

[YEAR][COUNTRY] Malaria Indicator Survey

Biomarker Manual

IMPLEMENTING AGENCY

SEPTEMBER 2015

PREFACE

In combination with classroom instruction and practical experience, this manual will be used to teach you how to collect biomarker data for the [YEAR] [COUNTRY] Malaria Indicator Survey. Before each training session, you should carefully study this manual and the Biomarker Questionnaire. You are encouraged to ask questions during training and to discuss problems encountered in order to avoid making mistakes during fieldwork. The stages of training are described below:

- During the first phase, we will review with you the chapters of this manual. You will learn how to identify eligible children, record information relating to biomarker collection in the biomarker questionnaire or on special field forms, handle the technical procedures involved in blood collection, testing, and transportation, and other related instructions.
- In the second phase, you will practice the procedures you've been taught by role playing with other trainees. This practice will include finger pricks for anemia and malaria testing.
- In the third phase, you will visit a clinic or a health center and, with their parents' consent, practice collecting blood samples for biomarker testing from infants and young children.
- In the final phase, known as field practice, you will be assigned to a MIS field team. During field practice, you will collect blood samples for biomarker testing from eligible children exactly as you will during the main survey. Households that you visit will be in clusters that are not part of the MIS sample.

Throughout the training, you may be given homework assignments and tests. At the end of the training, your overall performance will be assessed and those who have performed the best will be selected to work in the survey.

Your training does not end at the start of fieldwork. Rather, it is a continuous process. Your team supervisor and the MIS health and survey coordinators will play important roles in continuing your training and in ensuring the quality of data you collect throughout the survey. They will:

- Periodically observe your fieldwork activities to ensure that you are conducting yourself professionally, obtaining informed consent/assent from a parent/responsible adult and/or child, and following the biomarker data collection protocol correctly;
- Spot check that you collected biomarkers from the correct households and only from eligible children;
- Collect blood specimens for transport to the laboratory and consolidate the field record forms;
- Regularly meet with you to discuss your performance and give out future work assignments.

Any field staff member who is not performing at the level necessary to produce the high quality data required to make the [YEAR] MIS a success may be released from service.

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CHAPTER 1: GENERAL INFORMATION

OVERVIEW OF BIOMARKER COLLECTION

Note: References to microscopy should be removed in countries utilizing only Rapid Diagnostic Tests (RDTs) for parasite measurement.

Health technicians occupy a central position in the MIS because she/he collects information from respondents. Therefore, the success of the MIS depends on the quality of the health technician's work. The health technicians will:

- a) Confirm children's eligibility for biomarker sample collection
- b) Complete the Biomarker Questionnaire and return it to the interviewer.
- c) Administer consent to parents/guardians and to eligible children, according to the survey protocol, before collecting biomarkers
- d) Collect biomarker specimens for :
 - Malaria using Rapid Diagnostic Tests (RDTs)
 - Malaria using thick and thin smears
 - Anemia using the HemoCue 301
- e) Prepare the thick and thin smears for transport to the reference laboratory according to the protocol, including fixing the thin smears
- f) Give treatment to positive malaria RDT cases as per the study protocol
- g) Refer severe malaria and anemia cases to health care facilities
- h) Ensure that the biomarker supplies are well-stocked and appropriately stored
- i) Follow bio-safety standard operating procedures

A biomarker may be thought of as a characteristic that can be independently measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic response to a therapeutic intervention¹. Biomarker measurements can serve as diagnostic tools to identify diseases in their early stages and can be used as surveillance tools to track changes in disease patterns or to evaluate intervention programs. In population-based surveys, biomarkers help assess the prevalence or occurrence of diseases or conditions and can also be used at a macro level to measure the long-term effect of policies and programs. In MIS surveys, biomarkers are collected in order to report levels of specific disease and conditions on a population level. Specific to the [YEAR] [COUNTRY], the following biomarkers will be tested: **hemoglobin and malaria parasites in blood**. This training manual will discuss the proper collection techniques and the appropriate recording and result reporting of these biomarkers.

Biomarker collection should take place after the completion of the Household and Woman's questionnaires. A Biomarker Questionnaire will be used. Prior to collection, certain tasks must be completed. This chapter reviews the tasks that need to be completed prior to collecting biomarkers:

- Determining eligibility

¹ Biomarker Definitions Working Group, National Institutes of Health, 2001

- Obtaining informed consent.

ELIGIBILITY

Not all household members are eligible for biomarker data collection. Members of the household who are eligible for biomarker data collection are: children age 6 to 59 months old who are usual household residents or are visitors that have stayed in the house the night before the household interview took place. **It is the responsibility of the interviewer** to identify all children eligible for biomarker data collection and to enter their names and line numbers into the Biomarker Questionnaire. Individuals eligible for biomarker data collection can be identified by reviewing these columns from the Household Schedule:

- Column (1) Line number
- Column (2) Name
- Column (5) and (6) Residency (a 'yes' is necessary for either column)
- Column (7) Age of household member
- Column (9) Identification of eligible children

HOUSEHOLD SCHEDULE

LINE NO.	USUAL RESIDENTS AND VISITORS	RELATIONSHIP TO HEAD OF HOUSEHOLD	SEX	RESIDENCE		AGE	ELIGIBILITY	
				5	6		8	9
1	2	3	4	5	6	7	8	9
	<p>Please give me the names of the persons who usually live in your household and guests of the household who stayed here last night, starting with the head of the household.</p> <p>AFTER LISTING THE NAMES AND RECORDING THE RELATIONSHIP AND SEX FOR EACH PERSON, ASK QUESTIONS 2A-2C TO BE SURE THAT THE LISTING IS COMPLETE.</p> <p>THEN ASK APPROPRIATE QUESTIONS IN COLUMNS 5-9 FOR EACH PERSON.</p>	<p>What is the relationship of (NAME) to the head of the household?</p> <p>SEE CODES BELOW.</p>	<p>Is (NAME) male or female?</p>	<p>Does (NAME) usually live here?</p>	<p>Did (NAME) stay here last night?</p>	<p>How old is (NAME)?</p> <p>IF 95 OR MORE, RECORD '95'.</p>	<p>CIRCLE LINE NUMBER OF ALL WOMEN AGE 15-49</p>	<p>CIRCLE LINE NUMBER OF ALL CHILDREN AGE 0-5</p>
01		<input type="text"/>	M F 1 2	Y N 1 2	Y N 1 2	IN YEARS <input type="text"/>	01	01
02		<input type="text"/>	1 2	1 2	1 2	<input type="text"/>	02	02

The interviewer will now record the names and line numbers of children eligible for biomarker collection in the first rows of the Biomarker Questionnaire.

HEMOGLOBIN MEASUREMENT AND MALARIA TESTING FOR CHILDREN AGE 0-5

101	CHECK COLUMN 9 IN HOUSEHOLD QUESTIONNAIRE. RECORD THE LINE NUMBER AND NAME FOR ALL ELIGIBLE CHILDREN 0-5 YEARS IN QUESTION 102; IF MORE THAN SIX CHILDREN, USE ADDITIONAL QUESTIONNAIRE(S).			
		CHILD 1	CHILD 2	CHILD 3
102	CHECK HOUSEHOLD QUESTIONNAIRE: LINE NUMBER FROM COLUMN 9.	LINE NUMBER <input type="text"/> <input type="text"/> NAME _____	LINE NUMBER <input type="text"/> <input type="text"/> NAME _____	LINE NUMBER <input type="text"/> <input type="text"/> NAME _____
103	IF MOTHER INTERVIEWED: COPY CHILD'S DATE OF BIRTH (DAY, MONTH, AND YEAR) FROM BIRTH HISTORY. IF MOTHER NOT INTERVIEWED ASK: What is (NAME)'s date of birth?	DAY <input type="text"/> <input type="text"/> MONTH <input type="text"/> <input type="text"/> YEAR ... <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	DAY <input type="text"/> <input type="text"/> MONTH <input type="text"/> <input type="text"/> YEAR ... <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	DAY <input type="text"/> <input type="text"/> MONTH <input type="text"/> <input type="text"/> YEAR ... <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>
104 (2)	CHECK 103: CHILD BORN IN 2010-2015?	YES 1 NO 2 (SKIP TO 130) ←	YES 1 NO 2 (SKIP TO 130) ←	YES 1 NO 2 (SKIP TO 130) ←
105	CHECK 103: CHILD AGE 0-5 MONTHS, I.E., WAS CHILD BORN IN MONTH OF INTERVIEW OR 5 PREVIOUS MONTHS?	0-5 MONTHS 1 (SKIP TO 130) ← OLDER 2	0-5 MONTHS 1 (SKIP TO 130) ← OLDER 2	0-5 MONTHS 1 (SKIP TO 130) ← OLDER 2
106	LINE NUMBER OF PARENT/OTHER ADULT RESPONSIBLE FOR THE CHILD FROM COLUMN 1 OF HOUSEHOLD SCHEDULE.	LINE NUMBER <input type="text"/> <input type="text"/> (RECORD '00' IF NOT LISTED)	LINE NUMBER <input type="text"/> <input type="text"/> (RECORD '00' IF NOT LISTED)	LINE NUMBER <input type="text"/> <input type="text"/> (RECORD '00' IF NOT LISTED)

The Biomarker Questionnaire is then given to the health specialist to record all data related to biomarker collection. It is the responsibility of the health specialist to verify that the information recorded by the interviewer is properly recorded upon receipt on the biomarker questionnaire. Verify that the child's name and line number is written in Q. 102. Proceed to Q. 103 to record the exact date of birth (day/month/year). In Q. 104, check Q. 103 to see if the child was born anytime in the years 2011-2015. If so, record 1 and (s)he is eligible for testing. If a child was born prior to January 2011, record 2, s(h)e is not eligible for testing, go to Question 103 for the next eligible child or if no more children, end the interview.

The following are important points to keep in mind when completing the Biomarker Questionnaire:

1) Biomarker data should be collected from eligible children only after eligible woman's (child's mother or responsible adult) interview has been completed.

However, in a household with no eligible woman, the eligible child will be tested for anemia and malaria **only** after questions 101 to 110 of the Biomarker Questionnaire have been completed.

2) Collect biomarkers from one child at a time. All biomarker information collected for the MIS should be collected on one child before moving on to the collection of biomarkers from the next eligible child. For example, if there is more than one eligible child in a household, and the responsible adult has consented to biomarker collection, test the child for anemia and presence of malaria parasites before proceeding to the next child. Failure to do so may lead to results being recorded in the wrong columns of the questionnaire.

INFORMED CONSENT

One of the primary tasks before biomarker collection is to explain the purpose of the testing to the individuals involved, and to obtain their consent before collecting any blood samples. In order to ensure that these individuals can make an “informed” decision about whether or not to participate in the testing, there are consent and assent statements which you will need to read to the appropriate individuals. These consent and assent statements include the following basic elements:

- a description of the objectives of the test
- basic information on how the test will be conducted
- assurances about the confidentiality of the results
- a specific request for permission to collect the sample

Ask the parent/responsible adult or the child the age of the child.

For children age 0-5 years:

Read the document *PARENTAL CONSENT DOCUMENT FOR THE MALARIA AND ANEMIA TEST*.

You must obtain the consent of one of the child’s parents, or, in the absence of a parent, the consent of a responsible adult who is at least 18 years of age.

If the parent/responsible adult does not consent to the test, the test must not be performed.

Prior to performing the anemia or malaria test, you must also record the outcome of the parental consent request in the Biomarker Questionnaire. This is discussed in more detail in the upcoming chapters. You must also sign your name on the Biomarker Questionnaire to indicate that you read the consent statement to the parent/responsible adult and have recorded their response accurately and you must enter your fieldworker number.

KEY POINTS TO REMEMBER INCLUDE:

- 1. Read the applicable consent statements to each parent/responsible adult exactly as they appear.** When you arrive at the household and begin talking about the blood tests with the parent/responsible adult and with the children, you may informally discuss many of the items included in the informed consent statement. However, before beginning the testing procedures, you must still read the informed consent statements exactly as they are worded. If you feel that the parent/responsible adult or the children find the statements repetitive, tell him/her that you are required to read the statement to ensure that they are given all the appropriate information.
- 2. Read the informed consent statements clearly.** Practice reading the consent statements out loud so that you become comfortable delivering them in a clear, natural voice and manner. Avoid speaking rapidly or in a monotone.
- 3. Never attempt to force or coerce consent.** Some parents/responsible adults may be suspicious or fearful of having their child’s blood collected for testing. Others may

have questions or want to discuss the procedures before giving consent. Take time to patiently respond to all questions.

4. Some parents/responsible adults may be reluctant to allow testing without consulting someone not present at the time of your visit (for example, a woman may want to consult her husband before giving permission). **In such cases, make an appointment to return to the household later at an agreed upon time.** If you believe it will help, ask the team supervisor to visit a household where parents express fear or reluctance for their child (children) to be tested.

Once eligible children have been identified, the first part of the Biomarker Questionnaire filled out, and consent for biomarker testing granted, biomarker collection can take place.

Summary of Steps in Identifying Eligible Children and Seeking Informed Consent for Blood Collection

- Interviewers complete the Household and the Woman's Questionnaires.
- Check questions 104 and 105 to confirm children are eligible for biomarker testing:
 - Children 6 to 59 months, who are usual residents or who stayed in the household the night before the interview, are eligible for biomarker testing
- For children 6 to 59 months:
 - Obtain consent for blood collection and testing from parent/responsible adult.
 - Read consent exactly as written;
 - Record the outcome of the consent request and sign in the space provided;
 - If consent was granted, proceed with blood collection.

CHAPTER 2: GENERAL PROCEDURES FOR COLLECTING CAPILLARY BLOOD DROP SAMPLES

Capillary blood will be collected in the MIS to test for anemia and malaria. Capillary blood can be obtained from the palm side of the end of a finger or from a heel. For children 12 months of age and older, a finger should be used. For children 6 - 11 months, the heel should be used. However, if a child is 12 months or older but undernourished or skinny, the underlying tissue can be very thin and a lancet is likely to pierce the bone. For such children, a heel puncture is recommended. This chapter describes the materials needed for and, the steps involved in, capillary blood collection.

MATERIALS AND SUPPLIES FOR PERFORMING FINGER OR HEEL PRICK

The capillary blood drops collected for biomarker testing will be drawn from a finger or the heel. The following supplies and materials will be used in performing the finger or heel prick:

- **Disposable latex gloves:** used to reduce the risk of infection with blood borne diseases. Gloves must be worn by the health investigators and by anyone else who may assist with the blood collection.



- **Absorbent paper sheets:** the surface area where your supplies will be placed while you conduct the blood collection. Place the plastic/shiny side of the absorbent sheet down (the absorbent side without plastic on it should be up).
- **Alcohol preps:** used for cleaning the skin prior to pricking the finger or heel.



- **Safety lancets:** the lancet is a single-use, disposable device used to prick the fingertip or heel (Figure 2.1). The needle is retractable; when the trigger is pressed, a surgical blade quickly protrudes from the device, punctures the skin, and then automatically retracts. The Unistik 3 Normal lancets have a gauge of 23G and a depth of 1.8mm and can be used for children.



- **Preparation of lancet for use:**



- **Sterile gauze pads:** used to wipe away the first drop(s) of blood in order to stimulate a spontaneous capillary blood flow.



- **Adhesive bandages (plasters or band-aids):** applied to the puncture site to minimize the risk of infection.



- **Plastic bag for waste:** Large bags that are provided to hold all of the bio-hazardous waste generated during the day. All waste bags are to be clearly labeled “biohazard”.



- **Sharps Containers:** All biohazardous sharps wastes that have pointed tips like needle, microcuvettes and microscope glass slides.



STEPS IN OBTAINING CAPILLARY BLOOD FROM THE FINGER

The following describe the steps that are involved in obtaining a capillary blood drop sample from the finger.

1. Complete general preparation

- If possible, find an indoor site to encourage privacy. If possible, the site should have a table or other piece of furniture with a flat surface where you can lay out the supplies. A couch, bed or mat should be readily available if the child feels faint and needs to lie down.

- If you find you must do the test outdoors, find a site in the full shade and away from rain, dust, and other environmental elements that might affect the sample quality.
- When and where possible, wash and dry your hands. **Put on gloves** before beginning the collection of the blood sample from the child.
- Take out a clean **absorbent paper sheet** and spread it over a flat surface where you will lay out your supplies.
- Refer to the Biomarker Questionnaire to confirm the number of eligible children for whom blood samples will be collected. After you have established the number of children to test, take out the appropriate equipment and general supplies for the first child. You will want to have all general materials in easy reach when you begin collecting blood samples from the child.
 - **Note-** please do not remove the microcuvette until right before pricking the child. The microcuvette should be taken out on an individual basis. In other words, if three children are being tested for anemia, remove the microcuvette from the canister only one at a time, before pricking each child.
- Describe to the parent/responsible adult exactly what will be done during the collection of the blood sample and how they can assist by holding the child on their lap and holding the child's hand during the collection of the sample.
- The child may be fearful or anxious about what is going to happen. Therefore, using a calm and reassuring manner with the child is important as you begin to collect the blood sample. Remember that nonverbal communication is important, so maintain eye contact with the child as you prepare to take the sample.

2. Select and prepare the prick site

- Blood collection is usually easier if you **sit on the side of the child opposite to the hand** that you will collect blood from. For example, if you want to collect the specimen from the left hand, place yourself to the right side of the child.
- **Use the third or fourth finger** for collecting the blood (Figure 2.2). Do not use a finger with a scar, a wound or cut, an infection, swelling, a deformity, or a rash.

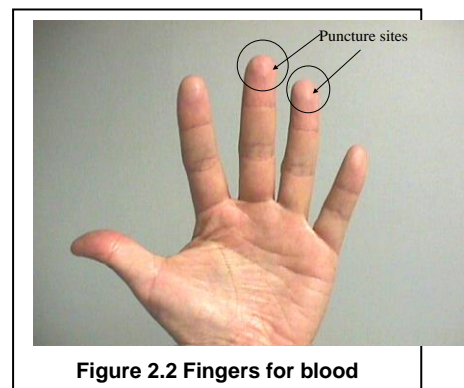


Figure 2.2 Fingers for blood

- **Warm the skin** over the puncture site by rubbing it. This will increase blood flow to the fingertip and improve the ease with which a sample can be obtained.
- **With an alcohol swab, clean the skin of the finger** thoroughly (Figure 2.3). If the skin is very dirty, use a second swab. Finish cleaning the finger before preparing it for the finger prick. Allow the alcohol to air dry. Do not blow on the area



Figure 2.3 Clean the finger

to dry the alcohol. Blowing may allow bacteria to contaminate the site.

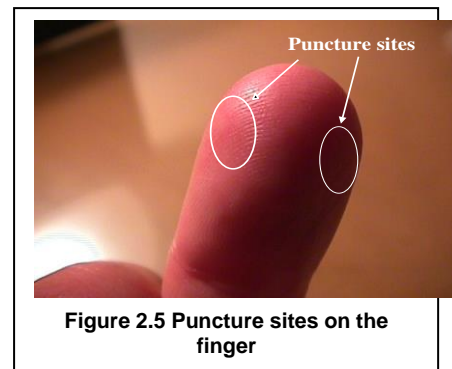
- **Ensure that the lancet is easily accessible.** Use the Unistiks 3 Normal lancet which pierces the skin to a depth of 1.8 mm.

- **Remove the blade slot cover**

- Remove the blade slot cover by first twisting it 360° and then pulling it (Figure 2.4).
- ***Do not remove the blade slot cover from the lancet other than as instructed above***, as this may cause the blade not to pierce the skin.

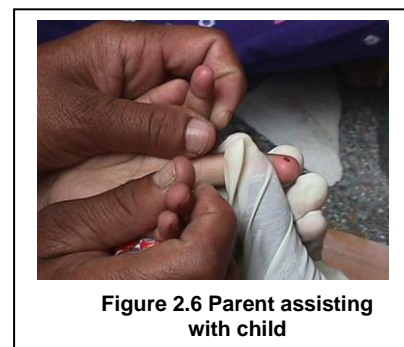


- Place the lancet at the puncture site (Figure 2.5), so that the wide body of the lancet faces up.
- Note: avoid placing the lancet on the very tip of the finger or the sides beyond the palmar area or you will risk piercing the underlying bone.



3. Prick the Finger

- It may be helpful if the parent/responsible adult assists you by holding the child's hand (Figure 2.6).
- **Make sure that the finger is below the level of the child's heart** to increase the flow of blood to the finger. Using a rolling movement of your thumb, **lightly press the finger from the top knuckle toward the tip**. This action will stimulate the flow of blood to the sample area.



- When your thumb reaches the fingertip, **maintain a gentle pressure to trap the blood in the finger tip**.
- Use the lancet to **prick the skin** by placing the blade-slot surface against the area and pressing the trigger. The tip of the blade ejects through the blade slot, producing a micro-incision in the skin, and immediately retracts into the device. After pricking the skin, set aside the lancet and turn the finger slightly to prevent blood from running into the grooves of the fingerprints.

4. Collect the blood drops

- When the blood appears, use a sterile gauze pad to wipe away ***the first one or two large drops of blood depending on the tests being performed***. Note: hemoglobin testing requires the first two blood drops be wiped away (see chapter 3 for more details on collecting blood for hemoglobin testing); blood collection for malaria parasite testing requires only the first blood drop be wiped away (see chapter 4 for more details on collecting blood for malaria parasite testing). If blood is being obtained for both malaria parasite and hemoglobin testing, only the first drop is wiped away and blood collection for malaria rapid test (RDT) precedes that for hemoglobin testing and lastly, thick and thin smears are prepared for malaria microscopy using the fourth and subsequent blood drops (See Chapter 5 for more details on collecting blood for both hemoglobin and malaria parasite testing).
 - If the blood stops flowing before you have done the hemoglobin testing and/or the malaria parasite testing, the pricking procedure may be repeated with the consent from the parent/responsible adult. ***Do not reuse any of the supplies used for the first finger prick.***
5. Discard all materials used in the blood collection procedure in a labeled bio-hazardous waste bag and sharps container.

STEPS IN OBTAINING CAPILLARY BLOOD FROM A CHILD'S HEEL

The **heel** is the puncture site for children age 6 to 11 months or malnourished (skinny) children whose fingers are very thin. A child lancet of 1.8 mm depth will be used to puncture the heel. The following describes the steps that are involved in obtaining a capillary blood drop sample from the heel.

1. The prick should be made outside a line drawn from the middle of the big toe to the heel or outside a line drawn from the area between the fourth and fifth toes to the heel (Figure 2.7). Take care to avoid the central area of the foot (to avoid injury to the nerves and tendons) or the center of the heel (to avoid piercing the heel bone).
2. Hold the heel firmly (Figure 2.8). Apply moderate pressure near the puncture site. This can be done by wrapping the heel using your thumb and second finger.
3. Clean the site with an alcohol swab. Make sure the site is dry before puncturing the skin with the lancet. In selecting a puncture site, avoid any areas of the skin that are broken or infected.
4. Use the lancet for the skin puncture by placing the blade-slot surface against the area and pressing the trigger. Ensure the free flow of blood.

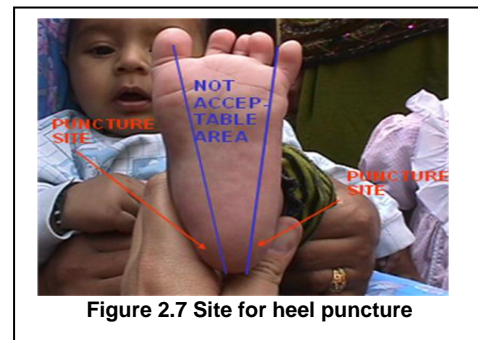


Figure 2.7 Site for heel puncture



Figure 2.8 Holding the infant's heel

- When the blood appears, use a sterile

gauze pad to wipe away ***the first one or two large drops of blood depending on the tests being performed.*** Note: hemoglobin testing in blood requires the first two drops be wiped away (see chapter 3 for more details on collecting blood for hemoglobin testing); blood collection for malaria parasite testing requires only the first drop be wiped away (see chapter 4 for more details on collecting blood for malaria parasite testing). If blood is being obtained for both malaria parasite and hemoglobin testing, only the first drop is wiped away and blood collection for malaria rapid test (RDT) precedes that for hemoglobin testing and lastly, thick and thin smears prepared for malaria microscopy using the fourth and subsequent blood drops (See Chapter 5 for more details on collecting blood for both hemoglobin and malaria parasite testing).

5. Discard all materials used in the blood collection procedure in a labeled bio-hazardous waste bag and the sharps container.

PRECAUTIONS TO OBSERVE WHEN COLLECTING BLOOD SAMPLES²

This section describes the universal (general) precautions to be followed during blood collection³. You should take precautions when collecting blood to prevent exposure to blood borne infections, such as hepatitis B, or human immunodeficiency virus (HIV). Under general precautions the following rules should be followed to ensure protection from acquiring blood borne infections.

Wear gloves. Gloves help to prevent skin and mucous-membrane exposure to blood. Gloves should be worn during blood collection, until the specimen(s) from a child is collected and all waste materials produced during the collection are disposed of. At that point, the gloves used should be treated as bio-hazardous waste. A new pair of gloves should be used with each child. **Gloves must never be reused! Avoid penetrating injuries.** Although gloves can prevent blood contamination of intact and non-intact skin surfaces, they cannot prevent penetrating injuries caused by the instruments used for finger or heel pricks. Safety lancet devices reduce the risk of penetrating injuries.

Lancets should not be used for purposes other than a single finger or heel prick to collect blood for the biomarker testing. The lancets should not be broken or destroyed for curiosity or other purposes. After the device is used, it should be placed in a puncture-resistant disposal biohazard bag.

If an accident occurs, any skin surfaces or mucous membranes that become contaminated with blood should be immediately and thoroughly washed with running water or copious amounts of standing water.

Never eat or drink during the testing. Since eating and drinking can lead to self-contamination, it is not permitted while collecting blood samples.

Properly dispose of all bio-hazardous materials. All materials coming in contact with blood must be placed in a bio-hazardous waste bag and sharps container after use and disposed of according to the survey's policy on infectious waste disposal (see Chapter 6). Take precautions when storing and transporting the waste bags during fieldwork.

² Adapted from National Committee for Clinical Laboratory Standards (NCCLS) 1997

³ For the universal precautions regarding bloodborne pathogens, see the U.S. Centers for Disease Control and Prevention guidelines and the U.S. Occupational Safety and Health Administration (OSHA) standards for protection from exposure to bloodborne pathogen.

GOOD BLOOD COLLECTION PRACTICES

Good position in relation to the child. Position yourself well before you make a puncture on the child's finger.

Do not prick the finger if the hand is cold! Warm the hands by asking the mother to rub the child's hands together vigorously.

Never "milk" the finger. Excessive massaging or squeezing of the finger or foot will cause tissue juice to mix with and dilute the blood. This will result in erroneous test results, particularly yielding low levels of hemoglobin concentration in the blood. Instead, the interviewer or health technician should employ only mild pressure by using the thumb and the second and third fingers to make a "pad" at the puncture site. This will make the connective tissue underlying the skin more porous and allow the capillary blood to flow easily after the incision.

Never mix alcohol with the blood. If the alcohol used to clean the puncture site mixes with the blood, it can cause hemolysis of the sample leading to errors in the testing results. To avoid this problem, the finger or heel must be air dried completely before being punctured.

Avoid obstructing blood flow. It is important to hold the finger properly to allow for the accumulation of blood in the puncture-site area. Holding the finger too tightly can obstruct the blood flow to the finger.

Avoid shallow punctures. A deep puncture should be made for better blood flow and to have a representative concentration of red blood cells.

Dispose of biohazard materials as they are used. Keep the biohazard bag open during blood collection and drop each disposable item into the bag as you finish using it.

Summary of Steps for Capillary Blood Collection

- Seek informed consent
- For children between 6 and 11 months, a heel prick should be performed.
- For children whose fingers are very thin, determine if heel or finger prick should be performed
- Set up capillary blood collection supplies
- Clean the surface of finger/heel
- Maintain gentle pressure to trap blood in the fingertip
- Apply moderate pressure near the puncture site of the heel
- Prick finger/heel using the child lancet
- Collect capillary blood
- Dispose of all testing materials in a clearly labeled biohazard bag or sharps container.

CHAPTER 3: ANEMIA TESTING

Red blood cells contain hemoglobin (Hb), an iron-rich protein that binds oxygen in the lungs and carries it to tissues and organs throughout the body. Anemia is defined as a reduction in the normal number of red blood cells or a decrease in the concentration of Hb in the blood. Symptoms of anemia range from pallor, fatigue and weakness, shortness of breath and heart problems. During the MIS, we will measure the amount of Hb in the child's blood. Children who have an Hb level below a defined cut-off of less than 8.0g/dl will be classified as severely anemic.

Common causes of anemia include:

- Iron deficiency from inadequate intake of foods containing iron, such as red meat;
- Intake of foods that contain non-bioavailable iron (for example iron fortified foods);
- Malaria and other parasitic infections (for example, schistosomiasis; hookworm);
- Blood disorders (for example, sickle cell anemia; thalassemia).

Anemia is a common and serious global health problem. Consequences of anemia include an increased risk of maternal and child mortality, impaired cognitive development in children, increased numbers of pre-term and low birth weight babies, and reduced work productivity in adults.

The measurement of Hb is the primary method of screening for anemia. Hb measurement provides an opportunity to:

- Estimate the prevalence of anemia in a nationally-representative sample;
- Link the levels of anemia with demographic data so as to examine the socioeconomic, residential, and demographic differences in the prevalence of anemia among populations;
- Design programs to prevent iron-deficiency anemia among the populations most in need of intervention (for example, iron fortification programs for women and young children living in rural districts).

Anemia testing in the MIS will be performed using a HemoCue photometer (Hb 301). This widely used system measures Hb concentration from a drop of blood obtained from a finger/heel prick. The test is rapid, allowing results to be reported immediately following the testing procedure. Children found to have severe anemia will be referred to a health facility for treatment. Anemia due to malaria is considered severe when the Hb level is below 8 g/dl for children.

This chapter discusses the materials needed and the procedure for anemia testing. In addition, directions are given regarding precautions to take during collection and testing, recording results in the Biomarker Questionnaire, and providing test results and anemia information to households.

MATERIALS AND SUPPLIES FOR ANEMIA TESTING

In addition to the Biomarker Questionnaire and supplies listed in Chapter 2, the following equipment and supplies are required for anemia testing:

- **Microcuvette:** a plastic disposable unit that serves as both a reagent vessel and a measuring device (Figure 3.1). The tip of the microcuvette contains a dry, yellow reagent (sodium azide). The microcuvette is designed to draw up the exact amount of blood needed for the test.
- **HemoCue Hb 301 photometer:** It is a device that uses the absorption of light to measure hemoglobin concentration from a single drop of blood collected in a microcuvette (Figure 3.2). Test results are presented on the photometer's electronic display. The HemoCue system is described in greater detail below.
- **Anemia and Malaria Brochure:** a two page document designed to educate the household about malaria and anemia, including its definition, symptoms, causes, and methods of treatment and prevention. In addition, the child's malaria and Hb results are recorded and classified within this document. See Appendix A for an example of the brochure.
- **Anemia Referral Slip for Severely Anemic Children:** on this form is written the name and Hb results of severely anemic children. It is to be given to parent/responsible adult of the child with an Hb result indicative of severe anemia. They can take the child and referral slip to a local clinic or health center to receive proper treatment for his/her anemia.



Figure 3.1 HemoCue microcuvette



Figure 3.2 HemoCue Hb301 photometer

The HemoCue Photometer System (Hb 301)

Although the HemoCue system has proven to be durable and reliable under field conditions, there are some technical limitations related to the fact that microcuvettes are sensitive to humidity. Follow these instructions for the proper handling and storage of microcuvettes:

1. Record on the microcuvette container the date the container is first opened;
2. Remove from the container only those microcuvettes required for immediate testing;
3. Remove the microcuvettes by holding the side opposite the tip;
4. Immediately after taking a microcuvette out of the container, snap the container lid back on tightly;
5. Keep the microcuvette container at room temperature and avoid exposing it to heat or strong sunlight.

Under these conditions, a microcuvette container can be stored for up to 3 months (90 days) after opening. Under field conditions, it is advisable to store the microcuvettes in the opened container for no more than a month. Microcuvettes from unopened containers can be used up to the expiration date on the container.

To ensure the HemoCue Hb301 system operates properly, allow the photometer to come to the ambient temperature and protect it from direct sunlight. The device operates optimally between 18 and 30° C. The photometer has an internal electronic “SELFTTEST”; every time the device is turned on, it automatically verifies the performance of its optronic unit.

The photometer’s black microcuvette holder has three operating positions: 1) pushed in, for measuring; 2) pulled out until “clicked,” for placing the microcuvette; 3) completely withdrawn for cleaning.

Clean the microcuvette holder (the drawer) at the end of each day’s fieldwork. For cleaning the holder, use an alcohol swab or cotton wool/cotton-tipped swabs moistened with 70% alcohol. Follow these procedures to clean the microcuvette holder:

1. Check that the analyzer is turned off and the display window is blank.
2. Pull the microcuvette holder out of its loading position. Carefully press the small catch positioned in the upper right corner of the microcuvette holder.
3. While pressing the catch, carefully rotate the microcuvette holder towards the left as far as possible. Carefully pull the microcuvette holder away from the analyzer.
4. Clean the microcuvette holder at the end of each work day with an alcohol swab or cotton wool moistened with 70% alcohol (ethanol or isopropyl alcohol).
5. The HemoCue cleaner swabs are used to clean the optronic unit inside the machine. Clean the optronic unit twice a week or when necessary by pushing the swab into the opening of the microcuvette holder (Figure 3.3). Move the cleaner from side to side 5-10 times. If the swab is stained (blood or dirt), repeat the cleaning procedure with a new swab. It is important that the microcuvette holder is completely dry prior to reinserting it in the photometer.



Blood may get on the optronic system if you do not wipe the outside of the microcuvette before placing the microcuvette in the holder. If this happens, you will get an error message (E01-E05; E09-E30). Clean the HemoCue machine as described above when you get an error message.

COLLECTING BLOOD AND TESTING FOR ANEMIA

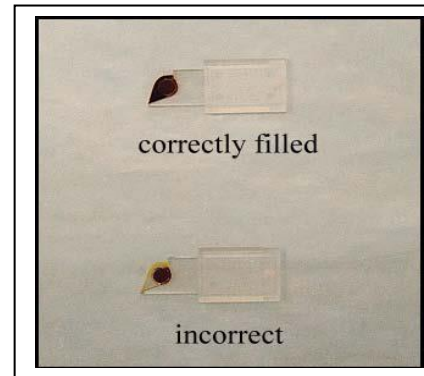
The instructions below describe the appropriate steps and procedures for testing children for anemia. When children are tested for both anemia and malaria, the steps will differ slightly from those in the instructions below; instructions for combined anemia and malaria testing are given in Chapter 5.

Follow the procedure below to test children for anemia:

1. Complete **Question 103** and record the child's day, month, and year of birth.
2. **Question 104** and **Question 105** will determine if the child is really eligible. For **Question 104**, refer to **Question 103** to determine if the child was born anytime in the years 2011-2015. If the child was born before 2011 (e.g. in 2010, 2009, etc), then the child is too old to be eligible for testing. For **Question 105**, refer to **Question 103** to determine if the child is less than 6 months old. If the child was born in the month of interview or in the 5 months before the month of interview, the child is not eligible for hemoglobin measurement. For example, if you are visiting the household in July, a child born in February, March, April, May, June, or July will not be eligible for testing because they would be too young. In other words, if the child is less than 6 months old, the child is not eligible for hemoglobin measurement, so you will record '1' and continue to the next child. If the child is 6 months or older, record '2' and proceed to **Question 106**.
3. In **Question 106**, record the name of the parent or other adult responsible for and responding to the consent process.
4. Seek consent as described in Chapter 1 (**Question 107**).
5. Record the outcome of the consent process in **Question 108**. Confirm that you read the statement to the parent/responsible adult and recorded their response accurately by signing in the space provided and by providing your assigned health tech's number.
6. If consent was granted, collect a finger or heel stick blood sample from the child, following the procedure described in Chapter 2. Use a sterile gauze pad to wipe away the **first two blood drops** from the finger (heel) prick.
7. Conduct the anemia test as follows:
 - Step 1: Collect the capillary blood in the microcuvette:
 - Apply the tip of the HemoCue microcuvette to the middle of the blood drop. The microcuvette chamber will fill itself automatically by capillary action. The chamber needs to be filled completely (Figure 3.4). Never "top off" the microcuvette. Instead, if the microcuvette is not completely filled, use a fresh microcuvette and fill it with the next blood drop that forms.



- Wipe any surplus blood off both sides of the microcuvette “like butter from a knife,” using the clean end of a sterile gauze pad. Ensure that no blood is sucked out of the microcuvette when wiping it – do not let the tip of the filled microcuvette touch the gauze.



- After filling the chamber, the microcuvette needs to be visually inspected for air bubbles (Figure 3.5a). Since air bubbles may cause inaccurate Hb measurements, any microcuvette containing air bubbles must be discarded. In such cases, with the permission of the parent/responsible adult, repeat the blood collection using a different finger (heel). Again, you must use new supplies and follow all of the steps described previously in collecting the new sample.

- Place the microcuvette in its holder and gently push the holder into the photometer (Figure 3.5b).



- Step 2: Stop bleeding at the site of the prick:

- After collecting the blood drop, wipe any remaining blood from the prick site with a sterile gauze pad. Press the gauze pad against the prick site until the blood flow has stopped completely.

- Take an adhesive bandage from its wrapper and apply it to the prick site (Figure 3.6). Advise the mother, especially when the child is a toddler, to watch carefully that child does not take off the bandage and put it in his/her mouth where the child may choke on it.



- Step 3: Obtain the hemoglobin level:

- **Reading the results:** The microcuvette should be analyzed immediately, and no later than ten minutes after being filled. The blood hemoglobin level in grams per deciliter (g/dl) is displayed 15 to 45 seconds after the drawer is closed (Figure 3.7).
- Step 4: Record the hemoglobin level and test result:

- Record the hemoglobin level shown on the photometer (Figure 3.7) in the appropriate box in **Question 113** of the Biomarker Questionnaire. If there is no value to record because the child was not present, the parent/responsible adult refused to consent to the test, or there was some other problem, record the appropriate code in **Question 113**. If more than one child in the household is eligible and is listed, check carefully that you are recording the hemoglobin level in the correct column of the schedule.

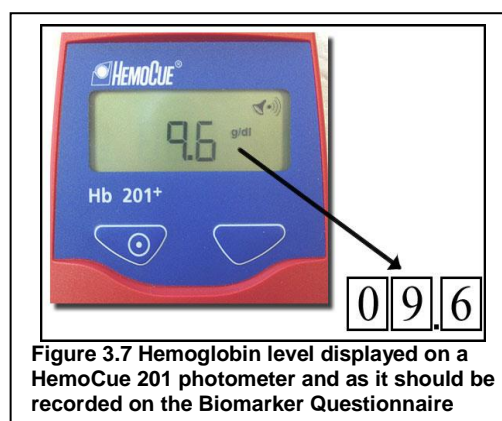


Figure 3.7 Hemoglobin level displayed on a HemoCue 201 photometer and as it should be recorded on the Biomarker Questionnaire

- Step 5: Collect bio-hazardous waste:
 - Place all bio-hazardous waste (lancets, microcuvettes, alcohol swabs, gauze, and gloves) into a plastic bag and sharps container provided for field disposal of these items. At the end of the day, follow the procedures described in Chapter 6 for the proper disposal of waste materials.
- 8. Verify the child's hemoglobin result in **Question 113** and on the Anemia and Malaria Brochure. Record the child's hemoglobin level in the Anemia and Malaria Brochure. Inform the parent/responsible adult of the results and provide the parent with the brochure (see Appendix A).
- 9. Proceed with the remaining part of the Biomarker Questionnaire.

PRECAUTIONS TO TAKE DURING ANEMIA TESTING

Please take the following precautions while doing anemia testing:

- **Never remove a microcuvette from the container with fingers wet with alcohol.** This can result in alcohol coming into contact with the reagents inside the microcuvette and destroying them. Using fingers wet with alcohol to handle other microcuvettes in the container can also affect them.
- **Never use the first two drops of blood for hemoglobin testing.** If the parent/responsible adult consents to the anemia test, **wipe away the first two drops** of blood and then collect the third drop in the microcuvette. This ensures the free flow of blood and allows for the collection of blood with a representative concentration of red blood cells. When testing for malaria in addition to anemia, follow the instructions provided in Chapter 5, and use the third drop of blood for hemoglobin testing.

- **Avoid inadequate filling or re-filling of the microcuvette.** The chamber of the microcuvette that contains dry reagents (yellow portion) has to be completely filled. The microcuvette should be filled with a drop of blood in one continuous motion. A microcuvette that contains air bubbles should be discarded.
- **Wiping off blood on the microcuvette.** Blood on the exterior of the microcuvette should be removed; failure to clean the exterior of the microcuvette can lead to an erroneously high hemoglobin reading.
- **Avoid keeping the microcuvette out for too long.** Keeping the microcuvette out of the container for too long before using it can lead to errors. Remove the microcuvettes from its container immediately before starting the testing procedure.
- **Avoid misalignment of the microcuvette in the photometer.** The microcuvette only fits into the photometer's microcuvette holder in one position. Therefore, place it carefully in the holder and slowly push the holder inside the photometer to obtain a reading. Slamming the microcuvette holder can cause blood to spray onto the optronic system, an action that can damage the photometer.
- **Old or improperly stored microcuvettes should not be used for testing.** While in the field, microcuvettes should not be used if more than 1 month has elapsed since the seal on the container was broken. The containers must be kept closed when not in use to avoid exposure to moisture, which can destroy the reagents.

PROVIDING ANEMIA TEST RESULTS AND REFERRALS FOR SEVERE ANEMIA

Before leaving the household, you will verbally report the results of the hemoglobin measurement for each child for whom an anemia test was completed. In addition to verbally reporting Hb results, Hb results will also be written in an informational brochure that will be left at the household. When reporting the results, briefly explain to the parent/responsible adult what his/her child's hemoglobin reading means, using the **Anemia and Malaria Brochure** as a guide. Please see Appendix A for an example of the **Anemia and Malaria Brochure**.

Parents of children with severe anemia should be informed about the effects of severe anemia and recommended to visit a health facility for follow-up medical attention. For each child with severe anemia, you will fill out an Anemia Referral Slip (Figure 3.8), on which you have recorded the hemoglobin level.

FIGURE 3.8 EXAMPLE OF AN ANEMIA REFERRAL SLIP*

[YEAR] [COUNTRY] MALARIA INDICATOR SURVEY
During the [YEAR] [COUNTRY] MIS, child _____ age ____ years/months, was tested for anemia on ____/____/____. His/her level of hemoglobin was _____.____ g/dl, which indicates he/she has severe anemia. This person needs medical attention to treat the anemia

Summary of the steps involved in testing for anemia:

- Seek consent as described in Chapter 1;
- Clean the finger or heel with an alcohol swab;
- Prick the finger or heel with the child lancet
- Wipe away the first two drops of blood;
- Collect the third blood drop in a microcuvette;
- Stop the bleeding and apply an adhesive bandage to the puncture site;
- Measure hemoglobin level in the blood sample using the HemoCue photometer;
- Record the hemoglobin level in the appropriate column of the Biomarker Questionnaire;
- Collect biohazardous waste in appropriate waste container;
- Inform the parent/responsible adult of child's hemoglobin level and provide an informational brochure on anemia;
- Provide a written referral for follow-up medical attention for a child found to be severely anemic.

CHAPTER 4: MALARIA TESTING

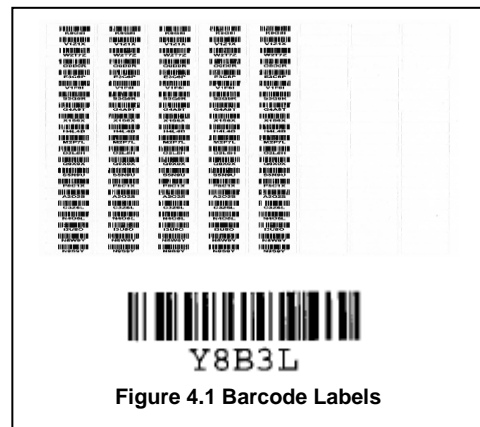
Malaria is a parasitic infection which is transmitted by the bite of a *Plasmodium* infected mosquito. Symptoms of malaria include fever, chills, headache, and vomiting, in addition to other flu-like symptoms. If left untreated, severe cases of malaria can quickly become life threatening. In the MIS, children will be tested in their homes for malaria with the rapid diagnostic test (RDT) [SPECIFIC RDT USED]. In addition, thick blood smears and thin blood smears will be prepared and sent to a laboratory where they will be stained and examined under the microscope for the presence, quantification and speciation of the malaria parasite.

This chapter discusses the [SPECIFIC RDT USED] RDT test kit, thick and thin blood smear preparation, and the detailed procedures of malaria testing. Specific details regarding the treatment protocol for malaria positive children, and the storage of thick and thin blood smears and their transfer to the laboratory, will also be discussed.

MATERIALS AND SUPPLIES FOR MALARIA TESTING

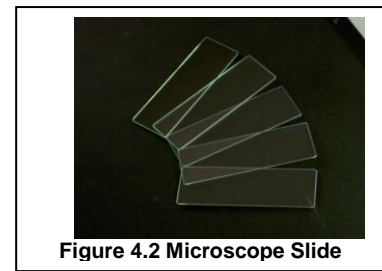
In addition to the supplies required for capillary blood collection (Chapter 2), and the Biomarker Questionnaire, the following materials and supplies are required for malaria testing:

- **[SPECIFIC RDT USED] RDT:** will be used for home-based malaria testing. [SPECIFIC RDT USED] RDT detects malaria antigens (*Plasmodium* proteins) and produces results in 15 minutes. This rapid test is discussed in greater detail below.
- **The Anemia and Malaria Brochure:** a one page document designed to educate the household about malaria and anemia, including its definition, symptoms, causes, and methods of treatment and prevention. In addition, the child's malaria and Hb results are recorded and classified within this document. See Appendix A for an example of the brochure.
- **Severe Malaria Referral Slip:** on this form is written the name and Hb results of children testing malaria positive and showing symptoms of severe malaria (Q. 118). It is to be given to parent/responsible adult of the child. They can take the child and referral to a local clinic or health center to receive proper treatment for his/her malaria.
- **Barcode Labels:** used to uniquely identify each child's thick blood smear. You will be provided with sheets of "peel-off" adhesive bar code labels. The barcodes are arranged in rows; each row includes labels with the identical bar code. A different row on the sheet should be used for each child (Figure 4.1).
- **Microscope Slide Transmittal Form:** accompanies the thick and thin smears to the laboratory. The purpose of this form is to ensure that the number of slides sent to the laboratory matches the number of slides collected in the field, and to track the slides throughout the transport process. A bar code with the same unique identifier as the bar code label attached to the thick smear slide and the thin blood smear slide is also attached to the Microscope Slide Transmittal Form and in the Biomarker



Questionnaire (Q. 112). See Appendix B for an example of the Microscope Slide Transmittal Form.

- **Glass slides:** used for preparing thick and thin blood smears (Figure 4.2). The slides are non-sterile but have been pre-washed in alcohol to remove grease and debris.



- **Slide cardboard tray.** The blood smears (i.e., glass slides on which you have made a thick or thin blood smear) will be placed in the cardboard slide tray for drying and safe transport from the field at the end of the day.

- **Cover slip or glass slide:** These are used for spreading the drop of blood to prepare a *thin* blood smear.



- **Sharps container:** This container is used for disposal of all used materials that have sharp edges capable of tearing the biohazard waste bag. Any microscope slides used in the field are contaminated with blood and considered to be bio-hazardous. They are to be disposed of in the sharps container.

- **Plastic microslide box:** Used for storage of blood slides collected in the field and transport of slides to the laboratory for analysis (Figure 4.4).



- **Zip-loc storage bags:** used in storing the cardboard microslide tray and microslide boxes.

- **Desiccant packets:** drying agents that absorb moisture from the air are used to keep the slides as dry as possible (Figure 4.5). The granules inside the packets change color from blue to pink as they absorb moisture.

- Change the desiccants when the granules change to a pink color or as indicated by the humidity indicator cards.
- Treat used desiccants as bio-hazardous waste and throw them away in a bio-hazardous waste bag.



THE [SPECIFIC RDT USED] RDT KIT

The [SPECIFIC RDT USED] RDT is a rapid qualitative test for malaria *Plasmodium falciparum* (Pf), the major cause of malaria in Kenya. It also tests for other species although these other species are not prevalent in Kenya.

Each [SPECIFIC RDT USED] RDT kit comes in a self-contained pouch which includes:

- Test device
- Desiccant packet
- Pipette
- Assay buffer in a dropper bottle
- Alcohol swab
- Instructions

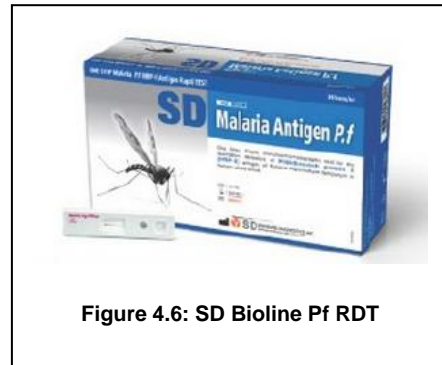
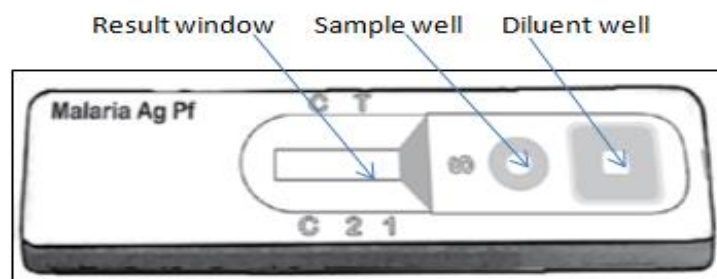


Figure 4.6: SD Bioline Pf RDT

In addition to the materials in the pouch, you will need a barcode label to ensure you correctly link the test result to the child whose blood is being tested. You will also need a timer to allow the correct time to elapse before you read the test results.

The [SPECIFIC RDT USED] RDT device involves several basic actions (Figure 4.7):

Figure 4.7: Example RDT



- (1) A **pipette** is used to collect and deposit the blood sample from the finger (heel) prick into the **sample well** in the test device.
- (2) Four drops of assay diluent are added to the **diluent well**.
- (3) The timer is set for **15 minutes**.
- (4) The test results are obtained by observing the **bands in the control (C) and test (T or P.f and other species) area in the result window**.

There are some points you should always observe when performing the [SPECIFIC RDT USED] RDT test:

- Do not re-use the device.
- Do not use the device after the expiration date.
- The test device must remain in the sealed pouch until use. **Once the device is opened, it must be used immediately.** Do not open the sealed pouch more than 5 minutes before doing the test as the device is sensitive to humidity.
- Take care not to touch the membrane area of the device.
- Do not mix reagents from different lots.

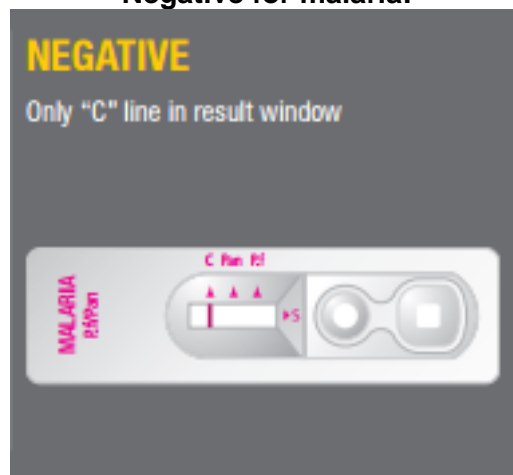
- Do not use the device if the pouch or device is damaged or if any lines are visible on the device before contact with the specimen.
- Remember to always set the timer for 20 minutes and read and record the test result immediately after the timer goes off.
- Do not miss 'faint positive' bands on the test device.

Interpreting the results of [SPECIFIC RDT USED] RDT

There are three possible outcomes of the [SPECIFIC RDT USED] RDT: negative, positive, and invalid. For a Pan (multispecies) RDT, a positive result might be 1) *P. falciparum* positive, 2) Non-*falciparum* positive, or 3) positive for both *P. falciparum* and non-*falciparum* (mixed infection).

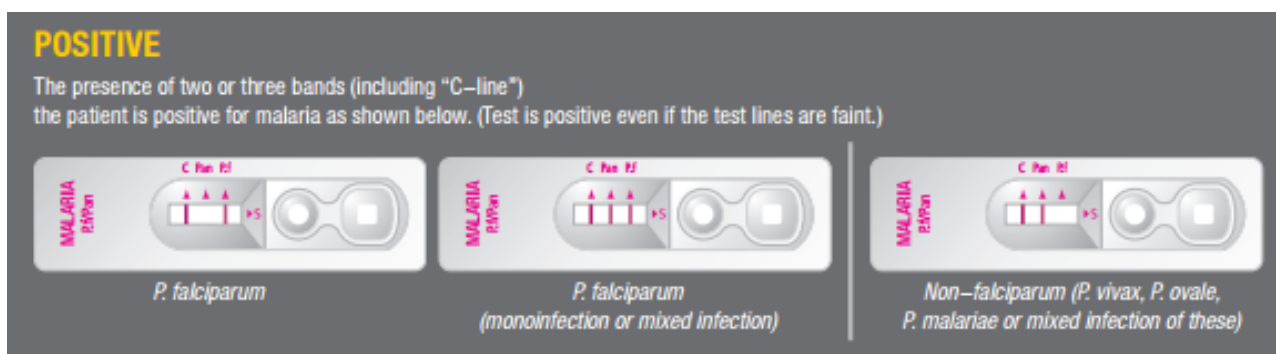
The result is **NEGATIVE** for malaria if **only a single pink/pink-purple band** corresponding to the **control "C"** is observed.

Negative for malaria:



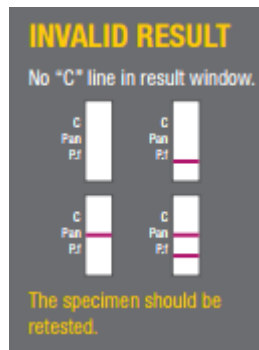
The result is **POSITIVE** for malaria if there is a **pink/pink-purple band** in **both the test and control** areas of the result window.

Positive for malaria:



If **no control band** is observed on the device, the test is **invalid**. It must be repeated with consent from the parent/responsible adult using a new device.

Example of invalid tests:



MALARIA TESTING IN THE LABORATORY

An objective of the [YEAR] [COUNTRY] MIS is to determine the national malaria prevalence in [COUNTRY]. To accomplish this, thick and thin blood smears will be collected in the field and transported to a laboratory for staining and reading. Thick smears are very sensitive for detecting parasitemia and parasite quantification because of the larger volume of blood. After blood smears are stained, they can be read in 20 minutes. If the thick smear is prepared correctly, when placed over newsprint, the paper's letters can be barely read. Thin films provide information on the morphology of the malaria parasites but are less sensitive than thick films in detecting parasitemia. Detailed instructions on how to prepare thick and thin blood smears are described in the next section.

COLLECTING BLOOD AND TESTING FOR MALARIA

The instructions below describe the appropriate steps and procedures for testing children for malaria. When children are tested for both anemia and malaria, the steps will differ slightly from those in the instructions below; procedure for combined anemia and malaria testing is described in Chapter 5.

Make sure that you have all of the documents you will need for the testing including the Biomarker Questionnaire, the Microscope Slide Transmittal Form and an Anemia and Malaria Brochure. As described in Chapter 1 of this manual, confirm the identity of all children eligible for the testing and obtain permission from a parent/responsible adult for the testing for each eligible child. After you have established the number of children you will test, take out the appropriate equipment and supplies for one child at a time. You will want to have all materials in easy reach when you begin collecting blood samples from each child.

Follow the procedure below to test eligible children for malaria:

1. Complete **Question 103** and record the child's day, month, and year of birth.
2. **Question 104** and **Question 105** will determine if the child is really eligible. For **Question 104**, refer to **Question 103** to determine if the child was born anytime in the years 2011-2015. If the child was born before 2011 (e.g. in 2010, 2009, etc), then the child is too old to be eligible for testing. For **Question 105**, refer to **Question 103** to determine if the child is less than 6 months old. If the child was born in the month of interview or in the 5 months before the month of interview, the child is not eligible for hemoglobin measurement. For example, if you are visiting the household in July, a child born in February, March, April, May, June, or July will not be eligible for testing because they would be too young. In other words, if the child is less than 6 months old, the child is not eligible for hemoglobin measurement, so you will record

'1' and continue to the next child. If the child is 6 months or older, record '2' and proceed to **Question 106**.

3. In **Question 106**, record the name of the parent or other adult responsible for and responding to the consent process.
4. Seek consent as described in Chapter 1 (**Question 107**).
5. Record the outcome of the consent process in **Question 108**. Confirm that you read the statement to the parent/responsible adult and recorded their response accurately by signing in the space provided and by providing your assigned health tech's number.
6. If consent was granted, prepare the materials required for blood collection and malaria testing.
 - Take the **first bar code label** from the first complete row on the sheet of bar code labels and paste it in the column of the Biomarker Questionnaire containing the line number of the child (Q. 112).
 - Take the **second bar code label** from the same row on the sheet of bar code labels and paste it on the Malaria RDT.
 - Take the **third bar code label** from the same row on the sheet of bar code labels and paste it on the slide (clean and grease free) that will be used for preparing the first thick and thin blood smear.
 - Take the **fourth bar code label** from the same row on the sheet of bar code labels and paste it on the slide (clean and grease free) that will be used for preparing back-up thick and thin blood smear (if applicable).
 - Take the **fifth bar code label** from the **same row** on the sheet of bar code labels and paste it on the Microscope Slide Transmittal Sheet for the cluster in which you are working.
 - **DO THE ABOVE STEPS CAREFULLY**. The bar code label is the only means of identifying the blood slides and for linking the malaria test results to the interview data. Mistakes will result in mismatches later on. **CHECK THAT THE FIVE MATCHING BAR CODE LABELS HAVE BEEN PLACED ON THE QUESTIONNAIRE, RDT, THE THICK AND THIN BLOOD SMEAR SLIDES and the TRANSMITTAL SHEET.**

COLLECT BLOOD DROPS FROM THE CHILD

1. Collect a finger or heel stick blood sample from the child, following the procedure described in Chapter 2. Use a sterile gauze pad to wipe away the first blood drop from the finger (heel) prick.
2. Collect the second drop of capillary blood in the pipette, loop/cup provided in the RDT kit. Be careful only to collect the required blood volume for the malaria RDT as recommended by the kit manufacturer.
 - Step 1: Dispense the blood from the RDT pipette into the RDT cassette (Figure 4.8).

- Check that that the blood sample has not clotted and immediately blot the specimen on **sample well**. Ensure the blood from the sample applicator pipette/loop/cup has been completely taken up by the sample pad.

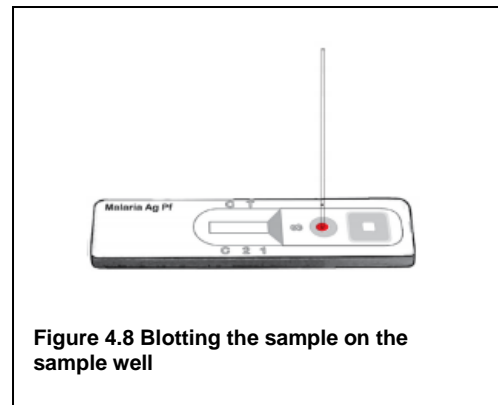


Figure 4.8 Blotting the sample on the sample well

- Step 2: Dispense **4 drops** of the buffer into the buffer well by holding the plastic dropper bottle **vertically** (Figure 4.9).

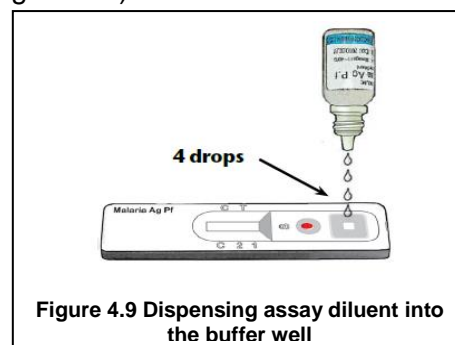
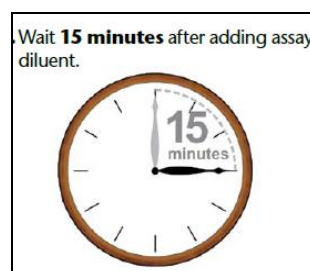


Figure 4.9 Dispensing assay diluent into the buffer well

- Step 3: Start the timer for 15 minutes
 - You will read and record the results of the test at the end of 15 minutes. **The test results should not be interpreted after 30 minutes.**



3. Collect additional blood drops to prepare the thick and thin smears. You would have **two separate barcode labeled microscope slides**, each with a thick and thin smear:
 - Turn the finger so that it is facing up. Wipe away residual blood from the second drop and apply gentle pressure to form a new medium-sized drop of blood.
 - Pick up the first slide (clean and grease free) with the barcode by holding the edges. Touch the slide to the blood drop near the center of the slide so that the barcode is facing down. Do not touch the slide to the finger.
 - For the thick smear, collect three small blood drops (each drop about 5 μ l or about this size●) at the center of the slide in the form of a triangle.
 - For the thin smear, collect one small blood drops (about 5 μ l or about this size●) at the center of the slide and place it on the absorbent paper sheet.
 - Pick up the second slide (clean and grease free) with the barcode by holding the edges. Collect drops for a thick and thin smear on this slide as well.
4. Preparation of thick blood smear:
 - Using the corner edge of a spreader slide, combine the three small (5ul) blood drops on the slide to make one circle, by spreading the drops in a circular motion to make a circle of 10mm in diameter (1cm). When you have completed the circle, return to the center of the blood circle without lifting the spreader. Once you reach the center of the blood circle, lift the spreader straight up. Do not 'stir' the blood; instead, the blood should be spread evenly in three to five circular motions.
 - Allow the smear to air-dry thoroughly in a horizontal ("flat") position. The slide should be left to dry overnight as necessary. You should place the slide face-up in the slide cardboard tray for drying. **Do not heat or fix the thick smear with alcohol.**
5. Preparation of thin blood smear:
 - Use a spreader slide and hold it at an angle of 30-45 degrees in front of, but not touching, the small blood drop (about 2 ul).
 - Pull back the spreader slide into the blood drop, allowing the blood to spread along the edges of the spreader slide.
 - With a smooth and continuous motion, push the spreader slide to the opposite end (away from the barcode label) of the slide to create a thin film of blood.
 - Place the slide in the slide cardboard tray and allow it to dry thoroughly.

Since both the thick and thin smear will be prepared on the same slide, the slide should look like this:



6. Dispose of spreader slides in the sharps container.
7. Stop bleeding at the site of the prick by holding a gauze pad against the prick site for at least one minute:
 - After preparing the malaria smear, wipe the child's finger a final time and then apply a bandage or plaster to ensure that the puncture site is protected from dirt and infection.
8. Collect bio-hazardous waste
 - Place all bio-hazardous waste (e.g., lancets, microcuvettes, alcohol swabs, gauze, and gloves) into a plastic bag or sharps container provided for field disposal of these items. At the end of the day, follow the procedures described in Chapter 6 for the proper disposal of these waste materials. Place all used slides in the sharps container.
9. Record the malaria results
 - Record the result code of malaria testing in **Q. 114**. If there is no value to record because the child was not present, the parent/responsible adult refused to consent to the test, or there was some other problem, record the appropriate code in **Q. 114**.
 - Record the result of the malaria RDT in **Q. 115** of the Questionnaire. If more than one child in the household is eligible and is listed, check carefully that you are recording the malaria result in the correct column of the Biomarker Questionnaire.
10. Screen malaria positive children for severe malaria or need for malaria treatment by recording the following information:
 - Ask the parent/responsible adult if the child has presented any of the symptoms listed in **Q. 118**. Verify if any of the symptoms are circled in **Q. 119** and follow the skip pattern to the next appropriate question if necessary.
 - Verify the child's hemoglobin result and record the appropriate code in **Q. 120**. Follow the skip instruction if applicable.
 - Record whether or not the child has received any ACTs to treat the malaria in the past two weeks in **Q. 121**. Follow the skip patterns to the next appropriate question.
11. Read the appropriate referral or treatment statements to the parent/responsible adult (**Q. 122**, **Q. 123**, **Q. 124**, **Q. 127**, or **Q. 129** as appropriate). Record whether or not

the medication was accepted by the parent/responsible adult or whether or not a referral was given for the child in **Q. 125** or in **Q. 126**, as appropriate.

12. Record the child's malaria result in the **Anemia and Malaria Brochure** inform the parent/responsible adult of the results and provide the parent with the brochure (see Appendix A).
13. Follow the treatment protocol instructions described in this chapter and in **Q. 127** if the child requires malaria treatment.

PROVIDING MALARIA TEST RESULTS

Before leaving the household, you will verbally report the test results of the measurement for each child for whom malaria testing was completed. In addition to verbally reporting the results, the malaria test result will also be written in the **Anemia and Malaria Brochure**, to be given to the parent/ responsible adult for the child. Briefly explain what the child's result means, using the brochure as a guide, and discuss what can be done to prevent malaria. Please see Appendix A for an example of the brochure.

SEVERE MALARIA REFERRAL FOR CHILDREN TESTING MALARIA POSITIVE

Each child testing positive for malaria will be screened with **Questions 118-120** for severe malaria. If the malaria positive child presents symptoms of severe malaria including extreme weakness, heart problems, loss of consciousness, rapid or difficult breathing, seizures, abnormal bleeding, jaundice or dark urine, he or she should not be treated with any ACTs. ACTs will not clear this type of malaria infection and the child will remain sick. Instead of treating the child, the child must be referred to a health facility right away.

When a child is malaria RDT positive and presents symptoms of severe malaria, read the following referral statement, found in **Q. 122** of the Questionnaire, to the parent/ responsible adult:

The malaria test shows that (NAME OF CHILD) has malaria. Your child also has symptoms of severe malaria. The malaria treatment I have will not help your child, and I cannot give you the medication. Your child is very ill and must be taken to a health facility right away.

In addition, for each child with symptoms of severe malaria, you will fill out a Severe Malaria Referral Slip on which you have recorded the symptoms the child has and the hemoglobin level, if less than 8.0 g/dl. Recommend to the parent/ responsible adult that the child visits a health facility for medical attention right away. The team nurse or health specialist should also confirm the child's signs and symptoms indicative of severe malaria.

Severe Malaria Referral Slip

[COUNTRY] MALARIA INDICATOR SURVEY: **Severe Malaria Referral**

During the 2011 [COUNTRY] MIS _____ (Name), age ____ years, was tested for malaria on ___/___/___, with a Rapid Diagnostic Test (RDT). He/she tested positive for malaria, and is displaying the following signs of severe malaria:

___ EXTREME WEAKNESS

___ HEART PROBLEMS

___ LOSS OF CONSCIOUSNESS

___ RAPID OR DIFFICULT BREATHING

___ SEIZURES

___ ABNORMAL BLEEDING

___ JAUNDICE OR YELLOW SKIN

___ DARK URINE

___ HEMOGLOBIN LEVEL OF ____ . ____ g/dl (LESS THAN 8.0G/DL)

He/she appears to be very ill and did not receive treatment for the malaria. **THIS CHILD NEEDS TO BE TAKEN TO A HEALTH FACILITY RIGHT AWAY.**

TREATMENT PROTOCOL FOR CHILDREN TESTING MALARIA POSITIVE

Malaria treatment will be provided to children testing malaria positive in the [YEAR] [COUNTRY] MIS. Following the national malaria case management guidelines of [COUNTRY], the team nurse and health specialist will distribute fixed doses of ACT to treat malaria.

Prior to treating children, the team nurse and health specialist must first identify whether or not the child is in need of treatment (**Q. 121**). If the child has taken any ACTs within the previous two weeks or is taking ACTs to treat the malaria, it is not appropriate to give him or her additional medication. Rather, if the child has already received medication, share the following with the parent/ responsible adult, found in **Q. 123** of the Questionnaire:

You have told me that (NAME OF CHILD) had already received ACTs for malaria. Therefore, I cannot give you additional ACTs. However, the test shows that he/she has malaria. You should take the child to the nearest health facility for further examination.

For children 6 months and older with a positive malaria RDT test result that do not present symptoms of severe malaria and are eligible for malaria treatment, a nurse or health specialist on the MIS team will request consent from a parent/responsible adult to provide ACT, using the following language included in **Q. 124** in the Biomarker questionnaire:

The malaria test shows that your child has malaria. We can give you free medicine. The medicine is called ACT. ACTs are very effective and in a few days it should get rid of the fever and other symptoms. You do not have to give the child the medicine. This is up to you. Please tell me whether you accept the medicine or not.

In order to ascertain the correct dose of ACT, the following weight/age guidelines for the ACT treatment are provided in the Biomarker Questionnaire (Table 1):

Table 1: Example dosing schedule for artemether-lumefantrine

DOSING SCHEDULE WITH ARTEMETHER-LUMEFANTRINE (AL)							
WEIGHT	AGE	NUMBER OF TABLETS RECOMMENDED AT APPROX. HOURS (EACH TABLET CONTAINS 20 MG. A AND 120 MG. LU)					
		0 h	8 h	24 h	36 h	48 h	60 h
5-<15kgs	6mos-3yrs	1	1	1	1	1	1
15-<25kgs	3-7yrs	2	2	2	2	2	2
25-<35kgs	8-11yrs	3	3	3	3	3	3
>35kgs	>12yrs	4	4	4	4	4	4

Note: Be sure to modify this schedule according to national recommendations

The nurse or health specialist should administer the first dose of ACT to the child in the household and advise the parent/responsible adult on how to administer the subsequent doses. The nurse or health specialist should tell the parent to make sure the child is given the **full** 3 days of medication so that the infection will not return. The team nurse or health specialist should also advise the parent that additional ACT tablets must be obtained at a health facility and given to the child if the child vomits within an hour of being given a tablet.

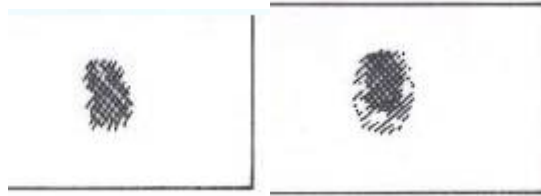
The team nurse or health specialist should tell each parent/responsible adult to take the child to a health facility immediately if the child experiences a fever for two days after taking the last dose of medication.

Summary of the steps involved in testing for Malaria:

- Lay out all required supplies and forms for malaria testing within reach.
- Open the pouch of the [SPECIFIC RDT USED] RDT malaria kit and make ready for use.
- Put barcode labels on the Biomarker Questionnaire, RDT device, three clean and grease free microscope slides, and the Microscope Slide Transmittal Form.
- Clean the child's middle or ring finger with alcohol.
- Prick the finger using sterile child lancet.
- Wipe away the first blood drop.
- Form a second blood drop. Take sample using pipette for malaria RDT (be fast to avoid blood clotting from the pricked finger)
- Perform the rapid malaria test:
 - Blot the blood sample into the sample well of the malaria RDT test device.
 - Add 4 drops of buffer in to the buffer well and start the timer for 15minutes
 - Read the RDT result after 15 minutes. Record result in **Q. 115** of the Biomarker Questionnaire.
- Wipe away residual blood on the finger and apply gentle pressure to the pricked site again to obtain another blood drop for the thick and thin smears. Place the slides on the absorbent paper sheet.
- Stop the bleeding at the prick site.
- Prepare the thick and thin blood smears and the back-up slide and put these in the tray to dry.
- Dispose of all materials used for testing in the biohazard bag or the sharps container.
- Record the test results in the **Anemia and Malaria Brochure**.
- Inform the parent/responsible adult of the child's test result.
- As appropriate, refer to a health facility any child with severe malaria or provide treatment for malaria to eligible children.

The following illustrate some **common errors in thick blood smear preparation** which you should avoid.

Edge of spreader slide chipped:



Blood film spread on a greasy slide:

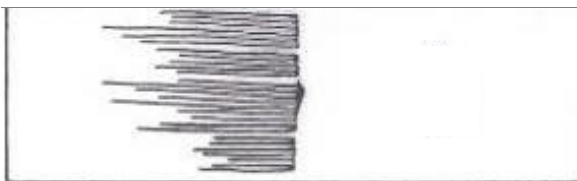


Too little blood:



The following illustrate some **common errors in thin blood smear preparation** which you should avoid.

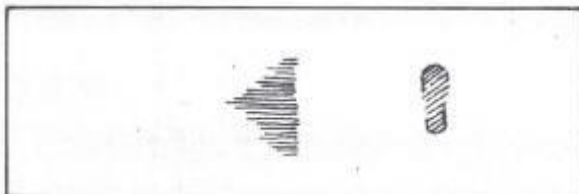
Edge of spreader slide chipped



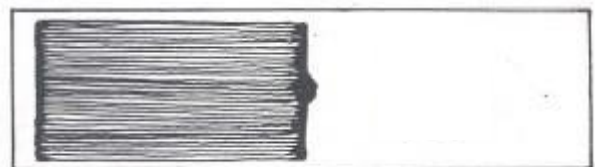
Blood film spread on a greasy slide



Too little blood



Thin film spread too long



STORAGE, FIXING, AND TRANSPORT OF THICK AND THIN BLOOD SMEARS

Processing and Storage of Malaria Smears in the Field

The blood smears you make in the field for malaria testing must be properly maintained. They must be allowed to dry thoroughly, be protected from dust, flies, debris and other

contaminants and, be kept from high levels of humidity. The thin smears must be fixed. Follow the guidelines below when storing to maintain the quality of the blood smears.

1. After returning from the field each day, you should inspect the slides you collected that day to check their quality.
2. You should also check that you have two slides per eligible child tested that day, each slide with a thick and thin smear. Check that the bar code label matches **Q. 112** in the Biomarker Questionnaire and the information on the Microscope Slide Transmittal Form. Note any discrepancies and try to resolve them. If you are missing slides for any child for whom you have recorded that smears were collected, you must go back to the household and ask permission to test the child again.
3. Make sure you do not touch the smears accidentally with your fingers or with any materials you carry in the field and that there are no dust or dirt particles embedded in the blood. If you touch the smear before it has dried thoroughly, you must go back to the household and ask permission to collect another blood smear.
4. **The next morning before going to the field, you must perform the following:**
5. Check the thick and thin smears to make sure that they have dried completely.
6. “Fix” only the thin smears using absolute methanol provided to each survey team:

To ‘fix’ the thin smears, follow these steps:

- ◆ Pour enough absolute methanol into a Coplin jar to cover the blood smear; do not put so much methanol in the Coplin jar that the methanol will come in contact with the barcode label.
- ◆ Remove the smears from the slide folder
- ◆ Dip the thin smear in absolute methanol contained in a coplin jar to cover $\frac{3}{4}$ of the film for 30 seconds. Allow to air dry on slide drying rack. The fumes will flow upwards to cover the remaining $\frac{1}{4}$ of the smear without reaching the thick film
- ◆ Place the dry slides into micro-slide box. You should change the methanol each day as you fix before fixing a new batch of slides.



Figure 4.11 Coplin jar used to fix thin smears

DO NOT

- Fix the thick smear
- Fix the smears near an open fire, lighted cigarettes, lighted stove (LPG or Electric)
- Handle the methanol in a closed room; whenever possible, open windows when handling the methanol.

Note!! Thick smears do not need to be further processed in the field before they are stored.

7. Transfer only the dried blood smears from the cardboard tray in to the slide slots of plastic slide storage box.

8. Place the plastic slide storage box in a zip-loc bag containing about 3 to 5 sachets of desiccants; place this bag into a cool box. It is very important that the zip-loc bag remained sealed. The buildup of humidity can damage blood smears. Monitor the desiccants for color change; if necessary, remove pink desiccants and replace with new desiccant packets. Continue adding blood slides to the slide box on a daily basis until the cluster is complete.
9. When the cluster is complete and all slides appropriately dried and packaged, the slides are ready for transport to the lab.

Transferring the Malaria Slides to the Laboratory

When fieldwork in a cluster is complete, the slides will be transported to the lab for microscopy. **You will transfer slides as a completed cluster only.**

In order to make sure that all of the slides are transferred, you must check the slides against the **Microscope Slide Transmittal Form** for each cluster and with the information in the Biomarker Questionnaires for that cluster. Please see an example of the **Microscope Slide Transmittal Form** in Appendix B. Follow these steps for transferring the slides:

1. Put on a clean pair of gloves. Remove the slide box from the zip-loc bag. Check the bar code on the slides against the barcodes on the Microscope Slide Transmittal Form. Put a check mark in the column labeled TECHNICIAN for each corresponding barcode found on the Microscope Slide Transmittal Form.
2. Count the total number of slides and record the number in the boxes provided in Column (3A) and (3B) on the front side of the Microscope Slide Transmittal Form in the row labeled TECHNICIAN. If there are any discrepancies between the record on the Transmittal form and your count, you must account for them; use Column (7) to explain.
3. Note that the count of slides for a cluster should be double the number of barcode labels on the transmittal form for that cluster, unless you were unable to collect a smear from a child or a slide was lost or damaged.
4. Sign your name in Column (4) and record your name in Column (5) and the date in Column (6).
5. The team supervisor will also re-verify that the barcodes on the smears match the barcodes on the Transmittal Form. Fold the Microscope Slide Transmittal Form along the dotted lines (so that the bar-coded labels are not folded), and keep it with the slides in the slide box or zip-loc bag.
6. After the checking is complete, the Microscope Slide Transmittal Form for a cluster should be packaged with the slides in the cool box and given to the courier service picking up the slides.

When the lab receives the slides from the teams, the lab personnel will recount the slides for each of the completed clusters and verify the bar codes on the slides against the bar codes on the Microscope Slide Transmittal Form.

PRECAUTIONS TO TAKE DURING MALARIA TESTING

Please take the following precautions when doing malaria testing:

- **Label the slides.** As the blood smear will be taken to another location to do the microscopic examination, it is critical that the glass slide is properly labeled so the result can match with the child when recorded. Similarly, the RDT device must be properly labeled so that there is no mistake in linking the result to the child tested.
- **Use free-flowing blood.** It is important that the blood be free flowing, especially the drops used for preparing the blood smear for parasite testing.
- **Fill the RDT system adequately.** The blood collection device should be filled correctly with the volume of blood recommended by the RDT kit manufacturer. The entire volume of blood should be blotted in the sample collection well.
- **Only used properly stored RDTs for testing.** RDTs may have specific storage requirements and should not be used if these storage requirements were not followed. The containers must be kept closed when not in use to avoid exposure to moisture, which may destroy the reagents or alter the properties of the test.
- **Avoid touching the glass slide with fingers wet with alcohol.** This can result in alcohol and dirt contamination of the glass slide preventing the proper spread and drying of the blood smear.
- **Avoid using slides with grease to prepare thick and thin smears.** Preparing blood smears on greasy slides results in smears with holes and streaks, as the grease does not allow the blood to spread evenly on the slide. These slides cannot be properly read.

CHAPTER 5: COMBINED ANEMIA AND MALARIA TESTING

The majority of children tested for biomarkers in the [YEAR] [COUNTRY] MIS will be tested for both anemia and malaria. The information in the preceding chapters on, recording and providing results, appropriate treatment and referral guidelines, and testing precautions is also applicable to combined testing. The procedures described in Chapters 2, 3, and 4 should be followed. However, the procedural steps of combined anemia and malaria testing differ slightly from the steps of individual testing. This chapter provides a summary of steps for combined testing.

MATERIALS AND SUPPLIES

All of the materials and supplies listed in Chapters 2, 3, and 4 will be required for combined anemia and malaria testing. Please see the aforementioned chapters for details. Below is a list of all the supplies that will be required. See also the supply list in Appendix E to assist with your daily supply counts for the field.

- General Blood Collection Supplies:
 - Disposable Latex gloves
 - Absorbent paper sheets
 - Alcohol preps
 - Safety child lancets
 - Sterile gauze pads
 - Plasters or Band-aids
 - Plastic Bag for Waste

- Anemia testing Supplies:
 - Microcuvette
 - HemoCue Hb 301 + photometer

- Malaria Testing Supplies:
 - [SPECIFIC RDT USED] RDT
 - Glass slides
 - Barcode Labels
 - Sharps container
 - Cardboard slide tray
 - Plastic microslide box
 - Ziploc storage bags
 - Desiccant packets
 - 100% (absolute) Methanol
 - Coplin Jar
 - Container for storing used methanol

- Required Paperwork:
 - Biomarker Questionnaire
 - The Anemia and Malaria Brochure
 - Microscope Slide Transmittal Form

- Severe Anemia Referral Slip
- Severe Malaria Referral Slip

COLLECTING BLOOD AND TESTING FOR ANEMIA AND MALARIA

When a parent/responsible adult consents to both anemia and malaria testing for his/her child, the child is only pricked one time and blood is collected for both tests. The order of steps in the combined testing is very important, and differs from the order steps involved in testing only for anemia or only for malaria.

Follow the summary steps below for combined anemia and malaria testing.

1. Complete **Question 103** and record the child's day, month, and year of birth.
2. **Question 104** and **Question 105** will determine if the child is really eligible. For **Question 104**, refer to **Question 103** to determine if the child was born anytime in the years 2011-2015. If the child was born before 2011 (e.g. in 2010, 2009, etc), then the child is too old to be eligible for testing. For **Question 105**, refer to **Question 103** to determine if the child is less than 6 months old. If the child was born in the month of interview or in the 5 months before the month of interview, the child is not eligible for hemoglobin measurement. For example, if you are visiting the household in July, a child born in February, March, April, May, June, or July will not be eligible for testing because they would be too young. In other words, if the child is less than 6 months old, the child is not eligible for hemoglobin measurement, so you will record '1' and continue to the next child. If the child is 6 months or older, record '2' and proceed to **Question 106**.
3. In **Question 106**, record the name of the parent or other adult responsible for and responding to the consent process.
4. Seek consent as described in Chapter 1 (**Question 107**).
5. Record the outcome of the consent process in **Question 108**. Confirm that you read the statement to the parent/responsible adult and recorded their response accurately by signing in the space provided and by providing your assigned health tech's number.
6. If consent was granted for both tests, prepare the materials required for blood collection for anemia and malaria testing. Remember to place the barcodes as appropriate prior to blood collection.
7. Collect a finger or heel stick blood sample from the child, following the procedure described in Chapter 2. Use a sterile gauze pad to **wipe away the first blood drop** from the finger (heel) prick.
8. **Collect the second drop of capillary blood in the collection device provided in the malaria RDT kit.** Be careful only to collect the recommended amount of blood and dispense it in to the sample well of the RDT. Add 4 drops of buffer solution in the buffer well, set timer and read results after 15 minutes.
9. Collect the **third drop of blood in the microcuvette**, following the instructions in Chapter 3.

- Step 1: Wipe any surplus blood off both sides of the microcuvette “like butter from a knife,” using the clean end of a sterile gauze pad.
 - Step 2: Visually inspect the microcuvette for air bubbles.
 - Step 3: Place the microcuvette in its holder and gently push the holder into the photometer.
 - Step 4: Obtain the hemoglobin level
10. Collect **additional drops of blood to prepare thick and thin blood smears** and the back-up slide as described in Chapter 4.
11. Stop the bleeding at the site of the prick with a piece of gauze.
12. Prepare the thick and thin smears following the steps described in Chapter 4.
13. Record the results of the tests as follows:
- Record the hemoglobin level and test result in **Question 113**.
 - Record the result code of malaria testing in **Q. 114**. If necessary follow the skip pattern. If not, record the result of the malaria RDT in **Q. 115** of the Questionnaire. If the malaria RDT is positive, follow the skip **in Q. 115 to Q. 118**.
14. Collect the bio-hazardous waste in to the appropriate biohazard container.
15. If the malaria RDT is negative, verify the child’s hemoglobin result and record the appropriate code in **Q. 116**. Follow the skip instruction if applicable and read the **Severe Anemia Referral Statement** if necessary in **Q. 117** (hemoglobin level less than 8 g/dl).
16. Screen malaria RDT positive children for severe malaria or need for malaria treatment by recording the following information:
- Ask the parent/responsible adult if the child has presented any of the symptoms listed in **Q. 118**. Circle codes for all symptoms mentioned. If the parent/responsible adult does not mention any of the symptoms, circle code Y. Verify if any of the codes A-H are circled in **Q. 119** and follow the skip pattern to the next appropriate question if necessary.
 - Verify the child’s hemoglobin result and record the appropriate code in **Q. 120**. Follow the skip instruction if applicable.
 - Record whether or not the child has received ACTs to treat the malaria in the past two weeks in **Q. 121**. Follow the skip patterns to the next appropriate question.
17. Read the appropriate **referral or treatment statement** to the parent/responsible adult. Record whether or not the medication was accepted by the parent/responsible adult or whether or not a referral was given for the child in **Q. 128**.

18. Record the child's hemoglobin level and malaria result in the ***Anemia and Malaria Brochure***. Inform the parent/responsible adult of the results and provide the parent with the brochure (see Appendix A).
19. Follow the treatment protocol instructions described in Chapter 4 if the child requires malaria treatment.

Summary of the steps involved in testing for Anemia AND Malaria:

- Lay out all supplies and place bar code labels on questionnaire, RDT device, microscope slide for thick smear and the Microscope Slide Transmittal Form.
- Turn on the HemoCue machine and take out a microcuvette.
 - Open the pouch of the [SPECIFIC RDT USED] malaria RDT kit and make ready for use.
- Clean the child's middle or ring finger with alcohol.
- Prick the finger using sterile child lancet.
- Wipe away the first blood drop.
 - Form a second blood drop. Take sample using pipette for the RDT (be fast to avoid blood clotting from the pricked finger)
- Perform the rapid malaria test:
 - Blot the blood sample into the sample well of the test device.
 - Add 4 drops of buffer in to the buffer well and start the timer for 15 minutes.
- Form a third drop of blood. Take a sample using a microcuvette for anemia testing.
- Perform the anemia test.
- Press the pricked finger again to collect additional blood drops for the thick and thin smears on and the back-up slides. Place the slides on the absorbent paper sheet.
- Stop the bleeding at the prick site using a piece of gauze.
- Prepare the thick and thin smears and put the slides in a cardboard tray to air dry.
- Dispose of all materials used for testing in the biohazard bag or sharps container.
- Record the Hb result in Q. 113 of the Biomarker Questionnaire.
- Read the malaria RDT result after 15 minutes. Record result in Q. 115 of the Biomarker Questionnaire.
- Record both test results in the ***Anemia and Malaria Brochure***.
- Inform the parent/responsible adult of the child's test result.
- As appropriate, refer to a health facility any child with severe anemia or severe malaria.
- Provide malaria medication to children who are malaria RDT positive and are eligible for treatment as described in chapter 4

CHAPTER 6: FIELD BIORISK MANAGEMENT

Any material coming in contact with blood or serum (lancets, microcuvettes, alcohol swabs, gauze, and gloves) is considered to be bio-hazardous (hazardous to other humans). Safe disposal of such material is very important to prevent the transmission and spread of various blood borne diseases, such as hepatitis B and HIV, among survey personnel and within the study community. Bio-hazardous waste has to be collected in a special container during the blood collection and testing, securely stored and transported, and safely disposed of at the end of each day of fieldwork.

There are commercially available bio-hazardous waste disposal containers which should be used for waste disposal. These types of containers are red and have a special logo warning about bio-hazardous content. They can be securely closed for safe storage and transportation during the fieldwork.

There will be only one option for waste management in the field; that is waste segregation and field biosecurity. Take the bio-hazardous waste to the nearest health facility for disposal in an incinerator (preferred option). The health facilities should employ standard procedures for bio-hazardous waste disposal.

National Environmental Management Authority (NEMA) does not encourage burning and burial of waste in open field for fear of environmental concerns.

Materials and supplies: sanitizers, PPE ziplock bags, sharps container, lockable, field phlebotomy bag, jik, dettol, soap, water ,liners, gauze pads, table towels and latex gloves.

The waste generated will be segregated into appropriate recommended containers:

1. Containers for the sharps, red –
2. Infectious material , yellow –
3. Non infectious and general waste–black

PROCEDURES FOR FIELD DISPOSAL OF BIOHAZARDOUS WASTE

1. Before each blood collection session ensure you sterilize the work space using 10% jik solution. Use the provided dettol to clean you hands before and after testing.
2. At the end of each blood collection and testing in a household, all materials used during the testing (gloves, micro-cuvettes, lancets, alcohol swabs, and gauze pads) are to be segregated as described above and transported using the biohazard plastic bag.
3. The daily waste generated is to be transported back to the cluster work station and emptied into the larger waste disposal plastic bag
4. At the end of the cluster. The bigger waste disposal bag is transported to the nearest health facility for disposal.

The team supervisor is responsible for providing the necessary information to his/her team members regarding segregation and safety of the bio waste materials until onward transportation to appropriate health facility for the recommended safe disposal.

APPENDIX A: Anemia and Malaria Brochure

MALARIA AND ANEMIA TEST RESULTS

Date: _____

HEMOGLOBIN		
Name	Hb Level: _____ g/dl	Hb Level: _____ g/dl
Name	Hb Level: _____ g/dl	Hb Level: _____ g/dl
Anemia diagnosis:		
Severe anemia (less than 8.0 g/dl)	Moderate anemia (8.0-9.9 g/dl)	Mild anemia (10.0-10.9 g/dl)
Severe anemia (less than 8.0 g/dl)	Moderate anemia (8.0-9.9 g/dl)	Mild anemia (10.0-10.9 g/dl)
Normal (11.0 g/dl or more)	Normal (11.0 g/dl or more)	Normal (11.0 g/dl or more)
MALARIA		
Name	Malaria diagnosis:	Malaria diagnosis:
Positive	Positive	Positive
Negative	Negative	Negative
TREATMENT FOR MALARIA PROVIDED:	TREATMENT FOR MALARIA PROVIDED:	TREATMENT FOR MALARIA PROVIDED:
YES	YES	YES
NO	NO	NO
Instructions for Administering Malaria Treatment		
[TO BE ADDED BASED ON COUNTRY RECOMMENDATIONS]		

What is anemia?

Anemia is a serious health condition in which there are not enough red blood cells or hemoglobin in the blood.

Hemoglobin is a substance in the blood that carries oxygen to the brain, muscles, disease-fighting organs and other parts of the body. Iron is important for making hemoglobin.

What are the symptoms of anemia?

Some of the symptoms of anemia are:

- tiredness
- headaches
- dizziness
- poor appetite
- heart palpitations
- shortness of breath

Why is anemia dangerous?

Anemia is dangerous because:

- it reduces one's resistance to infections
- severe anemia can lead to heart failure
- anemic children have poor learning capacity

What is malaria?

Malaria is a disease caused by a mosquito bite. A person can get this disease if bitten by an infected mosquito.

Can malaria be prevented?

- Malaria can be prevented by:
- Spraying the house with insecticides to kill the mosquitoes.
 - Getting rid of stagnant water in which mosquitoes breed.
 - Sleeping under a mosquito net which has been treated with insecticide.
 - Pregnant women can take an anti-malarial drug during pregnancy.

Why is malaria dangerous?

Malaria is dangerous because it can lead to serious health problems such as kidney failure, severe anemia, and death.

What are the symptoms of malaria?

Some of the symptoms of malaria are:

- Fever
- Headache
- Vomiting
- Muscle and joint pain
- Other flu-like signs

What do the anemia test results mean?

Severe anemia: The child has a seriously low level of hemoglobin in the blood. You need to take the child to your doctor or health centre immediately for treatment.

Moderate anemia: The child has a reduced level of hemoglobin. You need to take the child to your doctor or health centre as soon as possible.

Mild anemia: The child's hemoglobin level is slightly lower than normal. It is not necessary to take the child to a doctor or health centre, but you should take action to see that the child's diet includes more daily iron.

What causes anemia?

Anemia is caused by:

- loss of blood due to:
 - parasites, especially malaria and hookworms
 - excessive menstrual losses
 - chronic diseases such as ulcers or tuberculosis.
- lack of iron in the diet
- inability of the body to absorb iron from food
- Destruction of blood cells due to malaria

How can anemia be prevented or treated?

- Take iron tablets or syrup, if anemia is due to a lack of iron in the diet.
- Eat a diet adequate in iron-rich foods such as dark green leafy vegetables, liver, meat or fish, and fruits rich in vitamin C such as oranges, lemons and mangoes.
- Avoid giving tea or coffee to infants and young children.
- Prevent and treat worms.
- Prevent malaria by using mosquito nets, spraying the house with insecticide, removing stagnant water, and promptly treating any case in young children.

[NAME OF IMPLEMENTING AGENCY]

[LOGO OF IMPLEMENTING AGENCY]

Name of Household Head: _____

Date: _____

[IMPLEMENTING AGENCY] is conducting a study on anemia and malaria. The study will help us identify whether there are problems with anemia and malaria among young children in [COUNTRY].

We appreciate that we have had the opportunity to interview members of your household and to test children 6 months to 5 years old for anemia and malaria.

Thank you for your cooperation.

*Please look inside
for the results of the
anemia and malaria
testing.*

APPENDIX B: MICROSCOPE SLIDE TRANSMITTAL FORM

[YEAR] [COUNTRY] [MALARIA INDICATOR] SURVEY
 MICROSCOPE SLIDE TRANSMITTAL FORM (FRONT SIDE)

CLUSTER NUMBER

--	--	--

PERSON SENDING/ RECEIVING SAMPLES	TIME TO FILL IN FORM	TOTAL COUNT OF MICROSCOPE SLIDES	SIGNATURE (CONFIRMING THAT EACH SLIDE IS PRESENT - SEE BACK OF FORM)	NAME	DATE	NOTES (NOTE ANY DISCREPANCY IN NUMBERS OF SLIDES)		
(1)	(2)	(3)	(4)	(5)	(6)	(7)		
TECHNICIAN	WHEN CLUSTER IS COMPLETED	<table border="1" style="display: inline-table; width: 40px; height: 20px;"><tr><td style="width: 20px;"></td><td style="width: 20px;"></td></tr></table>						
TEAM SUPERVISOR	WHEN CLUSTER IS COMPLETED	<table border="1" style="display: inline-table; width: 40px; height: 20px;"><tr><td style="width: 20px;"></td><td style="width: 20px;"></td></tr></table>						
FIELD COORDINATOR	WHEN SAMPLES ARE PICKED UP IN FIELD	<table border="1" style="display: inline-table; width: 40px; height: 20px;"><tr><td style="width: 20px;"></td><td style="width: 20px;"></td></tr></table>						
CENTRAL OFFICE RECEIVER	UPON ARRIVAL AT [IMPLEMENTING ORGANIZATION]	<table border="1" style="display: inline-table; width: 40px; height: 20px;"><tr><td style="width: 20px;"></td><td style="width: 20px;"></td></tr></table>						
LAB	UPON ARRIVAL AT LAB	<table border="1" style="display: inline-table; width: 40px; height: 20px;"><tr><td style="width: 20px;"></td><td style="width: 20px;"></td></tr></table>						

INSTRUCTIONS: GENERAL: Count the microscope slides. Count bar codes on the Microscope Slide Transmittal Form. Check both against each other and make sure the counts match.

Technician: Upon completion of a cluster, verify that the unique bar code (identification) number on each slide collected in that cluster number corresponds to a bar code number pasted to the back of this transmittal form and vice-versa. Note any discrepancies in Column (7). Count and record the total number of slides in Column (3). Sign your name in Column (4) and record your name in Column (5). Write the date in Column (6). Fold and store this transmittal form in the box containing the slides.

Team Supervisor: After the technician has verified the slides, you will conduct a second verification. Verify that the unique bar code (identification) number on each slide collected in that cluster number corresponds to a bar code number pasted to the back of this transmittal form and vice-versa. Note any discrepancies in Column (7). Count and record the total number of slides in Column (3). Sign your name in Column (4) and record your name in Column (5). Write the date in Column (6). Fold and store this transmittal form in the box containing the slides.

Field Coordinator: Before returning to the Central Office after visiting a team in the field, you will count and record the total number of thick and thin slides in Column (3). Sign your name in Column (4) and record the date in Column (6). Note any discrepancies in Column (7). Fold and store this transmittal form in the box containing the slides.

[IMPLEMENTING AGENCY]: For each cluster arriving from the field, verify that the unique bar code (identification) number on each slide collected in that cluster number corresponds to a bar code number pasted to the back of this transmittal form and vice-versa. Note any discrepancies in Column (7). Count and record the total number of slides in Column (3). Sign your name in Column (4) and record your name in Column (5). Write the date in Column (6). Make a photocopy of the Microscope Slide Transmittal Form. Fold and store this transmittal form in the box containing the slides and send them to the Malaria Lab [NAME].

Laboratory: Upon receiving slides from [CENTRAL OFFICE], verify that the unique bar code (identification) number on each slide collected in that cluster number corresponds to a bar code number pasted on the back of this transmittal form and vice-versa. Count and record the total number of slides in Column (3). Sign your name in Column (4) and record your name in Column (5). Write the date in Column (6). Note any discrepancies in Column (7) and inform the [CENTRAL OFFICE]. Follow the [NAME OF COUNTRY] MIS protocol for storing and reading the slides.

[YEAR] [COUNTRY] [MALARIA INDICATOR] SURVEY
 MICROSCOPE SLIDE TRANSMITTAL FORM (BACK SIDE)

CLUSTER NUMBER

--	--	--

NO.	SLIDE BAR CODE	THICK SLIDE	THIN SLIDE	TECH.	LAB
1					
2					
3					
4					
5					
6					
7					
8					

NO.	SLIDE BAR CODE	THICK SLIDE	THIN SLIDE	TECH.	LAB
17					
18					
19					
20					
21					
22					
23					
24					

Fold here

9					
10					
11					
12					
13					
14					
15					
16					

25					
26					
27					
28					
29					
30					
31					
32					

APPENDIX C: SEVERE ANEMIA AND SEVERE MALARIA REFERRAL FORMS

[COUNTRY] [YEAR] MIS REFERRAL FORMS

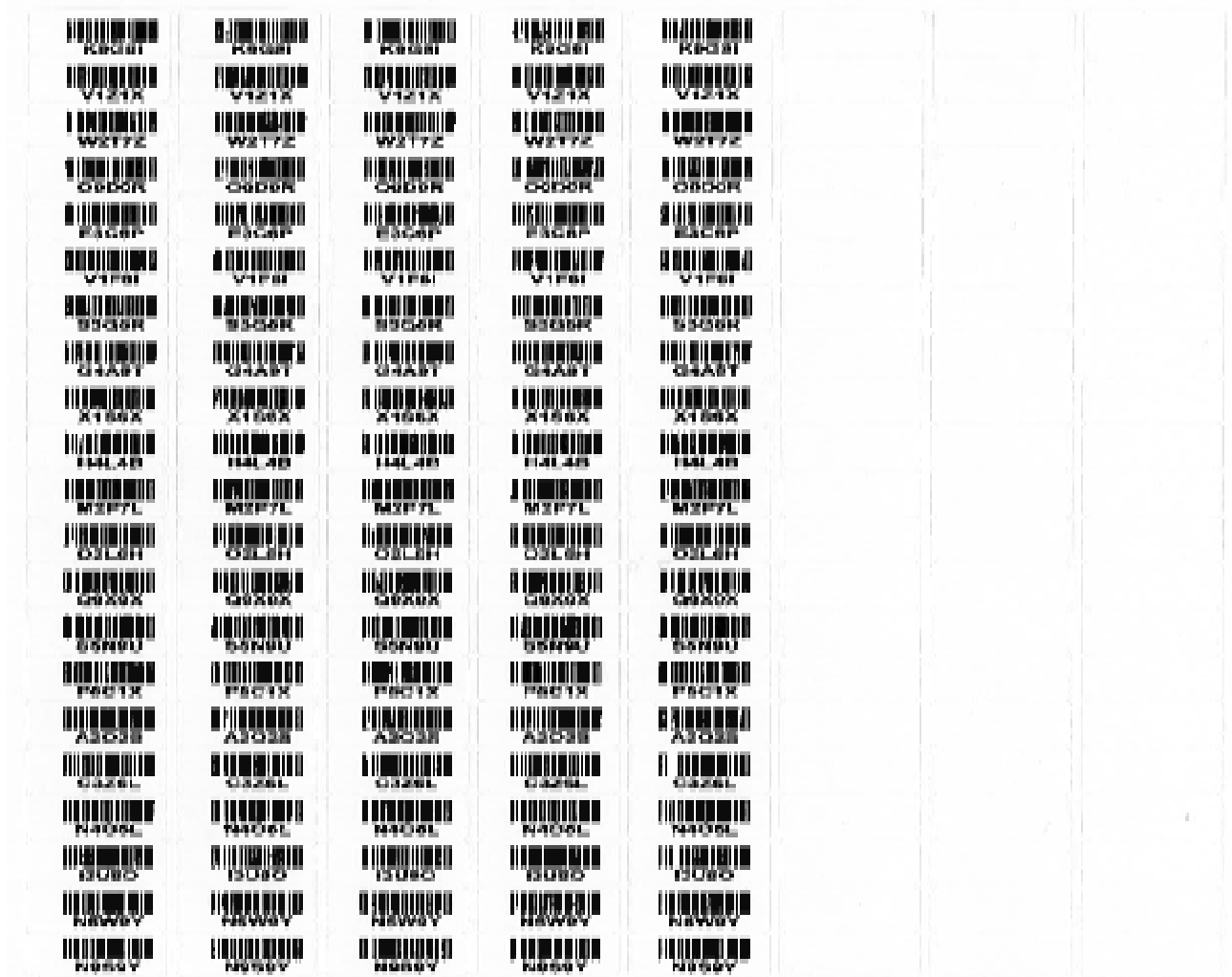
Anemia Referral to be given when child's hemoglobin level is less than 8.0 g/dL.

[COUNTRY] MALARIA INDICATOR SURVEY: Anemia Referral
During the 2011 [COUNTRY] MIS _____ (Name), age ____ _____ years, was tested for anemia on ____/____/____. His/her level of hemoglobin was ____. ____ g/dl, which indicates he/she has severe anemia. This child needs medical attention for the anemia.

Severe Malaria Referral to be given when child has a positive RDT and displays signs of severe malaria.

[COUNTRY] MALARIA INDICATOR SURVEY: Severe Malaria Referral	
During the 2011 [COUNTRY] MIS _____ (Name), age ____ _____ years, was tested for malaria on ____/____/____, with a Rapid Diagnostic Test (RDT). He/she tested positive for malaria, and is displaying the following signs of severe malaria:	
<input type="checkbox"/> EXTREME WEAKNESS	<input type="checkbox"/> HEART PROBLEMS
<input type="checkbox"/> LOSS OF CONSCIOUSNESS	<input type="checkbox"/> RAPID OR DIFFICULT BREATHING
<input type="checkbox"/> SEIZURES	<input type="checkbox"/> ABNORMAL BLEEDING
<input type="checkbox"/> JAUNDICE OR YELLOW SKIN	<input type="checkbox"/> DARK URINE
<input type="checkbox"/> HEMOGLOBIN LEVEL OF ____ . ____ g/dl (LESS THAN 8.0G/DL)	
He/she appears to be very ill and did not receive treatment for the malaria. THIS CHILD NEEDS TO BE TAKEN TO A HEALTH FACILITY RIGHT AWAY.	

Appendix D: Example barcode labels



APPENDIX E: EXAMPLE SUPPLY LIST FOR THE FIELD

KMIS Supply List for the Field							
Biomarker supplies				Interviewer, Supervisor supplies			
<i>-- quantities per health technician team --</i>				<i>-- quantities per individual field staff --</i>			
PER DAY	PER CLUSTER			PER DAY	PER CLUSTER		
20	120	Consent forms (parent/guardian)		6	32	Biomarker questionnaires	
10	57	Assent forms (children >6 years)		20	120	Consent forms (household interview)	
5	29	Referral forms		20	120	Consent forms (women's interview)	
6	32	Malaria/anaemia brochures		10	57	Consent forms (women, parental consent)	
20	120	Disposal latex gloves (pairs)		1	1	Tablet (fully charged)	
20	120	Alcohol swabs		1	1	Tablet charger (portable or for use in lodging)	
20	120	Sterile gauze pads		1	1	SD card	
10	60	Adhesive bandages		1	1	Airtime	
20	120	Retractable lancet		1	1	Blue Biro pen	
5	32	Absorbant sheet		1	1	Notepad	
1	1	Hemocue 301+ (with batteries)		1	1	Interviewer's Manual	
0	<1	Hemocue cleaner		1	1	Bag/backpack	
10	60	Microcuvettes		1	1	Supervisor's Manual (supervisor only)	
10	60	RDT packet		1	1	Modem (supervisor only)	
1	1	Timer		1	1	Data bundle (supervisor only)	
10	60	Slide spreader					
20	120	Slides					
1	1	Cardboard slide folder					
10	60	Malaria treatment					
1	1	Biohazard bag					
1	1	Sharps container					
5	20	Dessicants					
0	1	Cool box					
2	6	Slide boxes					
2	2	Zploc bag					
1	1	Airtime					
1	1	Bag/backpack					
1	1	Blue Biro pen					
1	1	Notepad					
1	1	Torch					
1	1	Biomarker Manual					
**the following to remain at lodging and not to be taken to the field							
1	1	Coplin Jar					
1	4	Absolute methanol (~100ml)					
1	1	Slide drying rack					

Assumes 1.89 children/HH (about 60 children/cluster), ~5 HH/day, 30 HH/cluster, 6% contingency