS000001	☞ BLS000001	☞ BLS000001 0007 RBC #1 BOX A
☞ BLS000001	☞ BLS000001	☞ BLS000001 0008 RBC #2 BOX B
☞ BLS000001	☞ BLS000001	☞ BLS000001 0009 SALIVA #1 BOX A
☞ BLS000001	☞ BLS000001	☞ BLS000001 0010 SALIVA #2 BOX B
☞ BLS000001	☞ BLS000001 0001 PLASMA #1 BOX A	☞ BLS000001 0011 SALIVA #3 BOX A
☞ BLS000001	☞ BLS000001 0002 PLASMA #2 BOX B	☞ BLS000001 0012 SALIVA #4 BOX B

### 6.3 Research Specimen Storage and Tracking [Paul needs to confirm that this will work as before]

- After specimen processing and aliquoting has been completed, transfer all vial aliquots to a secure storage location at the appropriate temperature that will best maintain specimen integrity and stability.
- Frozen tissue and blood specimens should be maintained in a -75°C mechanical freezer for storage unless otherwise noted.
- Paraffin embedded tissue should be maintained in a secure air conditioned room to prevent the paraffin from melting or softening. Paraffin blocks should be maintained in cabinets or containers that will prevent entry by rodents or other vermin.
- The labeling scheme for the plasma, buffy coat, RBC and saliva aliquots includes the notation of Box A or Box B. These aliquots are split into two groups to provide an efficient method for shipping complete sets of collected vials for each subject to the NCI repository while maintaining a backup set of specimens at the hospital. The freezer boxes are to be set up so that there is a Box A and a Box B for each specimen type (plasma, buffy coat, RBC, saliva). Each box should be numbered sequentially (e.g., Plasma Box 1A and Plasma Box 1B). Within the storage boxes, the aliquots for any particular subject should be in the same row/column locations across box types;

for example, if the subject's plasma samples Type A are in Row 1, Columns 1-4 in Plasma Box 27A, then his plasma samples Type B should similarly be located in Row 1, Columns 1-4 in Plasma Box 27B. If it should happen that a subject is missing one his vials, that space should be left empty in the box. Refer to Appendix 5-9 for more information on cryobox layout.

- Store each group of specimens (A and B) in a separate storage locations or separately designated areas within the same freezer. This will provide an efficient method for shipping a complete set of collected vials for each subject to the NCI Repository while maintaining a backup set of specimens at the hospital.
- Specimen tracking log information will be entered into the Specimen Tracking Log when aliquots are completed and transfer to the final local storage is in process.
- Manually record freezer storage temperatures twice daily to document and validate specimen storage.

### **6.3.1 Specimen Tracking Log**

The Specimen Tracking Log is the key tracking log for the research specimens. It provides the link between the Hospital, EMBLEM Subject and specimen BSI IDs, documents that the expected number of aliquots of each specimen type were created, and documents the box/row/column locations of the aliquots in the freezer.

The laboratory technician is responsible for affixing master BSI ID labels and Hospital ID labels to each copy of this 2-part NCR form. Once the form has been completed, the original copy should be returned to the EMBLEM office where the Subject ID will be applied. The second copy may be retained in the laboratory

Form SLF2 - EMBLEM Research blood and saliva specimen tracking log.



Staff Initials / Date

Infectious Diseases Institute (IDI)															Emblem Protocol (SLF1)																			
Emblem Protocol (015) STUDY ID															SLF1 (450) Patient Initials										Baseline									
<div style="display: flex; align-items: center;"> <div style="border: 1px solid black; padding: 2px 5px; margin-right: 5px;">EMB</div> <div style="border: 1px solid black; padding: 2px 5px; margin-right: 5px;">1</div> <div style="border: 1px solid black; padding: 2px 5px; margin-right: 5px;"> </div> <div style="border: 1px solid black; padding: 2px 5px; margin-right: 5px;"> </div> <div style="border: 1px solid black; padding: 2px 5px; margin-right: 5px;"> </div> <div style="border: 1px solid black; padding: 2px 5px; margin-right: 5px;"> </div> <div style="border: 1px solid black; padding: 2px 5px; margin-right: 5px;"> </div> </div> <div style="display: flex; justify-content: space-between; margin-top: 5px;"> <span>Site #</span> <span>Subject #</span> </div>															<div style="display: flex; align-items: center;"> <div style="border: 1px solid black; padding: 2px 5px; margin-right: 5px;"> </div> <div style="border: 1px solid black; padding: 2px 5px; margin-right: 5px;"> </div> <div style="border: 1px solid black; padding: 2px 5px; margin-right: 5px;"> </div> </div> <div style="display: flex; justify-content: space-between; margin-top: 5px;"> <span>Last</span> <span>First</span> <span>Middle</span> </div>										<div style="display: flex; align-items: center;"> <div style="border: 1px solid black; padding: 2px 5px; margin-right: 5px;"> </div> <div style="border: 1px solid black; padding: 2px 5px; margin-right: 5px;"> </div> </div> <div style="display: flex; justify-content: space-between; margin-top: 5px;"> <span>dd</span> <span>mm</span> </div>					<div style="display: flex; align-items: center;"> <div style="border: 1px solid black; padding: 2px 5px; margin-right: 5px;">2</div> <div style="border: 1px solid black; padding: 2px 5px; margin-right: 5px;">0</div> <div style="border: 1px solid black; padding: 2px 5px; margin-right: 5px;">1</div> <div style="border: 1px solid black; padding: 2px 5px; margin-right: 5px;"> </div> </div> <div style="display: flex; justify-content: space-between; margin-top: 5px;"> <span>yyyy</span> </div>				
Hospital ID															<input type="checkbox"/> Or Note Done																			

**FORM SLF1 - EMBLEM Lab-Link Form**

BSI-ID for blood and saliva specimens BLS-

BSI ID for tissue specimens BLT-

	Specimen	Collected	Not Collected	Date Collected	Comment
1.	Research blood (ACD)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
2.	Hospital blood (2 ml ACD)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
3.	Hospital blood (3 ml ACD)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
4.	Hospital blood (2.5 ml SST)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
5.	Research saliva	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
6.	Stool specimen	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
7.	Tissue specimen	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	

Place Label here

Staff Initials / Date

## VerFebruary2017

**Purpose:** These instructions are a guide for EMBLEM sites on how best to ship specimens in-country before shipment to the repository in USA.

**Supplies and requirements:**

- Cryo-boxes for shipping & storage (10x10 grid configuration; acquired locally)
- Shipping boxes and packing material for shipping Biological specimens.
- IATA certification
- Shipping Companies: It is the responsibility of the site to establish a contract and shipping arrangements with a reliable local shipping company that can handle frequent shipments of Biological substances from the study site to an in country shipment dispatch centre.

**Shipping Archived Aliquots/Samples to the country dispatch center (UVRI for Ugandan sites)**

1. All samples will be sent directly from the in country dispatch centres to the study central specimen repository. All archived aliquots stored at the local lab will be captured on the Specimen Tracking log and database. Aliquots will be stored in 2ml cryovials stored in freezer boxes (10x10 boxes).
2. Shipments will be scheduled by the NCI in conjunction with the in country central dispatch centre. Only completely filled or close to completely filled freezer boxes should be shipped to avoid shipping partially empty boxes.
3. When the shipment is ready, the local lab will contact the respective local shipping company one week in advance to allow time buy dry ice. In addition the lab will give appropriate information concerning the type and contents of the consignment to the courier as required by the protocol in order to facilitate their proper preparation for the shipment. All specimens are to be shipped as diagnostic specimens.
4. The lab contact person will call the courier two days earlier to confirm shipment.
5. On the day of shipment the local lab must then generate a shipping manifest for all samples to be shipped. An electronic copy of this manifest will be generated by the Specimen Tracking module in the EMBLEM database system. This manifest must be generated electronically and then a hard copy printed to be used for shipping. The electronic copy must be saved with a date name and waybill number (this electronic copy is to be email to both the Central repository at Fisher Bioservices in Frederick, Maryland, USA, and the local

in country dispatch centre). The email contacts for Fisher Bioservices are

[Christine.Demasco-Cody@Thermofisher.com](mailto:Christine.Demasco-Cody@Thermofisher.com) and [Judith-Franke@Thermofisher.com](mailto:Judith-Franke@Thermofisher.com)

6. Each archive 2ml vial sample (plasma, saliva, RBC, and buffy coat) must be packed according to IATA requirements packaging instruction 650. This involves placing a box of vials into a plastic bag into a SAF-T-PAK biohazard bag #STP711. This bag must contain an absorbent material pad, which will absorb any leakage that could occur from the enclosed tubes.
7. The biohazard bag is then placed into a SAF-T-PAK biohazard envelope Part # STP710.
8. The biohazard envelope containing samples is then placed into the courier supplied dry ice shipping box (World Courier Large Thermal Box)). Ensure the SAF-T-PAK biohazard bag is well surrounded by dry ice.
9. The shipping manifest is placed on top polystyrene lid inside the box, which is then closed and sealed simply by folding over the lids in the order of sequence as stipulated on the lids.
10. The completed waybill and proforma invoice is handed to the courier together with the shipment.
11. The in country dispatch centre must then be emailed the shipping manifests together with the way bill number (a copy of this email must be sent to Fisher Bioservices, Frederick, Maryland, USA to notify them of the shipment). This email must be sent on the date of shipment.
12. Shipping address and contact details in the USA:

Business Hours: Monday – Friday 8:00 am to 5:00 PM EST/EDT

Delivery Contact/Address Information

Business Contact: Judith Franke

Shipping Contact: Christine Demasco-Cody

Shipping Address: Fisher BioServices, Inc., 4600 Wedgewood, Blvd., Suite H, Frederick, MD 21703 USA

Phone: 301-694-5911

Fax: 301-694-2659

Schedule: Shipments of biological materials sent to and shipped from the NCIF-CRS are sent Monday – Wednesday only to ensure delivery by Friday (local deliveries excepted).

## **6.5 Standard Operating Procedures (SOP)**

There are several different types of Standard Operating Procedures (SOP). SOPs are site-specific documents that outline exactly how certain procedures will be carried out at the site. For example,

EMBLEM sites would use the Informed Consent SOP to outline which individuals are responsible for obtaining informed consent, how informed consent forms will be stored at the site, etc. Some SOPs may be used for multiple activities being conducted at the hospitals (for example, the Staff Training SOP). Others are specific to EMBLEM, such as the SOP covering blood separation procedures.

The process for developing and receiving approval of site SOPs is as follows: the study departments will develop respective SOPs that will be approved by the site Principal Investigators. After creating their own SOPs, the sites will submit them to the NCI within the specified timeline. The NCI will review each SOP and notify the site as soon as it is approved. If necessary, the initial SOP may go back to the site for changes before approval.

## **APPENDIX 6.1 - Procedure for preparation of 10% Neutral Buffered Formalin (NBF)**

**Purpose:** This is a good routine fixative with PH 6.8. Upon obtaining a tissue, prompt fixation is recommended in a volume not less than 20:1; hence the need for appropriate specimen containers. Avoid letting tissues to dry prior to fixation.

### **Reagents**

**NOTE: 10% NBF can be purchased ready for use.**

Sodium phosphate, monobasic	4.0gm
Sodium phosphate, dibasic	6.5gm
Formaldehyde 37%	100.0 ml
Distilled water	900.0 ml

Mix well, PH and label.

**Fixation time:** 1-4 hours minimum, longer for larger specimens

**Safety:** Adhere to universal precautions and handle according to good laboratory practice (GLPs)

## **APPENDIX 6.2 - Tissue Processing**

For new patients, two tissues are likely to be received - one for Research and the other for Clinical diagnosis. The tissues should be processed as described below, but filed separately, using color-coded cassettes as described in Section 5.2.3.

This entails dehydration, clearing, and wax impregnation, facilitated by the use of an automatic tissue processor, with the first schedule dedicated (optional) to further fixation; this ensures adequate fixation. Tissues of adequate size; determined by the Tissue Tek cassettes during cut up, are processed by use of the overnight schedule as shown below.

<b>Container</b>	<b>Fluid</b>	<b>Time (hours)</b>
1	10% formalin	0 (optional)
2	70% alcohol	½
3	95% alcohol	½
4	100% alcohol	½
5	100% alcohol	1
6	100% alcohol	1
7	100% alcohol	1
8	100% alcohol/Xylene	½
9	Xylene	1
10	Xylene	2
11	Wax	2 ½
12	Wax	4

**NOTE:** This schedule may be adjusted based on local needs; these could be manual or rapid techniques.

**QA/QC of reagents:** used reagents are regularly (Mondays or Fridays) replaced with fresh/new ones to assure adequate tissue processing.

**Embedding:** Tissues are embedded to enable sectioning of the largest area. This is done by use of embedding molds and molten paraffin wax; thus ensuring appropriate orientation of the tissue.



### **APPENDIX 6.3 - Paraffin section cutting**

A microtome and microtome knives (blades) are manipulated to produce flawless sections of 2-4  $\mu\text{m}$ .

Other items required include:

Water bath

Drying oven or hot plate

Fine pointed forceps

Camel hair brush

Scalpel

Slide rack

Clean slides

Ice tray

Pencil

The temperature of the water bath should be approximately 10° C below the melting point of the paraffin wax used.

Upon obtaining a well fixed and an adequately processed and appropriately embedded tissue block, a sharp microtome knife is set at a correct clearance angle.

## **APPENDIX 6.4 - Haematoxylin & Eosin staining technique**

Slides should be prepared for both the Research and Clinical tissue specimens. The research slides should be placed in a research slide rack, which will be available in the histology laboratory, while the clinical slides will be placed in the clinical slide rack. This technique essentially stains general tissue structures; the haematoxylin stains cell nuclei blue/black, with good intra-nuclear detail, while eosin stains cell cytoplasm and connective tissue fibers varying shades and intensities of pink, orange or red.

**Solutions:**      Haematoxylin  
                         0.5% acid alcohol  
                         Scott's tap water  
                         1% Eosin

### **Procedure:**

- |  |                      |
|--|----------------------|
| 1- De-paraffinize sections in two changes of xylene, | 5 mins each          |
| 2- Take sections to water                            |                      |
| 3a- Stain in Haematoxylin                            | 5 mins (progressive) |

### **Or**

- |  |                      |
|--|----------------------|
| 3b- Stain in haematoxylin  | 15 mins (regressive) |
| 4- Wash well in tap water  | 1 min                |
| 5- Agitate gently in 0.5% acid alcohol   | 5 secs               |
| 6- Blue in Scott's tap water   | 30 sec               |
| 7- Wash well in water  |                      |
| 8- Stain in eosin  | 1 min                |
| 9- Wash well in water  |                      |
| 10- Dehydrate and clear in 2 changes of each of 95% alcohol, absolute alcohol and xylene |                      |
| 11- Mount  |                      |

### **Expected results:**

Nuclei: blue

Cytoplasm: varying shades of pink

Red blood cells: pink

## **APPENDIX 6.5 - Giemsa Staining Technique**

The Giemsa stain is a combination of azure dyes (methylene blue and eosin) that gives a wide colour range; it differentiates cells present in haemopoietic tissues.

### **Reagents:**

Giemsa

Distilled water

Acetic acid (0.25%)

### **Procedure:**

- 1- Dewax sections in two changes of xylene, 5 minutes each and hydrate to distilled water.
- 2- Stain with Giemsa, duration varies with concentration; 20% stain for 1 hour, 50% stain for 10 mins. The concentration/duration should be adopted/optimized by the user laboratory.
- 3- Wash in distilled water.
- 4- Partly differentiate in glacial acetic acid; 3 to 4 drops in 100ml of distilled water.
- 5- Differentiate further in 96% ethyl alcohol, with microscopy control; to obtain pink/red blood cells (RBCs).
- 6- Dehydrate and clear in iso-propyl alcohol.
- 7- Mount coverglass.

## **Appendix 6.10 THE STANDARD OPERATING PROCEDURE ON CRYOPRESERVATION OF CELLS.**

### **Purpose.**

To ensure reproducible results and continuity in research and biomedical processes, today's scientists are faced with the task of genetically stabilising replicable materials such as nucleic acids and proteins are preserved unchanged. Serial sub-culturing of replicable materials is time consuming and can often result in contamination or genetic drift as smaller and smaller portions of a population are selected. The purpose of this SOP is to provide an insight on how to store mammalian cells at temperatures below  $-130^{\circ}\text{C}$ .

### **Principle.**

A population of living cells or suspension of subcellular components can be stabilized by subjecting them to cryogenic temperatures which, for all practical purposes, stops time. The process of stabilizing biological material- blood cells for our case, at cryogenic temperatures is called cryopreservation. Ice forms at different rates during the cooling process. Slow cooling leads to freezing external to the cell before intracellular ice begins to form. As ice forms external to the cell, water is removed from the extracellular environment and an osmotic imbalance occurs across the cell membrane leading to water migration out of the cell.

The rate of cooling has a dramatic effect on these phenomena. Rapid cooling minimises the solute concentration effects as ice forms uniformly, but leads to formation of more intracellular ice since water has not migrated out of the cell. Slow cooling on the other hand, results in greater loss of water from the cell and less internal ice being formed, but results in an increase in solution effects.

### **Scope.**

The viability of cells after cryopreservation depends on their ability to cope with the variety of stresses imposed on them during freezing and thawing procedures. This SOP details methods and precautions to take in Cryopreservation of mammalian cells for future studies.

### **Requirements**

Isolated cells in Thermoscientific Nunc tube, DMSO, Mr Frosty, Isopropanol,  $-80^{\circ}\text{C}$  Freezer. G

### **Procedure.**

1. Harvest the cells by spinning the cells as gently as possible.
2. Re-suspend the cells in growth medium at room temperature to a concentration of  $1 \times 10^6$  –  $2 \times 10^6$  cells per ml.
3. Count the viable cells.
4. To avoid damage to the cell during freezing, a cryoprotectant is added to the growth medium in which the cells are to be frozen. Glycerol or DMSO (Dimethyl Sulphoxide) in 10% concentration is most commonly used. Glycerol is nontoxic to the cells and to the personnel and can also be directly to the cells. Conversely DMSO enters the cells more rapidly than Glycerol.

### **References.**

## **Appendix 6.11**

### **SOP FOR ISOLATION OF PERIPHERAL BLOOD MONONUCLEAR CELLS.**

#### **Purpose.**

Peripheral Blood Mononuclear Cells are blood cells with a round shaped nucleus, such as Monocytes and Lymphocyte with lymphocyte population comprising T-Cells, B-Cells and NK cells which are vital components of the Immune system. This technique for separation of lymphocyte populations will be applied to studies of surface markers like NKG2D in disease states as compared to normal controls. The Isolated lymphocytes will also be assayed for intracellular  $Mg^{2+}$  and  $Ca^{2+}$ .

**Note.** Perform the isolation and freezing of PBMCs less than 8 hours after the blood collection. Best results are obtained if procedure is performed less than 2 hours after blood collection. Minimize the time the cells remain in a pellet or in contact with the Ficoll.

#### **Principle;**

The most common method for peripheral blood mononuclear cell isolation is a Method known as Ficoll-Paque separation. Ficoll-Paque is a high molecular weight sucrose polymer of  $1.077 \pm 0.001$  m/ml at  $20^{\circ}C$  that was developed by GE Healthcare. The Ficoll-Paque separation method is based on the principle of differential migration of blood cells through the media during the centrifugation stage of the process.

The combination of centrifugation and Ficoll causes the blood to fraction into separate layers. The bottom layer contains particles denser than Ficoll. It is mostly made up of Erythrocytes which are aggregated by the Ficoll medium and sink completely through to the bottom. The next layer from the bottom is primarily the granulocytes, which also migrate down through the Ficoll Solution. The upper layer contains particles which are less dense than Ficoll and is found in the interface between plasma and Ficoll solution. Cells found within this upper Layer include lymphocytes, monocytes and platelets. To recover the lymphocytes, this layer is carefully recovered, washed with salt solution to remove platelets, Ficoll and plasma then Centrifuged again.

#### **Scope;**

The use of PBMCs for gene expression profiling has become an important way to potentially identify biomarkers that are implicated in a variety of disease processes. The PBMCs isolated in

these experiments will be analysed for a cell surface marker NKG2D and measurement of intracellular Magnesium and Calcium ions.

### **Requirements;**

Ficoll, Sterile Balanced Salt Solution e. g Phosphate Buffered Saline(PBS), Centrifuge with swing out rotor (Brake should be off), Sterile Centrifuge tubes, Sterile Needles, and blood collection set (Butterfly needles 21G), Acid Citrate Dextrose tubes, Pipette Aide, Serological Pipettes.

### **Procedure;**

1. Spin down the blood in the 10ml ACD tube at 2000rpm for 10 minutes with acceleration at 9 and deceleration at 9
2. Remove plasma and using a pipette transfer it into four separate aliquots {0.5ml,0.5ml,1.5ml, and1.5ml}.
3. Re-suspend blood to replace the plasma removed in step 2 with PBS (for example, if you removed 2ml of plasma, then add back 2ml of PBS).
4. Transfer blood and PBS from the ACD tube to a 50ml conical centrifuge tube using a pipette aid and a 20.0ml serological pipette.
5. To the Blood/PBS mixture in the 50ml conical, add an equal volume of 1X PBS to dilute blood and mix thoroughly.
6. Carefully layer Your Blood/PBS mixture on to Ficoll in a separate 50ml conical. Volume depends on how much blood you have. For 10-15ml of blood, use 5ml of Ficoll
7. Cap the tube and centrifuge at 2000rpm for 20 minutes at room temperature with acceleration at 9 and deceleration at 1.
8. With a sterile plastic pipette, Collect PBMCs (the thin layer between PBS& Ficoll) and transfer to a 15ml conical centrifuge tube, add 1XPBS to wash the cells and centrifuge at 1200-1500 rpm for 5 minutes.
9. Aspirate the supernatant again and re-suspend the pellet in 15mls of 1XPBS to wash again. Centrifuge at 1200 rpm for 5 minutes.
10. After 5 minutes, Volume up to wash the cells in 1X PBS. Centrifuge again for 5 minutes.
11. Add 2mls of 1X PBS, Aliquote the cell suspension into four cryotubes {0.5ml each}. Keep two cryotubes of sample on ice for NKG2D and intracellular  $Mg^{2+}$  Basal Staining respectively

12. Re-suspend cells in the two of the four cryotubes in step 11 in freezing media. Proceed to store the two cryotubes of cells in Liquid Nitrogen the following day.
13. Aspirate off the Ficoll in the 50ml Conical in step 6, leaving you with red cells.
14. Wash the red cells in 1X PBS once and aliquote in to two separate cryotubes.
15. Record the Volumes of aliquots and Date of processing on the SLF2 form.
16. Store all aliquots; from the four Plasma aliquots in step 3 to the two red cell aliquots in step 14 in the -80 freezer.

**Note:** Freezing Media= 90% FBS, 10%DMSO.

**Rule of thumb for freezing Cells:**

Don't freeze more than 10 million cells in freezing media. Cells will otherwise die.

**References.**

- 1.PBMC isolation from buffy coats or whole blood PBMC cryopreservation.

## Appendix 6.12

### **COUNTING OF CELLS USING THE IMPROVED NEUBAUER COUNTING CHAMBER**

#### **Purpose.**

This Protocol is a systematic guide for counting cells after they have been isolated using Ficol -a density gradient blood separation medium.

It is almost routine to count cells when carrying out tests that involve cells; for example, measurement of intracellular Magnesium ions and staining cells for the NKG2D receptor. The counting chamber recommended for cells counts is a metalized surface (Bright line) double cell improved Neubauer ruled chamber. The chamber grid has an area of  $9\text{mm}^2$  and the depth of the chamber (space between cover glass and grid) is 0.1mm.

#### **Principle**

Trypan blue dye is unable to penetrate healthy or live cells because of the intact cell membranes, so they remain unstained thus exclude the dye. Dead cells have a compromised cell membrane that is permeable to the trypan blue dye. Dead cells are stained blue and display as dark cells in the haemocytometer.

In this test, a cell suspension is simply mixed with dye and then visually examined to determine whether cells take up or exclude dye. The cells are subsequently counted and recorded.

#### **Scope**

For use in applications dealing with cell counts.

#### **Requirements.**

Haemocytometer, Cover glasses, 0.4% trypan blue, Pipettes, Pipette tips, Cell preparation, Microscope.

#### **Procedure.**

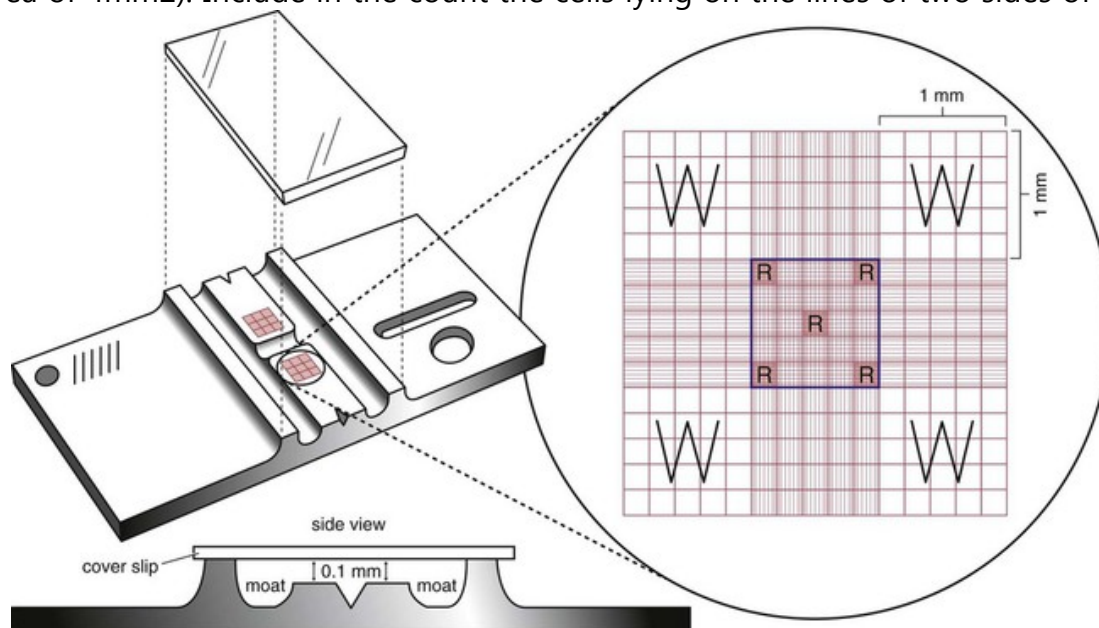
1. Measure 0.38ml of trypan blue and dispense it into a small container or tube.
2. Add 20  $\mu\text{l}$  of well mixed cells and mix: The Volume of the cells used in the test must be correct.
3. Assemble the Counting chamber.
  - Make sure the central grid areas of the chamber and the special haemocytometer cover glass are completely clean and dry.
  - Slide the cover glass into position over the grid areas and press down on each side until rainbow colours (Newton rings) are seen. Prior moistening of the chamber surface on each of the grid areas will help the cover glass to adhere to the chamber.
4. Re-mix the diluted blood sample. Using a pipette held at an angle of  $45^\circ$ , Fill one of the grids of the chamber with sample taking care not to overfill the area.

NB. The chamber must be refilled if the sample overfills into the channel beyond the grid or an air bubble forms in the grid.
5. Leave the chamber undisturbed for 2 minutes to allow cells to settle.

NB. To prevent drying of the fluid, Place the chamber in a petri dish with damp cotton wool, tissue or on ice.
6. Dry the underside of the chamber and Place it on the Microscope stage. Using the 10X objective with the condenser iris closed sufficiently to give good contrast, focus the rulings of the chamber and cells. Focus the cells until they appear as small black dots.



Count the cells in the four large corner squares of the chamber marked W, in *Figure 1* below (Total area of 4mm<sup>2</sup>). Include in the count the cells lying on the lines of two sides of the large squares.



squares.

*Figure 1* Rulings of the counting Chamber

7. Report the number of white cells per litre of cells using the following simple calculation.

-Divide the total No. of cells counted by 2

-Divide the No. obtained by 10.

The No. obtained  $\times 10^9$  is the cell count.

E.g. Cells counted in 4 squares = 84.

$84/2=42$

$42/2=4.2$ . Cell count is  $4.2 \times 10^9$  per litre.

After performing the count, before the sample dries, dismantle chamber, wash, and dry it. Store items in a safe place.

The expected PBMC yields from whole blood for healthy populations are (source: HIV AIDS Network coordination PBMC processing SOP)

Population	Mononuclear cell Yield Range (Cells/ml blood)
Adult	$(0.8-3.2) \times 10^6$
Pediatric – less than 6 months	$(3-10) \times 10^6$
Pediatric – 6 mo. To 2years	$(2-9) \times 10^6$
Pediatric- 2-5 years	$(1-6) \times 10^6$
Pediatric- more than 5 years	$(0.8-4) \times 10^6$
Pediatric – Unknown Age	$(1-10) \times 10^6$

**Counts higher than  $50 \times 10^9/l$**

When a count is higher than  $50 \times 10^9$ , repeat the count using 0.76 ml of 0.2% trypan blue and 20  $\mu$ l of cells. Multiply the result by 2.

Very high cells counts are found in some forms of Leukaemia.

Always examine a stained thin blood film.

**Counts lower than  $0.72 \times 10^9$ .**

When a count is lower than  $0.72 \times 10^9$ , repeat the count using 0.38ml of trypan blue and 40  $\mu$ l of cells. Divide the result by 2.

**Quality Control and Quality Assurance.**

Whenever possible perform WBC counts in duplicate. The difference between two counts (as a percentage of the mean) should not be more than 20%.

Check that the diluting fluid is free from particles which could be mistaken for WBCs. To do this fill the counting chamber with a sample of trypan blue and examine the grid areas. Microscopy using the 10X objective with greatly reduced condenser iris. If the fluid contains particles resembling Cells, filter it and recheck or discard the fluid and prepare fresh diluting fluid.

**Sources of Error.**

Incorrect measurement of cell Volume.

Insufficient mixing of cell preparation with diluting fluid.

Unclean chamber and cover glass.

Inappropriate cover glass.

Over filling the chamber

Not allowing sufficient time for cells to settle in the chamber.

**References**

- 1.Clinicalgate.com/routine-and point of care testing in hematology Manual and semi-automated methods/
- 2.District Laboratory practice in tropical countries Part 2. Monica Cheesebrough
- 3.PBMC Isolation from buffy coats or whole blood PBMC cryopreservation Immune Monitoring Platform.

## **Appendix 6.13**

### **Staining Cells for the Measurement of Baseline Intracellular Magnesium in Fresh or frozen PBMC Using the BD Accuri C6.**

#### **Purpose:**

This SOP is written to guide staff in the step by step handling of fresh cells in the estimation of intracellular Magnesium ions in Cells.

#### **Principal:**

The Magnesium ion( $Mg^{2+}$ ) is the most abundant divalent cation in eukaryotic cells and is required for many critical physiological processes. The vast majority of the total body's  $Mg^{2+}$  content is in a bound form either in the intracellular compartment or in the bone. Most intracellular bound  $Mg^{2+}$  is tightly associated with nucleotides and proteins, and plays vital roles in energy production, DNA replication, gene transcription and protein synthesis. Less than 5% of the intracellular  $Mg^{2+}$  exists in its free state. Intracellular  $Mg^{2+}$  regulation in the immune system participates in the cytolytic control of Epstein-Barr virus.

Accurate and Reproducible methods for the measurement of cytosolic free Magnesium ion concentration are required in order to assess its physiological role.

MagFluo<sub>4</sub> and Fura Red are two fluorochromes that are used for the ratiometric measurement of Magnesium.

#### **Scope.**

For our purpose, this SOP is about the Fluorescence Method for measurement of Intracellular magnesium ions.

#### **Requirements.**

RPMI, Mg Free medium, Power load, Fura Red, Mag Fluo<sub>4</sub>, HBSS, Centrifuge, Ice, FCR blocker, PBS, FACs buffer, CD8-PE/Cy7(1;100), FITC.

#### **Protocol.**

1. Thaw the frozen PBMCs, to achieve  $5 \times 10^6$  Cells/ tube.
2. Wash in  $Mg^{2+}$  free medium once.

3. Dilute cells in regular RPMI and re-suspend/wash the cells in Mg free medium.
4. or Count the fresh PBMCs using counting chamber \*Dilution is 1:1 (10µls of cells: 10µls of Acridine Orange or trypan blue).
5. Adjust at  $2 \times 10^6$  cells/ml in HBSS+1.2mM  $Mg^{2+}$  and  $Ca^{2+}$ . (This stabilises the extracellular surrounding of the cells so that  $Mg^{2+}$  does not leak out.)
6. Prepare loading Solution (Power Load 100X 80µl, Mag Fluo4 1mM 10µl, Fura Red 1mM 10µl).
7. Add 10 µl of loading solution for 1 ml ( $2 \times 10^6$  of cell suspension final 1M in HBSS+ 1.2mM  $Mg^{2+}$  and  $Ca^{2+}$ )
8. Incubate for 20 minutes at room temperature in the dark.
9. Add 10ml of HBSS+1.2mM  $Mg^{2+}$  and  $Ca^{2+}$ .
10. Centrifuge at 1500rpm for 5 minutes.
11. Re-suspend cells in HBSS+1.2mM  $Mg^{2+}$  and  $Ca^{2+}$ .
12. Keep on Ice in the Dark.
13. Add FCR blocker in 1:20 dilution (1000µl PBS to 50µl FcR blocker) allow to act for 20 minutes at room temperature.
14. Wash in PBS and dilute the cells in FACS (No  $NaN_3$ )
15. Add APC/Cy7 antihuman CD8 antibody (1:100) and APC-eFlour 780 anti-human CD3 on ice for 30 minutes.
16. Add 5ml FACS buffer, wash and spin.
17. Dilute the cells in 300µl FACS buffer then run on the Flow Cytometer.

## References.

1. Juan Ravell. Benjamin Chaigne-Delalande, Michael Leonardo X-linked immunodeficiency with magnesium defect, Epstein-Barr virus infection, and neoplasia disease: a combined immune deficiency with magnesium defect.

## Appendix 6.14

### THE STANDARD OPERATING PROCEDURE ON NKG2D STAINING.

#### Purpose:

To give a step by step guide on how to stain cells for the receptor NKG2D that is resident of the surface of Natural Killer Cells. A comparison in ratio-metric amounts of this receptor between patient and Normal Control will guide on severity of deficiency of the Receptor.

#### Principle:

Natural Killer(NK) Cells are an important element of the innate immune system as they can kill cells and virally infected cells. NK cells express many different cell surface receptors that deliver either activating or inhibitory signals. The relative balance of these signals regulates NK cell activity. Many of these receptors also occur on antibody T cells. NKG2D is an activating receptor that has recently generated considerable interest.

Human NKG2D was originally identified in 1991 as an orphan receptor that is expressed on NK cells and many T cells. NKG2D is a type II transmembrane protein with extracellular C-type (i.e.  $\text{Ca}^{2+}$  -binding) lectin like domain. Although the original proteotype for this domain, mannose-binding protein, binds a carbohydrate ligand, many receptors having a c-type lectin-like domain, lack the  $\text{Ca}^{2+}$  binding site and recognize protein ligands rather than carbohydrate. NKG2D appears to function in some cells as a co-stimulatory molecule. T cell co-stimulation refers to activation of signalling pathways that are complementary to those activated through the antigen specific T cell receptor. The potent T cell co-stimulatory molecule, CD28, has a cytoplasmic YXXM motif that activates PI 3-kinase via the DAP 10 adapter protein suggesting a similar co-stimulatory capacity.

The immune system relies on many signalling molecules to detect danger, and NKG2D appears to be an important contributor to this recognition. Up-regulation of the MIC proteins in transformed cells and upon virus infection will elicit a response via NKG2D. The HCMV-encoded protein, UL-16, probably serves to down-modulate the innate immune response by interfering with the recognition of NKG2D target ligands. Different alleles of MICA show large differences in binding affinity for NKG2D, and it is therefore also possible that the polymorphism of NKG2D ligands might define genetic determinants for Viral susceptibility.

#### Scope

This is SOP will be applied for research purposes only in comparison of patients and Normal controls.

#### Requirements

Phosphate Buffered Saline, Cell counter/(Haemocytometer), FACs Buffer, V-bottom Plate Micro wells, Isotype Control, Normal Control, Patient Sample, Multi-channel Pipettes, Incubator, Ice, Centrifuge.

#### Protocol

1. Isolate cells from whole blood using Ficoll or resuspend frozen cells using standards methods.

2. Wash cells (Centrifuge at 1200 rpm for 5 minutes) three times in 1X PBS then enumerate using a counting chamber or cellometer.
3. Resuspend cells in FACS (960ml 1XPBS,20ml FBS,10ml 100mM EDTA,10ml NaN<sub>3</sub>) buffer.
4. Resuspend cells so that you will achieve 300-500 thousand cells per 100 µls.
5. Plate 100 µls of cell concentration above into V bottom plate.
6. In a micro-centrifuge tube, prepare Isotype control and Normal staining buffer.
7. First add 500µls of FACS buffer to each tube then add the following.

A1	2	3	4	5	6	7	8	9	10	11	12
B											
C											
D											
E											
F											
G											
H											

A1 Normal Control # 1  
 B5 Single colour comp-add  
 1.0ml of (CD314) -NKG2D

A2 Normal Control # 2

A3 Patient

A4 Isotype Control (Use a Normal Control)

B1 Unstained Cells-Don't add any stain to this well.

B2 Single colour comp -add 1 ml of (CD3)

B3 Single Colour comp -add 1.0ml of (CD4)

B4 Single colour comp -add 1.0ml of (CD8)

**This Staining Scheme works well for Fresh blood or wPBMCs. You may add an additional NK marker if you are staining whole blood to assess NK%.**

8.Vortex staining buffers and keep on ice.

9.Add 100µls of staining buffer to each well of 300-500 thousand cells for a total volume of 200µls.

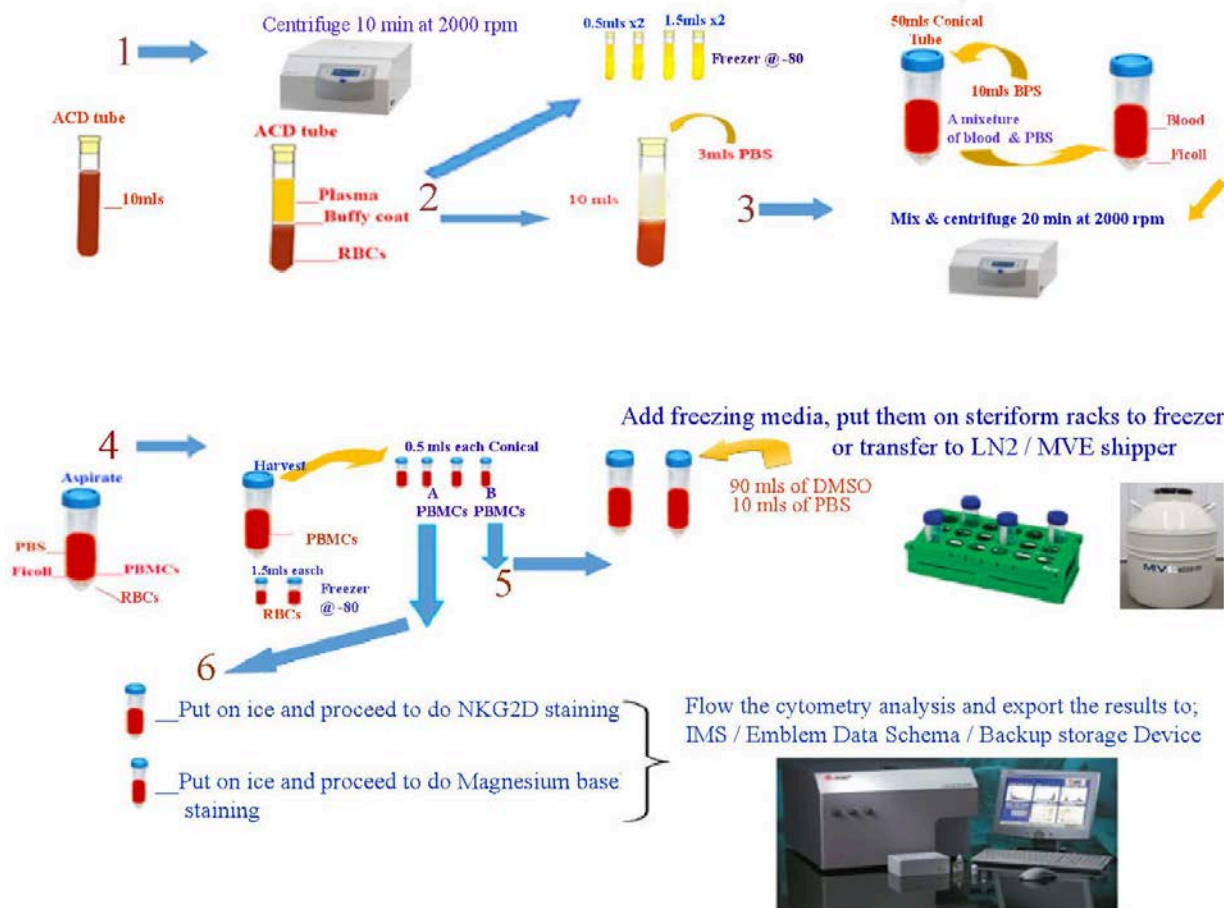
10.Gently mix by pipette.

11.Incubate either on ice or at 4<sup>0</sup>C for 30 minutes.

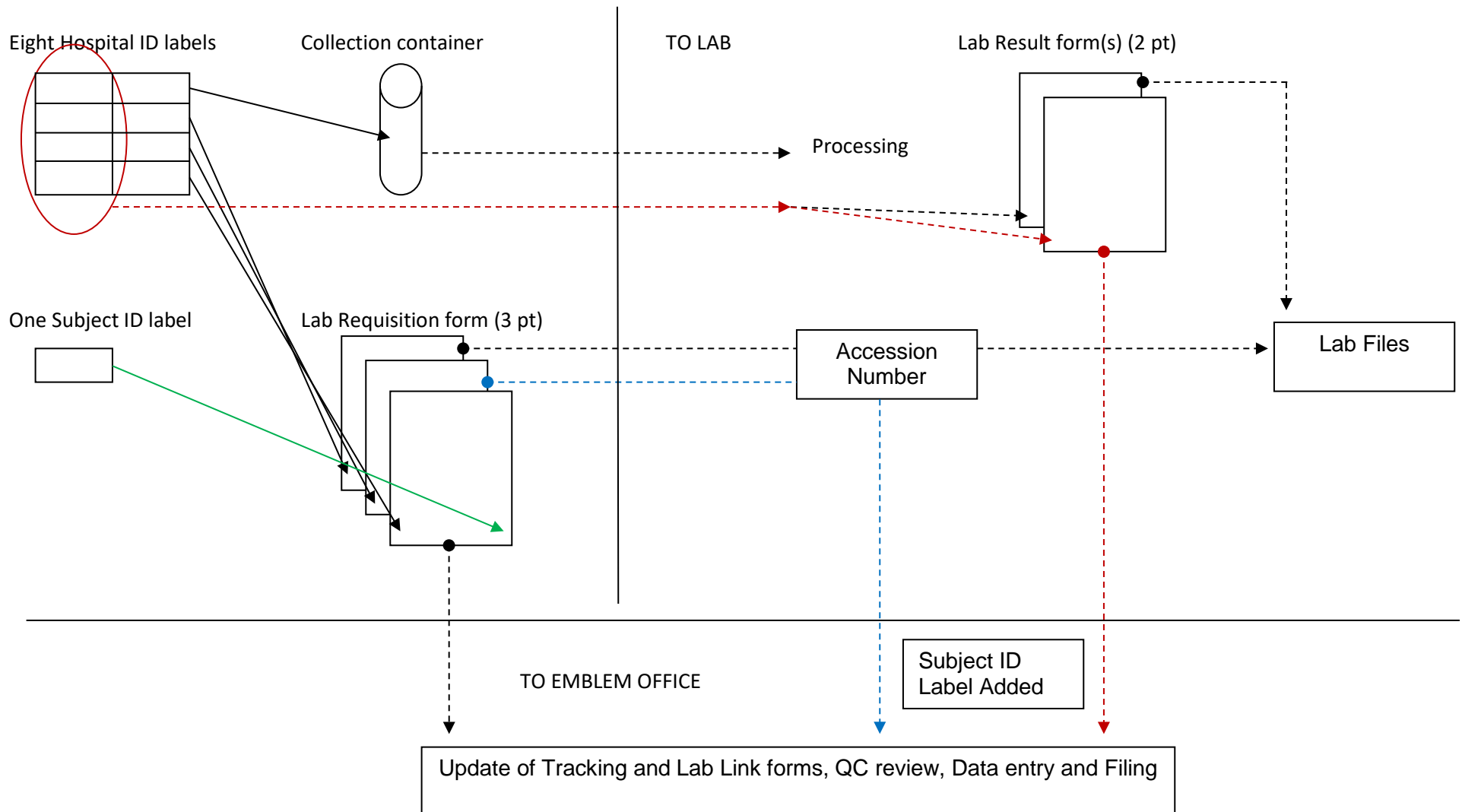
12.Prepare cells for flow cytometry by washing 2-3X in FACS buffer, Centrifuge at 1200rpm for 5 minutes.

13. After final wash, re-suspend cells in 300µls of FACS buffer in FACS tubes, chill on ice until acquisition.

## Appendix 6.6 - EMBLEM Specimen Collection and Processing Guide (Cases& Controls)

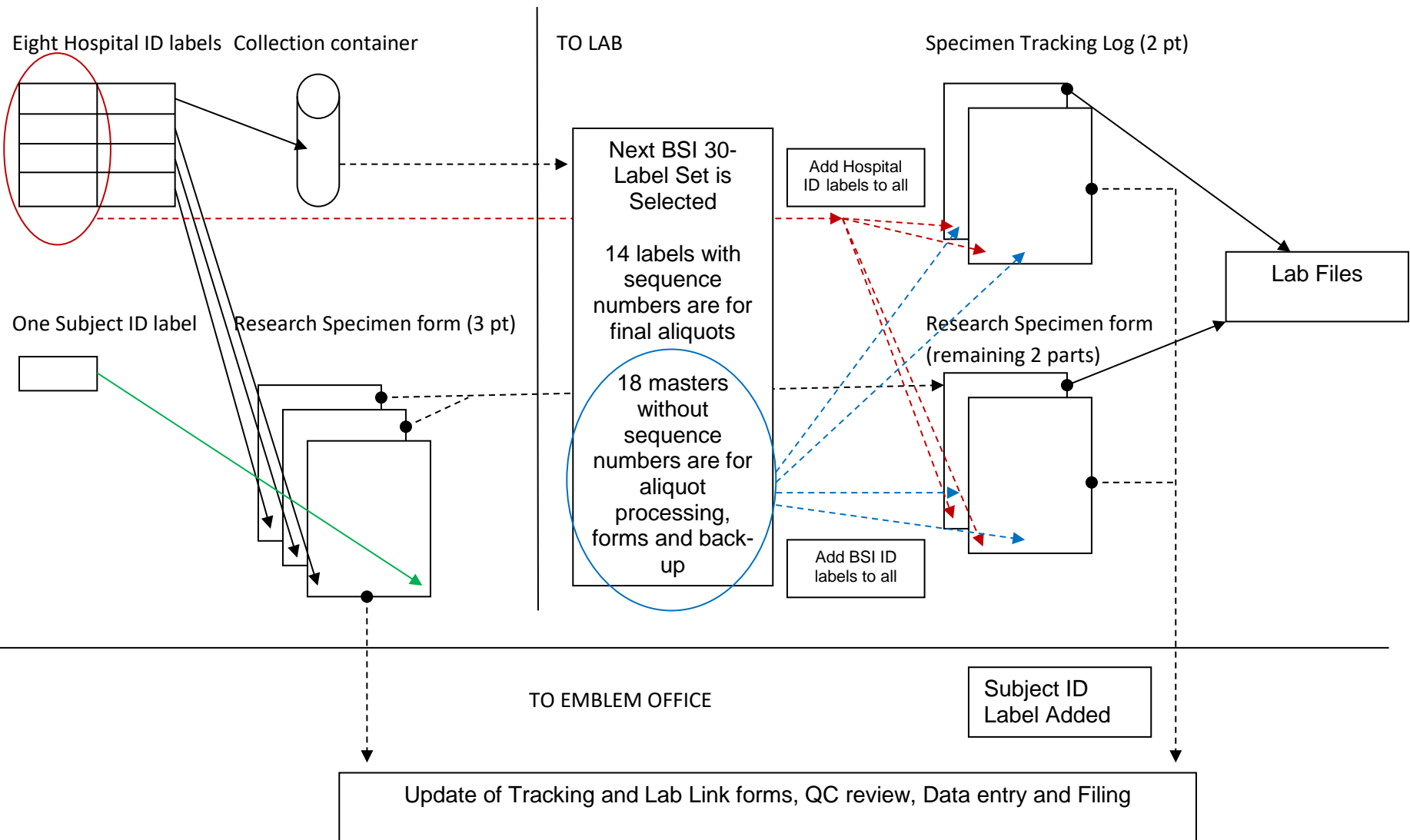


## Appendix 6.7 - Labeling for Hospital Specimens (each vial/container)





## Appendix 6.8 Labeling for Research Blood and Saliva Specimens (each vial/container)



## Appendix 6.9 Example 10x10 Cryobox Layout for Plasma

IF THE FOLLOWING SUBJECTS HAVE THE FOLLOWING ALIQUOTS OF PLASMA, THE ALIQUOT LOCATIONS WOULD BE AS ILLUSTRATED ON THE RIGHT:

ID AVAILABLE VIALS

- 1 0001 0002 0003 0004
- 2 0001 0002 0003 (missing 0004, insufficient sample)
- 3 0001 0002 0003 0004
- 4 0001 0002 (missing 0003 and 0004, insufficient sample)
- 5 0001 0002 0003 0004

PLASMA BOX 1-A (Note that vial sequence numbers in the A boxes are always odd numbers)

	1	2	3	4	5	6	7	8	9	10
A	0001									
B	0003									
C	0001									
D	0003									
E	0001									
F	0003									
G	0001									
H	empty									
I	0001									
J	0003									

PLASMA BOX 1-B (Note that vial sequence numbers in the B boxes are always even numbers)

	1	2	3	4	5	6	7	8	9	10
A	0002									
B	0004									
C	0002									
D	empty									
E	0002									
F	0004									
G	0002									
H	empty									
I	0002									
J	0004									

## **Chapter 7**

### **General Administrative Procedures**

#### **7.1 Quality Control Procedures**

##### **7.1.1 Site Visits and Retraining**

One of the key determinants of successful study implementation of EMBLEM is training and evaluation of field staff and their activities. The training program will provide the coordinator at the participating hospitals with the skills and knowledge required to successfully implement the study. While training is provided when each hospital is ready to begin the enrollment of study subjects, training and monitoring is a continual process. Each site will be monitored throughout the course of the study by the central coordinating agency, AFENET.

Site visits may be made for routine monitoring to ensure that the protocol and all study procedures are being followed, or for special purposes, such as training a new staff person. During a visit, the monitor will review all aspects of the study with the local coordinator. Activities include, but are not limited to, training in data collection for new forms and/or refresher training for current forms; training/retraining on shipping infectious substances; checking for signed informed consents for all subjects enrolled in the study; and updating field staff on new study procedures and recent findings.

For staff new to the project, training may include an orientation to EMBLEM, including a review of the study protocol; reviewing IRB requirements and informed consent procedures; enrollment of study subjects; shipping biospecimens and infectious substances; and completing data collection instruments.

These site visits also serve to gather information about how the study procedures are being followed and allow the opportunity to offer suggestions and make possible adjustments to decrease collaborator burden while maintaining the integrity of the study and the safety of human subjects.

##### **7.1.2 Monitoring Data Quality**

Project staff at IMS and AFENET will monitor the quality of data collection, abstraction, and entry throughout the course of the study. To provide a quality control component to the data entered through the EMBLEM data system, we may request that a sample of hard copy forms used for data entry be sent to us for verification. This process will not require additional effort on the part of study staff as the forms requested will be the same as those used for the original data entry and a copy of all forms should be kept in the participants study folder. During the course of the study a study manager would provide you with a list of the hard copy forms

that should be sent for the quality control check. If significant problems with abstraction or data entry are discovered, retraining may be required.

Monitoring the data will be performed via reports produced from the data management system. The data will be reviewed for apparent inconsistencies within form and across forms in order to make the data as meaningful as possible. To this end, periodically a list of items that need your attention to resolve will be sent. This may involve revision to the form or recheck of the actual medical record to confirm a value. In either scenario, timely resolution to these matters is critical.

## **7.2 Monitoring Enrollment Progress**

Although there is no set target for case enrollment, there are expectations of the number of newly diagnosed patients that will report to the hospital as eligible for inclusion in the study (as discussed in Chapter 3). An important function of supervision is close monitoring of how many eligible children are enrolled in the study. Through the data management system, authorized study staff as well as IMS and NCI will be able to produce reports to monitor accrual. In order for this data management system to be effective, data must be entered promptly. As discussed in Chapter 8, reports have been programmed that allow you to track various details of enrolled subjects and also assess the reasons that eligible patients are not being enrolled. Electronic reports based on actual study data allow for careful and effective study management by identifying problems early. The study coordinator is expected to produce reports on a weekly basis and track progress relative to established timelines and budget. In addition, study staff at IMS and NCI will support this effort, by providing feedback after examination of study reports. Weekly phone calls and email communication as frequently as helpful will be maintained throughout the study.

## **7.3 Routine Communications**

Study managers at IMS expect to work closely with the AFENET study coordinators and hospital staff, particularly at the start of data collection but also throughout the entire data collection period. IMS and NCI will be in regular contact, either by email or by telephone.

Standing teleconference calls will be held with EMBELM coordinators. Discussing problems as a group enhances the data collection effort and helps to provide meaningful resolutions.

## **7.4 Handling of Study Supplies in the Hospital Setting**

A plan for the storage, handling, and distribution of study supplies will be essential to the ease of subject enrollment and data collection. This includes not only the clinical and laboratory supplies, but the paper data collection forms and other material such as printed labels necessary to perform the study.

## Receipt and storage of supplies

Lacor Hospital has a computerized stock management system. All supplies destined for EMBLEM study will be processed using the system in place. The procedures will be that every stock delivered to Lacor Hospital will be accompanied by Goods Received Note (GRN), a delivery note, and packing list. The goods received note is a document which will be signed by the stores personnel acknowledging receipt of goods. The delivery note contains such information about the packages delivered such as; identity of the packages, quantity, quality, brand of goods supplied. In each package there is a packing slip that has the details of each and every item packed. The stores personnel receiving the goods will take note of any damages that might have occurred during transit and inform the relevant authorities accordingly.

Lacor supplies officer together with EMBLEM supplies officer will check each and every item on the packing slip to ensure that they are the right goods, received at the right destination, in the right quantity, right labels, right brand, right quality, right quantity and that there are no damages. It is only then that they enter the details of the received goods in a Bin Card. The Bin card has details of stock such as name of item, date of receipt, opening balance, quantity received, total good available, quantity issued, place of issue, requisition number, name of person collecting the item, issuing officer, and closing balance. This Bin card will be the document used for monitoring and stock control of supplies on a daily basis.

## 7.5 Study Supplies and Inventory Management

A list of supplies needed to conduct the study is included in **Exhibit 7-1 and 7-2**. Please monitor the quantity of supplies so that when any study materials run low, additional supplies can be sent before the supplies completely run out. Some of the supplies may need to be printed or ordered from outside vendors so please take this into consideration. In order to facilitate requests, a sample Supply Reorder Form (**Exhibit 7-2**) is provided and can be modified to meet the hospital requirements.

### 7.5.1 Stock ordering stores supplies.

For purposes of ease of stock tracking, EMBLEM will have departments like, stores, BL ward, laboratories, pathology, office and field departments. All these departments will be issued with stock requisition books. An officer identified by the EMBLEM study coordinator and designated as EMBLEM supplies officer will be in charge of ordering supplies for daily use in these departments. Every order issued by these departmental heads will be cross checked by the study coordinator or another person to whom this responsibility is delegated. This will be done in order to stream line ordering of supplies and easy monitoring of consumption and stock

level. The study staff making the requisition will indicate in the requisition book, the department ordering, serial number of item ordered, its particulars, units, quantity requested and purpose for the requisition.

### **7.5.2 Stock issuing**

On receipt of a requisition for any particular item needed for the study from the EMBLEM departments, the EMBLEM supplies officer will present this requisition to his counterpart at the Lacor stores who in turn will cross check the requisition against the available stock then issue the particular item to the EMBLEM supplies officer according to what is available. The EMBLEM supplies officer will receive the issued goods, cross check against a copy of the order and sign acknowledging receipt of the stock. The Lacor supplies officer at the store together with the EMBLEM supplies officer will proceed to enter record of the day's transaction in store's daily consumption book. Details to be entered in the store's daily consumption book includes, name of item, date of issue, total good available, quantity issued, place of issue, requisition number, signature of the person collecting the issue, issuing officer, and the closing balance. The stores daily consumption book will be kept with the EMBLEM supplies officer while his opposite number at Lacor stores enters record of this transaction in their computerized system which makes automatic deduction of the stock, get a print out and both personnel involved in the transaction sign and file copies both at the stores and EMBLEM office.

### **7.5.3 Stock monitoring**

In order to make sure that adequate stock is available and there is no stock outs that may hamper planned study activities, the EMBLEM supplies officer will carry out daily, monthly and quarterly stock level monitoring. For stocks that have high consumption rate, daily monitoring of stock level is recommended. Depending on demand and purchasing procedures established for EMBLEM supplies, the EMBLEM supplies officer will determine a re-order limit for every item meant for the study activities. This re-order limit is a level of stock below which the supplies officer is advised not to go. It is a warning that an order should be placed immediately. This is meant to create a buffer stock which will ensure that no study activities grinds to a halt because of unforeseen stock outs, increased demand, or delay in purchasing procedures. Physical stock taking whereby the EMBLEM study coordinator at Lacor together with the two supplies officers for EMBLEM and Lacor respectively physically count each item in the stores unit by unit and sign the stock or Bin card will be a regular stock control activity every six months..

### **7.5.4 Inventory book**

EMBLEM supplies officer will maintain simple inventory book of office equipments like filing cabinets, desks, tables, chairs, desktop and laptop. A unique label will be identified e.g AFENET/EMBLEM/2010-02. These label will be engraved on all equipments except the consumables. This book will be ruled in columns to capture, particulars date the item was received, serial number, make or model, quantity, name of user, date of issue, signature of user and status.

### **7.5.5 Computerization of the system**

The Lacor stock management system is computerized. It makes checking stock level, very simple. However, the system will work well if every item entered in it has a cost attached to it. It is recommended that this system runs parallel to the book system kept and maintained by the

EMBLEM supplies officer. In case of loss of Bin cards, the computerized system can trace the last transaction and therefore the stock level. Besides, this system makes auditing easy especially when it comes to wrap up of the project

## Exhibit 7-1 - Supply List

DESCRIPTION	PLANNED USE	COLLECTION/TEST SUPPLIES NEEDED PER SUBJECT
<b>Data Collection Forms (See Study Manual)</b>	Collection of study data from subjects and laboratory results	See Chapter 3 of the Study Manual
<b>Specimen Collection and Lab Supplies</b>		
Whole blood specimens	NCI storage specimen Buffy coat x 2 x 1.0 ml 10 ml K2EDTA tube Plasma x 2 @ 0.5 ml + 2 @ 1.5 ml  Hospital test specimens 1 x 2 ml and 1 x 3 ml K2EDTA tube CBC w/diff RBC Sed rate Malaria rapid test Malaria thick smear 1 x 2.5 ml SST tube Liver panel  Renal panel	Malaria rapid test and buffer 10 ml K2EDTA vacutainer tube x 1 3 ml K2EDTA vacutainer tube x 1 2 ml K2EDTA vacutainer tube x 1 2.5 ml SST tube x 1 Vacutainer tube holder x 1 Butterfly needles (21ga) x 1 Butterfly needles (22ga) x 1 Disposable gloves non-latex x 2 Alcohol pad x 1  Bandaid x 1 2 x 2 gauze pads x 2 Tourniquet x 1 (probably don't need 1/person) Frosted glass slides Sharps containers Specimen transport bags x 4
Saliva specimen	NCI storage specimen	Small plastic medicine cup w/lid (>10 ml) Disposable graduated transfer pipettes Standard Transport Media (STM)
Tissue biopsy specimen	NCI storage specimen paraffin block x 1 RNA later sample x 2  Hospital test specimen Paraffin block	Biopsy aspirate needle (Tru-cut?) Biopsy gun (Type?) RNAlater prefilled vials Paraffin wax Tissue cassettes Small prefilled buffered Formalin containers
Stool specimen	Hospital test specimen (wet mount microscopy)	Stool collection cup Wooden applicator sticks Cover slip x 1 Frosted glass slide x 1
Bone marrow specimen	NCI storage specimen	Biopsy/aspiration needle (size/length?)



DESCRIPTION	PLANNED USE	COLLECTION/TEST SUPPLIES NEEDED PER SUBJECT
CSF specimen	Slide smear x 1	Iodine swab sticks Gauze sponges Elastic bandage
	Hospital test specimen Slide smear x 3	Lidocaine hydrochloride, 1%, Fenestrated drape Frosted glass slides Scapel blade w/handle Disposable syringes and needles
	Hospital test specimen	Lidocaine hydrochloride Specimen tubes with cap Providone iodine swab sticks Gauze sponges, 2" x 2" Fenestrated drape Puncture site bandage Spinal needle 22G x 1 1/2" needle Disposable syringes and needle
General Lab supplies	Hospital specimen lab processing	Cryovials 2ml - graduated - 12/patient + spares pipettes pipettor Tube racks Sample collection tray Plastic apron
<b>General Supplies</b>	Hospitals and Field Sites	
Staff clothing / equipment White coats Wellington boots Flashlights & Batteries Mosquito netting Biospecimen Handling Supplies Hand washing / disinfecting supplies Water / power bars Sharps disposal containers Bins for biospecimen supplies Garbage bins / bags De-worming medicines Anti-malarials		
ID Label Printer Sheets	Hospitals	White Mini Laser Labels (46 x 11.11 mm)
Specimen/EMBLEM ID Labels	Hospitals and Field Sites	IMS to provide

DESCRIPTION	PLANNED USE	COLLECTION/TEST SUPPLIES NEEDED PER SUBJECT
<b>Study-Specific Tools/Equipment</b>		
Freezer	Kuluva Hospital	
CBC equipment for hospitals	Hospitals	
Hood	Hospitals	
Centrifuge	Hospitals	
Dry shipper	Hospitals	
Scales	Hospitals	
Microscope	Hospitals and Field Sites	
Bar Code Scanner	Hospitals and Field Sites	
Racks	Hospitals and Field Sites	
Cold boxes	Hospitals and Field Sites	
Stadiometer / tape measures, etc	Hospitals and Field Sites	
Blood pressure monitor with age appropriate cuffs	Hospitals and Field Sites	
Thermometers	Hospitals and Field Sites	
CBC equipment for field efforts	Field Efforts	

(EXAMPLE ONLY)

**Fax number:**

## REQUESTOR INFORMATION

Allow up to 2 weeks for delivery, depending on type of supplies ordered.

Form Name	Quantity Needed
Enrollment packets (pre-ID)	
Enrollment packets (blank)	
Study brochures	
Whole blood collection tube w/ACD Solution A (8.5 ml)	
Whole blood collection w/ freeze dried K2EDTA (10 ml)	
Whole blood collection w/ freeze dried K2EDTA (6 ml)	
Whole blood collection tube w/ACD Solution A (6 ml)	
White die-cut corrugated box, 8x8x2.75	
Specimen transport bag – leakproof / self sealing (5"x9")	
Aqui-Pak 4 tube holder absorbent pouch	
IATA Exempt human specimen labels	
Oragene saliva collection kit	
Oragene mail kit	
Oragene swab kit (5 swabs)	
Other:	
Other:	
Other	

TT

## **Chapter 8**

# **DATA APPLICATION FOR BURKITT LYMPHOMA HOSPITAL-BASED REGISTRY**

**AT**

**St. MARY's HOSPITAL, LACOR, GULU, UGANDA**

**By**

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**EMBLEM Field Office, St. Mary's Hospital, Lacor, Gulu**

**Version date: January 2010**

## **1. Background**

The Data Entry Application for the BL registry was developed with support from the epidemiology of Burkitt lymphoma in East African children and minors (EMBLEM) study. The application may be shared with other hospitals through collaborative arrangements. This User's Guide provides step-by-step explanations of how to use the application and to computerize data captured on BL chart abstraction forms (see Appendix). The User's Guide has two sections touching briefly on the history of St. Mary's Hospital, Lacor, and why the registry was implemented and on the specific modules to capture data on new and old BL cases.

### **1.1 St. Mary's Hospital, Lacor**

St. Mary's Hospital, Lacor, is a private non-profit catholic hospital owned by Registered Trustees of Gulu Diocese and operated in line with the Uganda Ministry of Health policy. The hospital, founded by missionaries in 1959, has grown from a small health center to a referral hospital with a capacity of 483 beds and supporting three Health Centers III units namely (Opit, Pabbo, and Amuru) each with 24 beds. The hospital supports two training program for nurses, Enrolled comprehensive nurses and Registered Nurses training programs a training program for laboratory assistant technicians, and also provides clinical training to Gulu University Medical School. In addition, the hospital is an accredited site for training intern medical officers for the Uganda Ministry of Health. The hospital primarily serves residents of Gulu District, where it is located, but patients come from far flung districts, including those bordering Gulu: Amuru in the West, Pader in the East, Kitgum and Lamwo in the North-East, Lira and Apach districts in the South- East. The hospital also receives patients from other parts of Uganda and in the neighbouring countries, especially the Southern Sudan and the Democratic Republic of Congo. The hospital offers preventive, promotive, curative and rehabilitative health care services. The hospital has, despite being at the center of a very disruptive civil war in northern Uganda, a

devastating HIV epidemic, episodic epidemics of meningitis and Ebola, managed to gain national and international recognition for high-quality and comprehensive medical services. The establishment of BL registry will complement and consolidate its work to improve data capture and archiving and facilitate accurate and timely reporting.

In 1992, the hospital started providing systematic treatment for children with lymphoma, the majority of which were Burkitt lymphoma, which is endemic in northern Uganda. The decision to provide treatment was influenced by realization that children who were diagnosed at the hospital and referred to the Uganda Cancer Institute in Kampala a distance of, 340 kilometres away, rarely undertook the journey because of lack of money and, for those few who went, many returned with advanced disease because they were unable to get curative drugs at hospitals in Kampala. This program to treat children with BL is the basis of the BL registry at St. Mary's Hospital, Lacor.

### ***1.2 Burkitt lymphoma at St. Mary's Hospital, Lacor***

To keep a record of children diagnosed and treated for Burkitt lymphoma at St. Mary's Hospital Lacor, information on cases was recorded in a casefile register –essentially a log book -- maintained at the Burkitt lymphoma ward. In 2004, the case file register was computerized to facilitate an epidemiological analysis on BL cases treated at the hospital during 1997-2006 (Ogwang *et al.*, IJC 2008). This analysis showed that 90% of the cases came from Gulu district and neighbouring districts (an area described as within a radius of about 100 miles from Lacor Hospital). Even so, case distribution was not directly related to distance from the hospital or by urbanization, suggesting heterogeneity in case activity. The basis for these heterogeneous patterns could not be determined, but incompleteness of registration of cases, incompleteness of the information collected, duplication of records, inconsistent coding of some information, and the general inability to implement routine data quality control were identified as weaknesses of the BL registry.

In 2005, the hospital established a basic computer-based BL register using the chart abstraction form that was used to computerize the BL registry. In

2009, partly in response to the need to improve data quality to facilitate a large, high-quality, epidemiological study of Burkitt lymphoma funded by the United States of America National Cancer Institute, the hospital decided to improve both the content and quality of collected data on Burkitt lymphoma patients. The changes made included the developing data forms to collect: a) demographical; b) clinical symptoms and signs; and c) laboratory and limited treatment data on all subjects, and to change the process of collecting information from passive to active, and to develop an improved data entry application.

To minimize delays in registering information and, therefore, increase the potential for correcting errors, it was decided that a BL Registrar should be appointed. The responsibilities include collecting completed forms from the BL ward, keying the forms, and resolving any queries with the different departments involved in BL care. Given that Burkitt lymphoma diagnosis, investigation, and treatment takes place over a relatively small area within the hospital, it was decided that the different BL registration forms should be completed at the point where they are generated and returned to the BL registry for keying. The BL Registrar was to be trained and supervised collaboratively by St. Mary's Hospital, Lacor, and the EMBLEM study. The procedure to collect data at point of generation and subsequent computerization is described below.

### ***1.3 Consultation, Diagnosis and Registration of Burkitt lymphoma patients***

The first point of contact with hospital for patients is the Out Patient Department (OPD), where patients are registered and given a unique number called an Out Patient number. Basic information, including names, age, sex, and address is collected and recorded in a log book and a medical form given to the patient. Doctors evaluating the patient collect and record additional information on the patient's form and may request medical investigations to assist them reach a diagnosis. Some patients, and invariably those with cancer, including BL, are referred for admission wards. Patients who are admitted receive another unique number called an IN Patient number on the ward. Children are admitted to the Paediatric ward and adults to one of the

adult wards. Burkitt lymphoma, because it is mostly a paediatric condition, are admitted to the paediatric ward and when confirmed, transferred to the Burkitt lymphoma ward. All information obtained during this process is recorded in a patient's file and in patient ward registers books

The Burkitt lymphoma registry is to capture information about patients with, or suspected to have, Burkitt lymphoma. The registry uses standardized forms to collect demographic, clinical, and laboratory data. Data are collected primarily through chart abstraction of patient files, logs, and review of investigation requests and result stubs conducted by BL registrar. All completed forms are returned to the BL Registry, logged in a book, reviewed for completeness, errors and then keyed into the BL Registry Data Application. Queries arising about the information on the forms are resolved by the registrar approaching the appropriate unit head to review the questionable information and to make corrections in the registry record and patient file. The following sections describe the type of information that is routinely collected and the point of collection, and may be useful when searching for information missing on the BL registration forms.

### **1.3.1 Outpatient department (OPD)**

Children with BL may first be seen in either the children's or adult's OPD. Information about today's date, serial number, name of the patient, place of residence (village, parish), age, sex, weight, tribe, classification of the patient (New, re-attendant), new diagnosis, drugs/treatments are collected and recorded in the OPD Register and on the patient's Medical Form. Returning patients carry their OPD form so they can use the same OPD number. Patients who require admission are referred to the appropriate wards and this information is recorded in the OPD register. If this information is missing on the files on the ward, queries should be resolved by checking the OPD logs.

### **1.3.2 IN Patient Departments**

Children admitted to the hospital ward have their information recorded in a book called the "ward register". There is a separate book for each ward. Information recorded in the ward register includes date of admission, name of



patient, village, tribe, sex, age, date of discharge, working diagnosis and final diagnosis. Each patient receives a unique in-patient number. The date of discharge and final diagnosis are recorded in the ward register when the patient is discharged or dies from the ward. More detailed information about inpatients, including the history of the disease, the results of physical examination, laboratory requests and findings and treatment, can be obtained from inpatient charts. The ward books offer more opportunities to ensure BL registration is complete.

### **1.3.3 The Paediatric Ward**

Children with BL are admitted on the Paediatric ward first and their information recorded in the ward register. The huge caseload typical of the paediatric ward may cause much information not to be recorded, thus information about BL cases is duplicated on the BL ward and this is likely to be more complete. The register captures basic information, including name, age, sex, country, county, parish and village, Tribe, date of admission, Date of discharge, patients OPD and IPD number, BL number-which is sequential, and a narrative summary about anatomic location of tumour and stage of BL, histological results and results from other investigations. This information is of high quality because it is recorded by a paediatrician. Its major drawback is lack of standardization so the patient summaries vary in type, detail, and sequence. The improved BL registration forms offer standard data capture and their consistent use reduces queries from BL registry.

## **2. *Computerization of Burkitt Lymphoma Registry at St. Mary's Hospital, Lacor***

### **2.1 Introduction**

The Burkitt lymphoma registry has the following specific objectives:-

1. To capture demographic, clinical, laboratory, and follow-up data on BL cases treated at St. Mary's Hospital, Lacor
2. To computerize the data

3. To produce regular reports about BL patients admitted at the hospital.

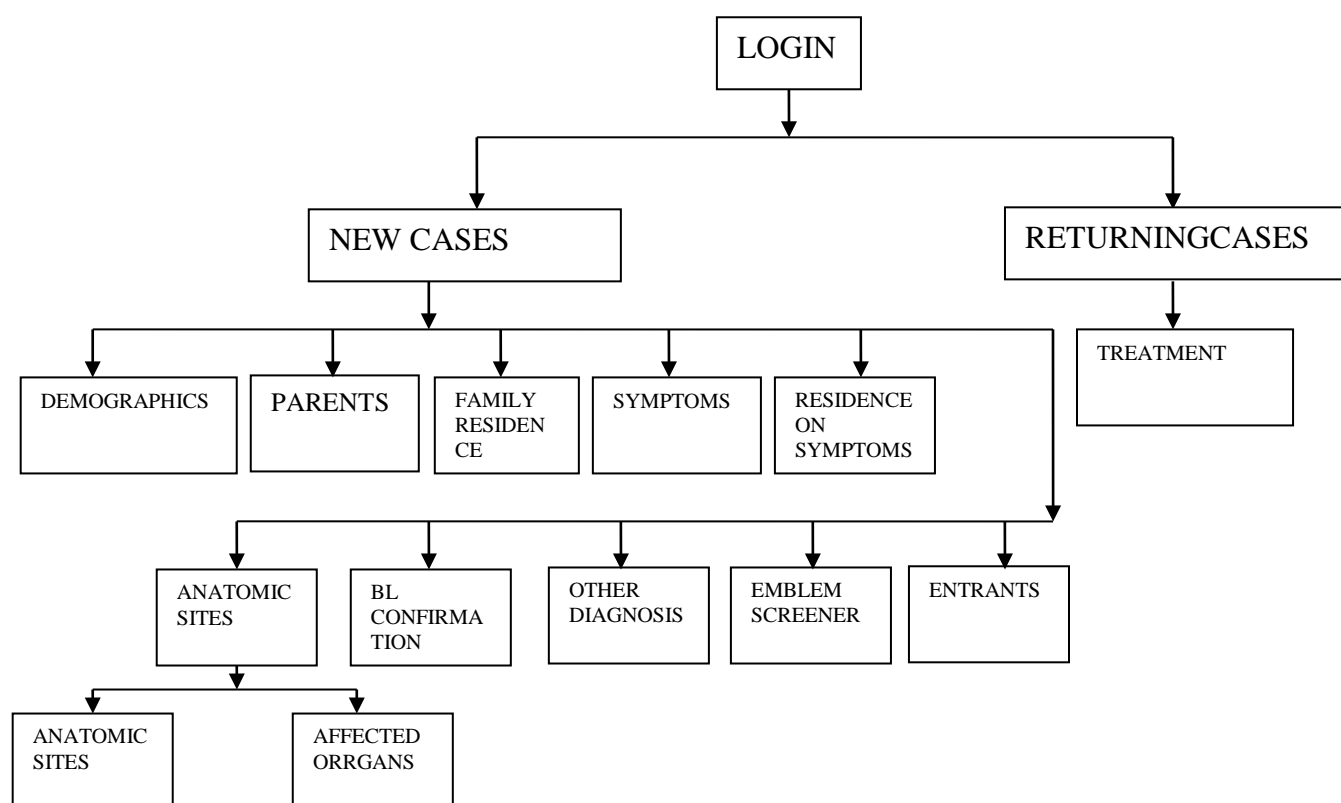
## ***2.2 BL Registry Structure***

The BL registry will be physically located in the EMBLEM Office (also known as the Research Office) and the BL Registrar will work as one of the EMBLEM staff when fulfilling his duties on the registry. The BL registry data entry application has four main modules

1. A demographic module to capture demographical data, including name of the patients and the parents/caregivers, age, sex, address;
2. A clinical module to capture clinical data, including symptoms, anatomic sites and organ involvement.
3. A laboratory module to capture laboratory data
4. Treatment module to capture the drugs administered

## ***2.3 BL Data Entry Application Overview***

The BL registry data entry application was developed in MS Access and Visual Basic. This package is widely available on most personal computers (PCs) and requires basic computer skills to be used effectively. The following section shows the schematic structure of the BL Registry Data base and provides definitions of terms used in the registry.



**New cases:** A “new” BL case (also called an incident case) is any patient that has received a new diagnosis of BL at St. Mary’s Hospital, Lacor, or at another hospital and has not started receiving specific BL treatment. A diagnosis of BL, for purposes of registration, means clinical diagnosis before cytological/histology report. For every new case, the following data items are captured:-

- **Inpatient- number:** A unique in-patient number given to the patient when they are first admitted on the ward. This number links a patient to all other numbers that are used in the hospital. The IP number will be the primary number used by the registry and data entry will not be possible when the in-patient number is missing.
- **Demographical information:** This information includes, names, date of birth, gender, age and tribe of the patient.
- **Parent’s Names:** Refers to names of the biological parents (father and mother) or the guardian.
- **Address of Family residence:** Refers to the address of the residence where the patient was living when they developed the symptoms that led to a diagnosis of BL. The address should include the country,

district, county, Sub County, parish and village. In addition, the registry captures limited information about:

- Symptoms and their duration
- Anatomical sites and organs involved
- Laboratory results including the method of BL confirmation, type of biopsy if done, bone marrow results, HIV serology and cytology results etc
- Any other diagnoses made

## ***2.4 Detailed Instructions on the Use of the BL Registry Data Base***

### ***Application***

The registry handles confidential patient data, thus, the registry data entry application is password protected to maintain confidentiality. You must log in using a User ID and a password to start using the application. You will be assigned a User ID and a temporary password by the BL Registrar (or EMBLEM Study Manager). If you forget your password, the Registrar will assign you a new temporary password. When you open the application, you will be prompted to enter your User name and password, and the application will open and display the main menu when the requested information is entered correctly. The main menu is where you can navigate between patient's records and modules.

#### **2.4.1 New cases**

Select the new patient option from the Main menu to begin keying data for new patients. You are now ready to transfer information from the paper form into the Data Entry Application. The application is similar in arrangement to the BL registry forms.

#### **Section A: BL Basic Diagnosis information**

The first field (Q1) captures **the date the patient was admitted at Lacor hospital**. The date is keyed as date, month and year, respectively, using two digits for the date and month and four digits for the year e.g. for 3<sup>rd</sup> of April 2009 is keyed as follows: 03/04/2009. Date is captured as one field. The application will not accept dates that are greater than today's date, i.e., dates

in the future. You can key in the date by selecting a date from the calendar displayed by the application. Make sure you select the correct month and year.

The second field (Q2) captures **the unique In Patient number** for the patient. The In Patient ID number is unique, it is not allocated to two different patients, and thus, the data application system uses the IN patient ID numbers as the unique subject identifier. Records cannot be keyed without this number.

Question 3 (Q3) captures information of **whether the patient is newly diagnosed or not**. Please note that “new” does not refer to patient the attending St. Mary’s Hospital, Lacor, for the first time, but to patient newly diagnosed with BL. Select the appropriate choice from the drop down menu.

The fourth field (Q4) captures **treatment history for the patient**. This question has two sub-questions: the first sub-question is for **the date the diagnosis was made** and the second sub-question is for the **institution where the diagnosis was made**. The date is keyed as previously explained with two digits for the date and month and four digits for the year e.g. for 3<sup>rd</sup> of December 2009 key in as follows: 03/12/2009. A date in the future will not be accepted. You can select the date from the drop down calendar. For the second sub-question, please choose the name of the institution where the patient was diagnosed from drop down menu.

### **Section B: DEMOGRAPHIC Module**

**In field 5(Q5)** Enter the patient’s surname in the first field and the patient’s other names in the second field. The Application requires you to specify at least one of the names. Numerals will not be accepted in the field for names, so a name such as Junior 1 will not be accepted but Junior one will. The system will change and store all the names entered in small alphabet to capitals letters.

The sixth field (Q6) is for the patient’s **date of birth** if this is known. If the date of birth is not known, key in the estimated age in the next field. The field takes date of birth as for other dates, i.e., date, month and year, dd/mm/yyyy

respectively, keyed in as two digits for the date and month and four digits for the year e.g. for 3<sup>rd</sup> of April 2009 key in as follows: 03/04/2009. This date cannot be greater than today (current date). You can choose the dates from the displayed system calendar. When the full date of birth is not known, you can enter the “15”, and for month “07” and the year. If none is known, enter the estimated age. The system will display an error message if both the date of birth and the estimated age are not keyed.

Field (Q7) is for the **patient’s gender**. Select the gender from the drop down menu in the Application. This field must be keyed otherwise the application will display an error message.

The eighth field (Q8) is for the **patient’s tribe**. Select the tribe from the drop down menu.

Field 9 (Q9) is for the **patient’s mother’s surname and other names** respectively. The computerized form requires you to specify at least one of the names and, as for the patient’s names numerals will not be accepted in the names field.

Field 10 (Q10) is for the **patient’s father’s surname and other names** respectively. The computerized form requires that you to specify at least one of the names and as for the patient’s names numerals will not be accepted in the names field.

Field 11 (Q11) is for the **patient’s guardians surname and other names** respectively. The details of the guardians may be missing, so you may skip these fields, however, as for the patient’s names numerals will not be accepted in the names field.

Field 12 (Q12) is for the **residence of the family** where the patient normally lives. The fields to be keyed include village, parish, sub-county, county, district, country and telephone contacts (two 10-digit mobile phone numbers if available). Because some caretakers may not clearly and correctly provide details of the location of their residences, you can use the drop-down menu to

find correct administrative hierarchy that fits in the patient's village and parish. Enter the country first from a drop down menu, then select the district from the drop down menu of districts then select the county. Difficulties may arise as some guardians/patients may not correctly report their counties, sub-counties, parishes and villages. Thus, you will be able to type in the corresponding village, Parish, sub-county. All entries will be converted and stored in capital letters.

### **Section C: SYMPTOMS AND SIGNS module**

Field 13 (Q13) is for **the present symptoms reported by the patient** or observed. The application (and form) has a list of reported symptom to select from. You can key in a symptom that is not on the list in the field specifying "other symptoms".

Field (Q14) is for **duration since the onset of the first symptoms**. Select the appropriate time period from the drop down menu.

Field 15 (Q15) has three sub questions. Question 15a (q15a) seeks to find out if **the patient was living at the same address as he/she does now** (as recorded in field 12) four months prior to the onset of symptoms. If not, then question 15b (q15b) requires that you specify **the address where the child was living onset of the symptoms** (village, parish, sub-county, county, district and country) while in question 16 (Q16) **enter the length of stay of this child at this address**. For field 15a, select Yes or No from a drop down menu. If you select "Yes", then the application will skip address fields of q15b and Q15c because that information is not required. However, if you select "No", then, the fields of question 15b and question 16 will be active requiring entries.

The attributes captured by field 15c village, parish, sub-county, county, district, country and some telephone contacts (two 10-digit mobile phone numbers if available). Key in the country first from the drop down menu, followed by district selected from the drop down menu for the districts, followed by county from the drop down menu in the districts in Uganda. Difficulties may arise as some guardians/patients may not correctly report

their counties, sub-counties, parishes and villages. Thus, you may type in the corresponding village, Parish, sub-county. All entries will be converted and stored in capital letters.

Question 16 has two fields for **duration the child stayed at the residence where the symptoms started**, the first field is for **the years** while the second field is for **the months**. These entries are numeric with no digits allowed.

#### **Section D: ANATOMICAL SITES module**

Field 17 (Q17) is for the **affected anatomical sites** and the **side of the body affected**. For example, for maxilla involvement, you will record the left, the right or both sides affected from the drop down menu. .

Field (Q18) is for **the organs involved**, and **the side where applicable**. Slect by clicking the organ involved. If a cranial nerve is affected, specify the level of involvement in addition to stating the sides affected. Select the correct answer from a drop down menu which is in Roman numbering running from I to XII.

#### **Section E: BL CONFIRMATION module**

*Question 19(Q19)* has two sub questions. Under the first sub-question (Q19a) paediatrician will specify **how the cancer/BL was confirmed**. The method of confirmation is either by biopsy or clinical. Select the response from the drop down menu. For cases confirmed by *biopsy*, key in the date the biopsy was done, biopsy number, institution that did the biopsy and the type of biopsy (Incision/excision, fine needle aspiration or trucut). To capture the date when the biopsy was done, you must enter two digits for the date and month and four digits for the year e.g. for 3<sup>rd</sup> of April 2009 key in as follows: 03/04/2009. Date is entered as one field. The application will not accept dates that are in the future at the time when you are keying in the data, i.e., the data cannot be greater than today's date. The system displays a calendar from which you can choose the appropriate dates (date, months and year). The name of the institution and type of biopsy done shall be chosen from a drop down menu. If diagnosis was made *clinically*, specify the date of clinical diagnosis. You must enter two digits for the date and month and four digits for the year e.g.



for 3<sup>rd</sup> of April 2009 key in as follows: 03/04/2009. Date is entered as one field. Do not enter dates that are in the future at the time when you are keying in the data, i.e., the data cannot be greater than today's date. The system displays a calendar from which you can choose the appropriate dates (date, months and year).

In the second sub-question (Q19b), enter the exact type of cancer finally diagnosed. Among the list of likely diagnoses include Burkitt Lymphoma, Hodgkin Lymphoma, Non Hodgkin Lymphoma, Acute Lymphocytic Leukaemia, Wilm's Tumour, Sarcoma, etc. though the registry is purposely for capturing BL cases. Given the fact that all cancer children are kept in the same Burkitt Lymphoma ward, this variable is meant to help the hospital track all the cancers admitted. I choose from a drop down menu the type of cancer diagnosed.

Field Q20, Q21 and Q22 is for the **results of C.S.F Cytology, bone marrow and HIV serology tests**, respectively. For each of the questions, select the appropriate option from a drop down menu.

Field Q23 is for **any other illnesses diagnosed on the patient**. You must type these illnesses as presented on the hard-copy form.

If you are the one completing the hardcopy of the form, you must complete the fields for your name and the dates you completed the forms. This information will be captured by the application. Names of people authorized to complete forms can be selected from a dropdown menu while the dates require two digits for the date and month and four digits for the year e.g. for 3<sup>rd</sup> of April 2009 key in as follows: 03/04/2009. Date is captured as one field. The application will not accept dates that are in the future at the time when you are keying in the data, i.e., the data cannot be greater than today's date. The system displays a calendar from which the user can choose the appropriate dates (date, months and year)

### ***Appending items on a drop down menu***

The system gives you an opportunity to append to a list a new item. For example, if you came across a new health unit or a new country. In case you

encounter a new “valid entry”, such as a tribe that is not part of the existing list, then you have an option of appending on the list by clicking on the funnel like icon on the right of the screen. To do this, specify what you want to append to say tribe and then click on the funnel-like icon.

Once you have clicked on the funnel-like icon, the system opens a table with the already existing items to which you append the new item. Appending an item requires you to assign it a unique ID number and stating its Name or description. Once you have appended the item close, the form so as to access in the application for use.

Drug ID	Drug Name
1	Cyclophosphone
2	Vinicristine
3	Methotrexate
4	Prednisolone
5	Dexamethasone
*	

**Entering information into the corresponding application sections**

I Enter the corresponding information of each section by clicking on the appropriate tab. Enter the appropriate inpatient unique ID number to begin data entry for a new subject.

Tab	Information captured	Summary guide
<b>Demographics</b>	The tab has variables that capture the information on the patients' names, age, tribe, and sex.	Choose from a drop down menu to enter the tribe and sex. If the tribe is not among the displayed list, Append on the list of tribes by clicking on the funnel like icon on the left of the screen, specify as to what item to append to (in this case- tribe) and the form for tribes will be displayed to allow you append. Appending requires you to assign a unique number for the tribe and the description of the tribe
<b>Family</b>	country, district,	Choose from the drop down menu for

<b><i>residence</i></b>	county, Sub County, parish and village, address location description	each. Append on the list of appropriate variable by clicking on the funnel like icon on the left of the screen, specify to what variable to append to and the form will be displayed to allow you append. Appending requires you to assign a unique number and the description. You also enter the narrative description of the address location
<b><i>Symptoms</i></b>	Reported symptoms and time since onset of first symptoms	Click against each reported symptom, for example if bleeding was a reported symptom; then click against bleeding. A symptom is selected if a tick appears on it and de-selected if there is no tick. Choose from the dropdown menu the duration of symptoms
<b><i>Residence on symptoms</i></b>	country, district, county, Sub County, parish and village, general description of address	This requires specifying whether the child/patient was living in the family residence four months prior to the emergency of symptoms. If not specify the residence of the child (country, district, county, Sub County, parish and village). There is a provision for describing the general location of the residence. Choose from the drop down menu for each. Append on the list of appropriate variable by clicking on the funnel like icon on the left of the screen, specify to what variable to append to and the form will be displayed to allow you append. Appending requires you to assign a unique number and the description. In case yes, the address details will be inactive (requiring no

		entry).
<b>Anatomical sites</b>	This tab has two other tabs, one for specifying the anatomical sites and the other for the organs affected.	Specify an anatomical site/affected organ by clicking against it. For some of the anatomical sites/affected organs, for example Maxilla swelling you are required to specify the location/affected side (left or right). If both sides are affected, then both left and right icons are selected. In case an organ or site other than those in list is affected, the user types it under others.
<b>BL Confirmation</b>	Confirmation method, dates and institutions; actual final diagnosis, C.S.F histology, Bone marrow and HIV serology,	Choose from a drop down menu for the appropriate possible results. In case the institution is not among the list, click on the funnel-like icon to append.
<b>Other diagnosis</b>		Provide a descriptive description of any other diagnosed illnesses
<b>Entrants</b>	Form and application entrants	Enter details of the people who filled the registry form and those who transferred the information into the computerised registry
<b>EMBLEM</b>	EMBLEM Screener	Capture information as is on the EMBLEM screener. This information is to be used to verify against the Registry information

#### 2.4.2 Returning cases

A patient on BL specific treatment is considered a returning case. On the Main menu, select the Returning patient option, a screen where you can key-

in data is opens. You are now ready to transfer information from the paper form into the Data Entry Application. The application is organized to follow the same sequence as the paper data collection forms.

Enter the unique IN Patient ID to begin keying data for an old subject who is returning for review. Since this number is the one given to the patient when they are first identified and registered by the Registry, a number will not be accepted if it was not registered in the new cases, as this implies that the preliminary information on this patient as a new patient to the hospital has never been entered.

After entering the inpatient number, the next step is to enter the current date (the date this patient returned), the recorded temperature ( $^{\circ}$  C), and the weight (Kgs). This will be followed by capturing any other new diagnoses made.

The application user will then tick against the drugs administered and for each drug; the entrant will specify the dosage and route. You enter the number of cycles given, the name of the institution giving the treatment and the date the treatment was given.

If you are the one completing the hardcopy of the form or transferring the information you must write down your name. This information will be captured by the application. Select from a dropdown menu while the dates require two digits for the date and month and four digits for the year e.g. for 3<sup>rd</sup> of April 2009 key in as follows: 03/04/2009. Enter date as one field. The application

will not accept dates that are in the future at the time when you are keying in the data, i.e., the data cannot be greater than today's date. The system displays a calendar from which you can choose the appropriate dates (date, months and year)

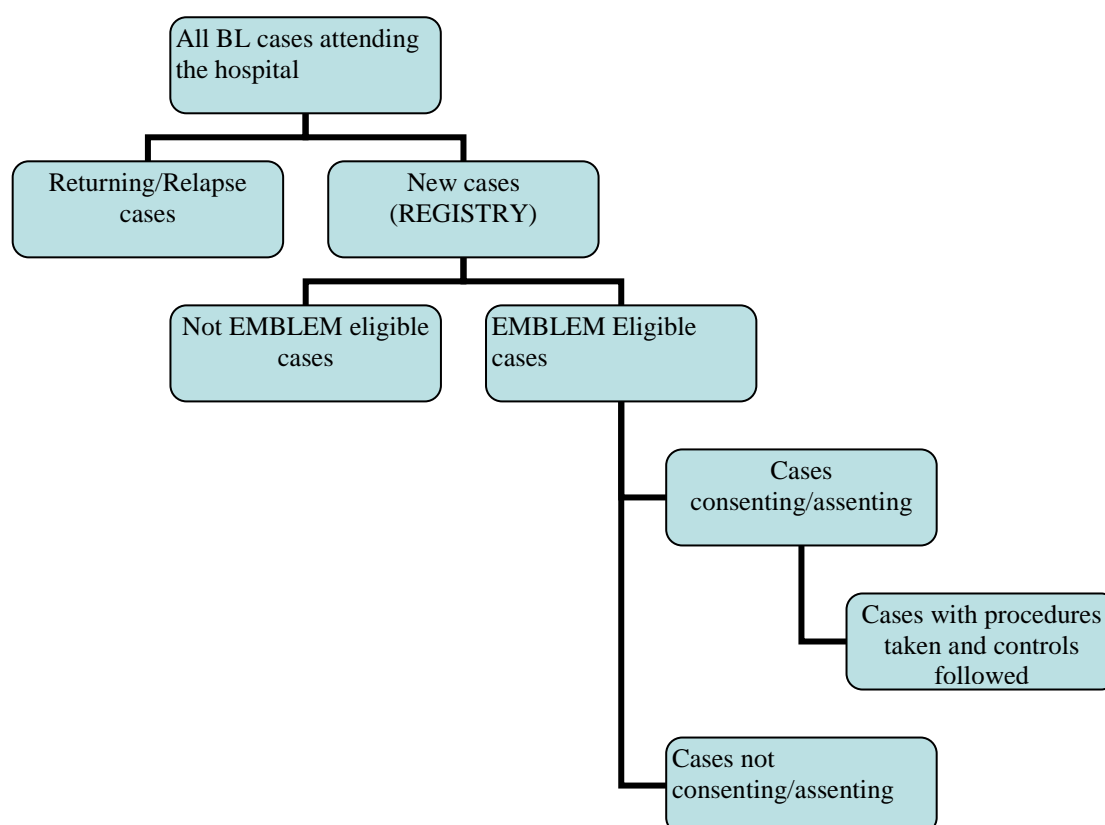
## **2.5 Analysis**

You can run queries in MS ACCESS to generate results of your choice. In addition, you can export the data to other statistical packages such as SPSS or MS Excel for detailed analysis and cross tabulations. The information attained from the registry includes:-

1. demographic summaries e.g. sex and gender summaries
2. distribution of BL cases (areas where cases come from)
3. main symptoms reported by BL patients
4. BL anatomic sites and affected organs
5. BL confirmation results (BIOPSY, Cytology, HIV serology etc)

The registry captures some information important for the screening process of EMBLEM. The registry captures information on every new hospital BL patient admitted at the hospitals. These patients shall be eligible to EMBLEM if:-

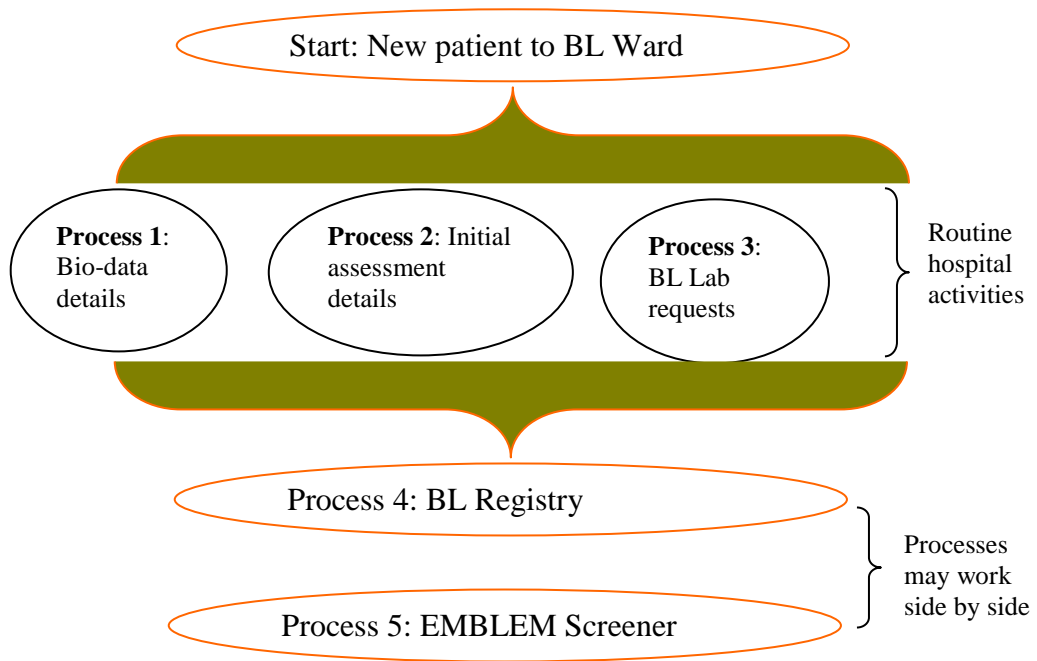
- Age  $\leq$  14
- Resides in catchment area
- No prior chemotherapy
- Clinically stable



Through running of queries from the data base, eligible EMBLEM cases shall be identified (automatically), and their results shall be compared with the EMBLEM screener form which was completed manually. This registry has a page where the EMBLEM screener form (completed manually) will be entered and these results shall be counter checked against those run automatically through queries. This is aimed at ensuring consistency in screening.

However, because information completed in the BL form is summarised from the routine hospital forms and filling of the BL forms depends on the availability of the paediatrician, it takes a while to have the registry form filled. Sometimes, the registry form may be completed after the patient is discharged. There is likelihood of delayed entry of information in the registry forms. Subsequently, the manual screening of cases will work side by side with the Registry to ensure that EMBLEM eligible cases are identified in time and the required samples for the study are taken before they begin BL specific chemotherapy.





3. *Appendix (BL Form)*

**BURKITT LYMPHOMA (BL) HOSPITAL REGISTRY FORM**

**COMPLETE THE FOLLOWING INFORMATION FOR EVERY NEW BL PATIENT TO LACOR HOSPITAL**

**SECTION A: BL HISTORY**

1. DATE OF ADMISSION:  /  /   
DAY MONTH YEAR
2. INPATIENT'S NUMBER:  /   
NUMBER YEAR
3. TYPE OF BL PATIENT: ☐ NEW (*skip to Question 5*)  
☐ ON TREATMENT  
☐ RELAPSE
4. IF **TREATMENT OR RELAPSED**,  
a. DATE FIRST DIAGNOSED:  /  /   
DAY MONTH YEAR
- b. HEALTH UNIT: \_\_\_\_\_

**SECTION B: DEMOGRAPHIC**

5. PATIENT SURNAME: \_\_\_\_\_ OTHER NAME: \_\_\_\_\_
6. PATIENT DATE OF BIRTH:  /  /  OR AGE:   
DAY MONTH YEAR
7. PATIENT'S GENDER: ☐ MALE ☐ FEMALE
8. PATIENT'S TRIBE: \_\_\_\_\_
9. MOTHER'S SURNAME: \_\_\_\_\_ OTHER NAMES: \_\_\_\_\_
10. FATHER'S SURNAME: \_\_\_\_\_ OTHER NAMES: \_\_\_\_\_
11. GUARDIAN'S SURNAME: \_\_\_\_\_ OTHER NAMES: \_\_\_\_\_

12. RESIDENCE OF THE FAMILY (where child usually lives):

LCI/Village:

LCII/Parish:

Sub-county:

County:

District:

Country:

TELEPHONE:  /

Description of address: \_\_\_\_\_



18. ORGANS INVOLVED (PHYSICAL EXAM AND RADIOLOGY)(Select ALL that apply:

**ORGAN**

- a. Cranial nerve ☐ Left ☐ Right Specify level: \_\_\_\_\_
- b. Kidney ☐ Left ☐ Right
- c. Ovaries ☐ Left ☐ Right
- d. Paraplegia or Paraparesis ☐
- e. Incontinence (stool/urine) ☐
- f. Spleen ☐
- g. Liver ☐
- h. Lungs/Pleura ☐
- i. Mediastinum masses ☐
- j. Bowel ☐
- k. Pancreas ☐
- l. Others, specify \_\_\_\_\_

**SECTION E: BL CONFIRMATION**

19. a) METHOD OF CONFIRMATION OF BL DIAGNOSIS? ☐ BIOPSY  
☐ CLINICAL

**IF BIOPSY,**

- a. DATE OF BIOPSY:  /  /   
DAY MONTH YEAR
- b. BIOPSY PERFORMED AT: \_\_\_\_\_
- c. BIOPSY NUMBER:
- d. TYPE OF BIOPSY: ☐ INCISION/EXCISION  
☐ FINE NEEDLE ASPIRATION  
☐ TRUCUT BIOPSY

**IF CLINICAL,**

- a. DATE OF CLINICAL DIAGNOSIS:  /  /   
DAY MONTH YEAR
- b) FINAL CANCER DIAGNOSED: \_\_\_\_\_

20. C.S.F CYTOLOGY RESULTS: ☐ NOT DONE  
☐ NEGATIVE FOR MALIGNANT CELLS  
☐ POSITIVE FOR MALIGNANT CELLS

21. BONE MARROW RESULTS: ☐ NOT DONE  
☐ NEGATIVE FOR MALIGNANT CELLS  
☐ POSITIVE FOR MALIGNANT CELLS

22. HIV SEROLOGY: ☐ NOT DONE  
☐ NEGATIVE  
☐ POSITIVE

23. LIST OTHER DIAGNOSES:

- a. \_\_\_\_\_
- b. \_\_\_\_\_
- c. \_\_\_\_\_

FORM FILLED BY: \_\_\_\_\_ DATE: \_\_\_\_\_

NAME OF ENTRANT INTO COMPUTER: \_\_\_\_\_ DATE: \_\_\_\_\_

# BURKITT LYMPHOMA (BL) HOSPITAL REGISTRY FORM (TREATMENT)

**COMPLETE THE FOLLOWING INFORMATION FOR EVERY PATIENT ON  
TREATMENT OR RELAPSE**

1. INPATIENT'S NUMBER: \_\_\_\_\_  
NUMBER
YEAR

2. TODAY'S DATE: \_\_\_\_\_  
DAY
MONTH
YEAR

3. ROUTINE INVESTIGATIONS,
- a. Temperature: \_\_\_\_\_ (°C)
  - b. Weight: \_\_\_\_\_ (Kilograms)
  - c. Height: \_\_\_\_\_ (Centimeters)

4. LIST OTHER DIAGNOSES:
- a. \_\_\_\_\_
  - b. \_\_\_\_\_
  - c. \_\_\_\_\_
  - d. \_\_\_\_\_

5. TICK ALL DRUGS ADMINISTERED

a) DRUGS

	<i><b>DRUG</b></i>	<i><b>DOSAGE</b></i>	<i><b>ROUTE</b></i>
a.	Cyclophosphone		
b.	Vinicristine		
c.	Methotrexate		
d.	Prednisolone		
e.	Dexamethasone		
f.	others, specify _____		

b) NUMBER OF CYCLES GIVEN \_\_\_\_\_

c) INSTITUTION GIVING DRUGS: \_\_\_\_\_

d) DATE DRUGS GIVEN: \_\_\_\_\_  
DAY
MONTH
YEAR

FORM FILLED BY: \_\_\_\_\_ DATE: \_\_\_\_\_

NAME OF ENTRANT INTO COMPUTER: \_\_\_\_\_ DATE: \_\_\_\_\_