

# **AIDS Indicator Survey**

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## **HIV Testing Manual**

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**Macro International Inc.  
Calverton, Maryland**

**April 2007**

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The AIDS Indicator Survey was developed as part of the MEASURE DHS project. MEASURE DHS assists countries worldwide with the collection and use of data to monitor and evaluate population, health and nutrition programs. Funded by the U.S. Agency for International Development (USAID), MEASURE DHS is implemented by Macro International Inc. in Calverton, Maryland.

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Suggested citation:

Macro International. 2007. *AIDS Indicator Survey: HIV Testing Manual* MEASURE DHS Calverton, Maryland, USA.: Macro International.

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# SECTION 1: INTRODUCTION

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## 1.1 General Background

This manual discusses in detail the techniques and procedures for collecting, handling and packaging blood samples for HIV testing during the AIDS Indicator Survey (AIS). In order to collect the blood samples for HIV testing, the interviewers for the AIS will act as both interviewers and as medical staff who will also be responsible for taking the blood samples from eligible women and men. In addition to their duties as interviewers, they will be responsible for:

- locating all eligible women and men for HIV testing;
- informing eligible women and men about the test and asking for consent;
- collecting and labeling blood samples;
- filling the necessary forms;
- leaving a brochure with HIV information;
- telling respondents where they can go for HIV testing if they want to know their HIV status;
- giving them a voucher for free testing.

The AIDS Indicator Survey will collect information on demographic characteristics, reproduction, marriage and sexual activity, HIV/AIDS and sexually transmitted infections (STIs) through individual interviews with a representative sample of women and men in the reproductive ages. In addition to individual interviews, blood samples will be collected in the survey households from men and women age 15-49 years for HIV testing.

## 1.2 HIV Testing

Acquired immunodeficiency syndrome (AIDS) is a condition caused by human immunodeficiency virus (HIV). It is a disorder of the immune system in which the body's normal defenses against infection break down, leaving it vulnerable to a host of life threatening infections, including unusual malignancies.

HIV testing in the AIS will involve the collection of dried blood spots (DBS) samples on a filter paper card. This is performed using capillary blood from a finger stick. After the DBS samples and questionnaires have been transported to the central office for logging and checking, the DBS samples will be transported to the laboratory where the testing will be conducted. The testing will be anonymous, i.e., personal identifiers of individual respondents will not be retained with the DBS samples. Because the testing is anonymous, respondents will not receive the results of the testing. However, they will be offered a coupon to obtain free HIV counseling and testing at a collaborating VCT center(s) near their community.

### Testing of Men and Women

Identify all men and women from the household schedule who are eligible for testing. Women and men age 15-49 years will be eligible for HIV testing;

Check the age of the respondent recorded in **question 103**. Then go to the **HIV Test** section and record whether the respondent is age 15-17 or age 18-49 for **Question 701**. If the respondent is age 15-17, record

whether the respondent has ever been married in **Question 702**. You will do this because if the respondent is age 15-17 and has never been married, consent for testing will have to be obtained from the respondent's parent or responsible adult;

If the respondent is between ages 15-17 and has never been married, record name and line number of the parent or adult responsible for the youth in **Question 703**;

If the respondent is age 15-17 and has never been married, seek consent for HIV testing from the parent/responsible adult before seeking consent directly from the respondent. If the eligible respondent is age 18 or over or has ever been married, seek direct consent of the respondent for HIV testing. Record outcome of the consent request in **Question 704**. If the parent refuses to consent, circle 2. If the respondent refuses to consent, circle 3. If consent is granted, circle 1. The signature in **Question 704** will be your signature, confirming that you read the statement to the responsible adult;

After obtaining the consent, identify the next available complete set (row) of bar code labels. Paste the first bar code label in **Question 705**, the second bar code label on a new filter paper card, and the third bar code label on the transmittal sheet;

Obtain a capillary blood sample from the finger prick. Use enough blood drops to fill as many of the preprinted circles on the filter paper card as possible for HIV antibody testing in the laboratory. At least four of the circles must be filled.

Place the filter paper card with the blood spots in the drying rack;

Record the result for HIV blood sample collection in **Question 706**;

Seek consent to store part of the blood sample for future research. If the respondent is age 15-17 and has never been married, seek consent for blood storage from the parent/responsible adult before seeking consent directly from the respondent. If the eligible respondent is age 18 or over or has ever been married, seek direct consent of the respondent for HIV testing. Record outcome of the consent request in **Question 707**. If the parent refuses to consent, circle 2. If the respondent refuses to consent, circle 3. If consent is granted, circle 1. The signature in **Question 707** will be your signature, confirming that you read the statement to the responsible adult;

Provide an informational brochure on HIV/AIDS, a coupon for free VCT services, and a list of the nearest participating VCT centers.

Subsequent sections in this manual will describe in detail the procedures you will follow in accomplishing the above tasks.

### **1.3 Training of Interviewers on HIV Testing**

Your training to conduct the HIV testing will consist of a combination of classroom training and practical experience that will provide you with the background necessary to perform these tasks during a field survey.

During the first phase of training, we will review with you various sections of this manual. You will learn how to identify eligible respondents, record information relating to the testing in the household questionnaire on special field forms, handle the technical procedures involved in specimen collection, testing and transporting, and other related instructions.

During the second phase of the training, there will be role-playing for HIV testing in which you will practice by collecting blood samples from other trainees. During the third phase of the training, we will visit a health center and practice testing on consenting clients.

During the final part of the training, you will be assigned to an AIS trainee team and will practice both interviewing and collecting blood samples from eligible adults in some pretest areas outside of those chosen for the AIS sample.

Before each training session, you should study this manual carefully along with the household questionnaire, writing down any questions you may have. You are encouraged to ask questions at any time in order to avoid mistakes during actual fieldwork. You can learn a lot from each other by asking questions and talking about problems encountered in practice and actual situations.

Like the rest of the AIS training, throughout the blood collection training, you will be given homework assignments for the evenings. You may be given tests to see how well you are progressing during your formal training period. At the end of the training course, your overall performance during the training will be assessed and we will select those of you who have performed the best to work in the main survey.

#### **1.4 Supervision of Interviewers During Blood Collection**

Training is a continuous process. Observation and supervision throughout the fieldwork are a part of the training and data collection process. Your team supervisor and the AIS survey coordinators will play very important roles in continuing your training and in ensuring the quality of data. They will:

- observe some of your fieldwork activities to ensure that you are conducting yourself professionally, asking the questions in the right manner, and following the survey protocol correctly;
- spot check some of the eligible respondents selected for testing to be sure that you collected the blood specimen from the correct households and respondents;
- consolidate the field record forms, samples, and both the household and individual questionnaires;
- meet with each member of the team on a regular basis to discuss performance and give out future work assignments;
- help to solve any problems that you might have with finding the assigned households, understanding the concepts in the questionnaire or dealing with difficult respondents.

The survey director may release from service any field staff member who is not performing at the level necessary to produce the high quality data required to make the AIS a success.

#### **1.5 Organization of the Training Manual**

The remaining sections of this manual are organized to assist you in learning how to conduct these tasks. They include:

Section 2: Introduction to the equipment and supplies for the testing

Section 3: Recording information on the testing

Section 4: General procedures to follow in obtaining a capillary blood sample

Section 5: Specific procedures to follow in collecting blood samples for HIV

Section 6: Precautions to follow in collecting samples

Section 7: Disposing of biohazardous waste

These sections should be studied carefully during the training and used as a reference in the field when you have questions about how you should proceed in conducting the various tasks involved in testing.

## SECTION 2: MATERIALS AND EQUIPMENT FOR TESTING

This section of the manual introduces the various materials and supplies which you will use for HIV testing. More detail on the use of these items is presented in Sections 4-6, which describe how the testing will be performed.

### 2.1 Materials and Supplies for Performing Finger Prick

The capillary blood drops used for HIV testing will be drawn from a finger. The following supplies and materials (Figure 2.1) will be used in performing the finger prick:

- **Non-powdered disposable latex gloves** are used to reduce the risk of blood-borne diseases. Gloves must be worn by the interviewer and by anyone else who may assist with the blood collection.
- **Alcohol preps** are used for cleaning the skin prior to puncturing the finger.
- **Sterile gauze pads** are used to wipe away the first drops of blood to stimulate a spontaneous capillary blood flow.

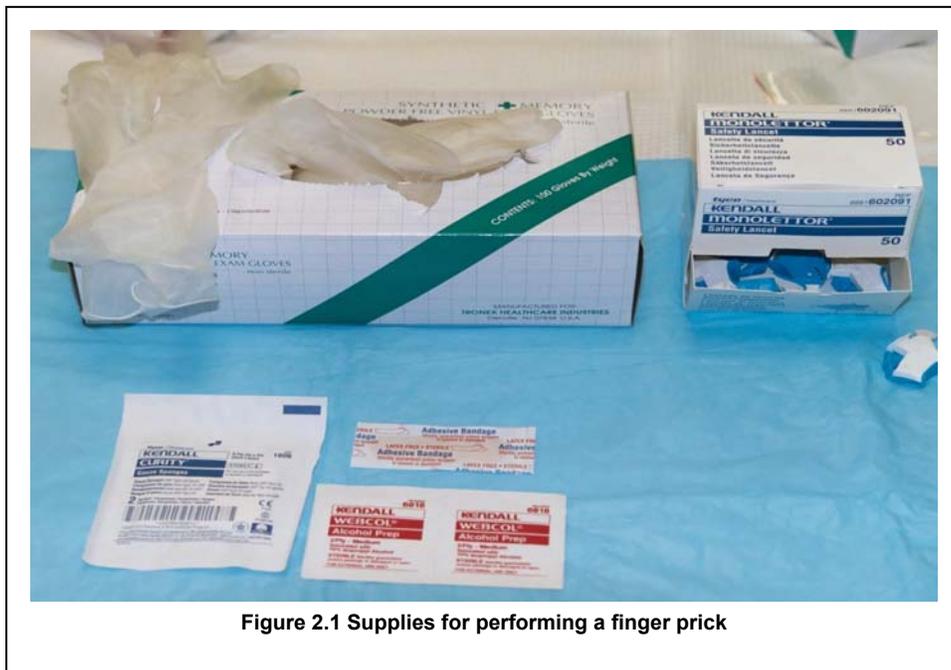


Figure 2.1 Supplies for performing a finger prick

- **Adhesive bandages (Band-aids).** After the blood collection, an adhesive bandage has to be applied on the puncture site to avoid infection.
- **Retractable lancets.** The lancet is an automated, disposable incision device used to puncture the fingertip. The device is specially shaped to fit easily on the skin surface, thus minimizing skin indentation; the angle of the blade is set for maximum blood flow. When the trigger is pressed, a surgical blade quickly protrudes from the device and then automatically retracts and the action of the blade is so fast it cannot be seen.

## 2.2 Materials and Supplies for HIV Testing

The following special equipment and supplies are used in the collection, storage and transport of the dried blood spot (DBS) samples.

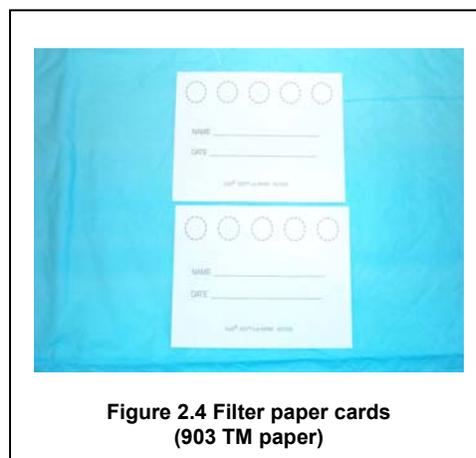
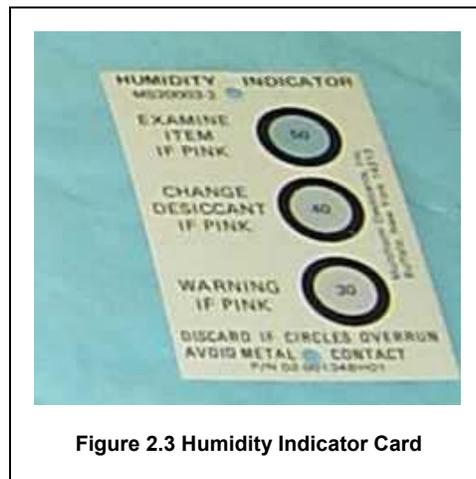
- **Desiccants (drying agents)** (Figure 2.2). Desiccant packets, which absorb moisture from the air, are used to keep the materials used for collecting the DBS samples as dry as possible. Non-regeneratable desiccant packets are transparent packets in which the granules are visible. The granules change color as they absorb moisture. You should change the desiccants as indicated by the humidity indicator cards or when the packet has changed color.
- **Humidity indicator cards** (Figure 2.3). The non-regeneratable desiccant packets will turn color at the point of saturation. The humidity indicator card allows you to more closely monitor the level of moisture. This will enable you to add additional desiccant packets in conditions of high humidity.

There are three circles on the humidity indicator card. If the circle at the bottom of the card (labeled 30%) turns pink, it indicates a relatively high level of humidity and is a warning to begin to carefully monitor the humidity level. If the middle or top circles (labeled 40% and 50%, respectively) turns pink, you should replace the desiccant packets in the bag with fresh packets. Replace the humidity indicator card with a fresh card if the circles merge.

- **Filter paper card** (Figure 2.4). You will use special filter paper cards to collect the blood samples. Each of the cards has five preprinted circles that contain approximately 100  $\mu$ l blood when fully filled. You must fill at least four of the circles for the HIV testing.

The preprinted circles of the filter paper cards must be kept clean and dry at all times as water, dust, sweat from your hands, or other environmental contaminants can affect the HIV test results. Thus, you should use gloves at all times when handling the filter paper cards.

- The filter paper cards come in packets of 100 cards. When you need a new packet of filter paper cards, put on gloves first. Open the packet and place all cards in a large zip-loc bag. They must be stored stacked so as to avoid compressing the filter paper. **Place two non-regeneratable desiccant packets and a humidity indicator card in each bag before sealing it.** Later, if the humidity indicator card changes color, follow the above instructions. If you do not have humidity



indicator cards and the crystals in the desiccant packets change color before you have used up all of the 100 filter paper cards, you should replace the desiccant packets with fresh packets.

- **Bar code labels.** Because the HIV testing is anonymous, bar code labels will be used to identify the DBS samples. These peel-off, preprinted bar code labels are provided on special sheets. Each row on a sheet includes a number of labels with the same bar code. A different row on the sheet will be used for each respondent for whom a DBS sample is collected. Instructions for using the bar code labels are included in Section 6 of this manual.
- **Drying Box.** After collection, the blood spot samples must be placed in a specially designed box where they will be stored overnight to dry. The plastic drying box has a cardboard paper drying rack that holds the sample filter paper cards in a horizontal position when the box is standing vertically. The plastic boxes are to be used for collection and overnight drying only. They are not intended for long-term sample storage. Pouches of non-regeneratable desiccants should be kept at all times at the bottom of the drying box (as it stands vertically) to hasten the drying process, particularly in high humidity environments. A humidity indicator card should be placed in the drying box to allow you to monitor the level of humidity.
- **Low gas-permeable bags (small zip-loc bags).** You will be given a supply of special small zip-loc bags to use for storing samples and supplies in the field. These bags are specially manufactured to reduce the exposure of their contents to air and moisture. These bags are expensive and should never be used for other purposes, like carrying food or adhesive bandages. The bags have a sliding “zipper” that is used to close and seal the bag. Each morning, you will transfer the DBS samples from the previous day’s fieldwork, which have dried overnight, to these bags (See Section 6).
- **Glassine paper.** Thin, glossy, semi-opaque paper squares. The glassine paper is used to protect the dried blood spots on the filter paper cards during storage.
- **Large zip-loc bags.** A large zip-loc bag will be provided to you for each of the AIS sample clusters in which you will work. These large zip-loc bags will be used to hold the DBS samples from the cluster during storage and transport.
- **Plastic bags for biohazardous wastes.** These are big bags that are provided to hold all the biohazardous waste materials during the day and will be discarded appropriately (see Section 7).

## **SECTION 3: COMPLETING QUESTIONNAIRES AND OTHER TESTING DOCUMENTS**

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You will be responsible for the accurate recording of information that will be used to track the outcome of the testing procedures. Documents which you will complete during the testing are:

- Blood Spot Collection page for women and men in the Individual Questionnaire.
- Blood Sample Transmittal Sheet.
- Voucher/coupon for free VCT services.

In addition, you will be provided with informational brochure on HIV/AIDS – to be left with the households that you visit for blood collection for HIV testing.

After the fieldwork and data entry and cleaning have ended, the Blood Spot Collection page, which includes the unique barcode assigned to the respondent's HIV blood sample, will be removed from the questionnaire in the central office and destroyed. This is to ensure the anonymity of the HIV test results. It will be impossible to identify which respondent gave a particular blood sample for testing once the page is removed and destroyed.

This section reviews the various tasks that are associated with using these various materials. The key tasks include:

- Identifying eligible respondents.
- Obtaining informed consent for the testing.
- Recording information relevant to the outcome of the testing in the Individual Questionnaire and other tracking documents.
- Providing informational brochures and written referrals for HIV VCT.

Specific activities that are involved in performing these tasks are described in detail below.

### **3.1 Identifying Eligible Respondents**

For eligible women and men (age 15-49 years), information relating to HIV testing will be recorded on the **HIV Test** page found in the Individual Questionnaire.

The first step in the testing process will be to identify members of the household who are eligible for HIV testing. All women and men age 15-49 years are eligible for collection of dried blood spot samples for later HIV testing in the laboratory. The age of the respondent has to be recorded and checked from question 103 of the individual questionnaire.

**All eligible consenting respondents should be tested after their individual interview has been completed.**

### 3.2 Obtaining Informed Consent for the Testing

One of the primary tasks before blood collection is to explain the purpose of the testing to eligible respondents and to obtain their consent before collecting any blood samples. In order to ensure that these individuals can make an ‘informed’ decision about the testing, the AIS questionnaire includes statements which you will read as appropriate. These ‘informed consent’ statements (Figures 3.1 and 3.2) include the following basic elements:

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

You must read the informed consent statements to each eligible respondent age 18 and over and obtain the respondent’s consent to the testing before any blood collection is done. The approach for obtaining consent differs slightly when the eligible respondent is a never-married youth (15-17 years old). If the respondent is a never-married youth, you must first obtain the consent of one of the respondent’s parents, or, in the absence of a parent, the consent of another responsible adult who is at least 18 years of age. For these respondents, after obtaining consent from the parent/responsible adult, you must read the consent statement to the youth. The only exception is if the youth lives alone or in a household in which there are no adults. In such cases, the consent of the youth is sufficient.

In all cases, you must record the outcome of the consent request before performing the test on a respondent.

The informed consent process for HIV testing **for youths** involves several steps. The following information must be recorded on Blood Spot Collection/ HIV Testing Page:

- **Question 701:** Verify the age of the respondent.
- **Question 702:** Verify that the respondent has never been married. As noted above, if the respondent is age 15-17 and not married or not living independently, check the appropriate boxes in Questions 701 and 702. You must obtain permission from his/her parent/responsible adult before seeking the respondent’s own consent for the testing.
- **Question 703:** For never-married respondents age 15-17, record the line number of the parent/responsible adult whose consent for the testing will be requested.

Record whether both the parent/responsible adult and the respondent consent to the HIV test in **Question 704**. If both the respondent and the parent/responsible adult consent for the test to be conducted on the youth, circle 1. If the parent refuses, circle 2. If the respondent refuses, circle 3, even if the respondent’s parent consented.

You must sign your name to indicate that you read the consent statements to both the respondent and the parent/responsible adult in the case of non-married, non-independent youths and have recorded their response accurately.

**Figure 3.1 Consent Statement for HIV Testing**

704	<p>READ CONSENT STATEMENT TO EACH RESPONDENT. CIRCLE CODE '1' IF RESPONDENT CONSENTS TO HIV TEST AND CODE 3' IF HE/SHE REFUSES.</p> <p>FOR NEVER-IN-UNION MEN/WOMEN AGE 15-17, ASK CONSENT FROM PARENT OR OTHER ADULT IDENTIFIED AS RESPONSIBLE FOR THE ADOLESCENT (SEE QUESTION 703) BEFORE ASKING FOR HIS/HER CONSENT).</p> <p>CIRCLE CODE '2' IF THE PARENT (OTHER ADULT) REFUSES. CIRCLE CODE '1' ONLY IF BOTH THE PARENT (OTHER ADULT) AND THE ADOLESCENT CONSENT.</p> <p>As part of this survey we also are asking people all over the country to take an HIV test. HIV is the virus that causes AIDS. AIDS is a very serious illness. The HIV test is being done to see how big the AIDS problem is in (COUNTRY).</p> <p>For the HIV test, we need a few more drops of blood from a finger. The equipment being used in taking the blood is clean and completely safe. It has never been used before and will be thrown away after each test.</p> <p>No names will be attached so we will not be able to tell you the test results. No one will be able to know your test results either.</p> <p>If you want to know whether you have HIV, I can provide you with a list of [nearby] facilities offering counseling and testing for HIV. I will also give you a voucher for free services for you (and your partner if you want) that you can use at any of these facilities.</p> <p>Do you have any questions?</p> <p>You can say yes to the test, or you can say no. It is up to you to decide.</p> <p>Will you (allow NAME OF ADOLESCENT to) take the HIV test?</p>	<p>GRANTED ..... 1</p> <p>PARENT/OTHER RESPONSIBLE ADULT REFUSED ..... 2 → END</p> <p>RESPONDENT REFUSED ..... 3 → END</p> <p>SIGNATURE OF INTERVIEWER: _____</p> <p>DO NOT FORGET TO SIGN</p>
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The following are some key points to remember in obtaining consent for the testing:

1. **Read the applicable consent statements to each eligible respondent exactly as they appear in the questionnaire.** When you arrive at the household and begin talking about the blood tests with an eligible respondent, you may informally discuss many of the items included in the informed consent statement. However, before beginning the testing with any respondent, you must still read the informed consent statements exactly as they are worded in the questionnaire. If you feel that the respondent may find the statements repetitive, tell him/her that you are required to formally read the statement to ensure that respondents are given all the appropriate information.
2. The only exception that is allowable is in cases when you have already requested consent for testing from an adult who also happens to be the parent or other responsible adult who must give consent to test a youth in the household. In such cases, you can simply ask the parent/responsible adult: “Do I also have your permission for (NAME OF YOUTH) to participate in the study?”.
3. **Be sure that you read the informed consent statements clearly.** Practice reading the consent statements so that you become comfortable reading them in a clear, natural manner. Avoid using a monotone when reading the statements or reading them so rapidly that they cannot be understood.
4. **Never collect blood from a never-married youth (15-17 years) before obtaining the consent of the parent/responsible adult** unless the youth lives alone or in a household where there are no adult members.



- Do not attempt to peel an incorrect label off of any form or filter paper card. If you make a mistake with the labels, use the next complete set of labels and paste them over the incorrect ones.

More detail on this procedure is provided in Section 6, which describes the process of collecting blood for HIV tests.

### **3.4 Providing VCT Information**

Along with an informational brochure on HIV/AIDS that will be left with the household, a list of participating VCT centers where vouchers distributed by the AIS can be taken for free HIV Voluntary Testing and Counseling services will be provided.

All eligible respondents and their spouse/partner, regardless of their participation or non-participation in the HIV testing, as well as anyone requesting such information should be provided with voucher(s) for free HIV VCT services, a list of participating VCT centers, and an informational brochure about HIV/AIDS.

**Figure 3.3 Blood Transmittal Sheet – Front**

TEAM  
NUMBER

--	--

CLUSTER  
NUMBER

--	--	--

PERSON SENDING/ RECEIVING SAMPLES	TIME TO FILL IN THE FORM	TOTAL COUNT OF BLOOD SAMPLES	SIGNATURE (CONFIRMING THAT EACH SAMPLE IS PRESENT—SEE BACK OF FORM)	SIGNATURE (CONFIRMING THAT THE NUMBER OF BLOOD SAMPLES MATCHES COL. 3)	DATE	NOTES (NOTE ANY DISCREPANCY IN NUMBERS OF SAMPLES)		
(1)	(2)	(3)	(4)	(5)	(6)	(7)		
INTERVIEWER AND TEAM SUPERVISOR	WHEN CLUSTER IS COMPLETED	<table border="1" style="width: 40px; height: 20px;"><tr><td></td><td></td></tr></table>						
FIELD COORDINATOR	WHEN SAMPLES ARE PICKED UP IN FIELD	<table border="1" style="width: 40px; height: 20px;"><tr><td></td><td></td></tr></table>						
TECHNICAL DIRECTOR OR MEDICAL COORDINATOR	UPON ARRIVAL AT THE CENTRAL STATISTICAL OFFICE	<table border="1" style="width: 40px; height: 20px;"><tr><td></td><td></td></tr></table>						
HEAD LABORATORY TECHNICIAN	UPON ARRIVAL AT LABORATORY	<table border="1" style="width: 40px; height: 20px;"><tr><td></td><td></td></tr></table>						

**INSTRUCTIONS**

**Interviewer:** Upon completion of a cluster, verify that the unique bar code (identification) number on each blood sample (filter paper card) collected and stored in the large zip-loc bag labeled with that cluster number corresponds to a bar code number pasted to the back of this transmittal sheet and vice-versa. Note any discrepancies in Column (7). Count and record the total number of blood samples in Column (3). Sign your name in Column (4) and the date in Column (6). Fold and store this transmittal sheet in the large zip-loc bag.

**Field Team Supervisor:** After the interviewer has verified the blood samples, you will conduct a second verification. Verify that the unique bar code (identification) number on each blood sample (filter paper card) collected and stored in the large zip-loc bag labeled with that cluster number corresponds to a bar code number pasted to the back of this transmittal sheet and vice-versa. Note any discrepancies in Column (7). Count and verify the total number of blood samples in Column (3). Sign your name in Column (5) and the date in Column (6). Refold and store this transmittal sheet in the large zip-loc bag.

**Field Coordinator:** Before returning to the General Statistical Office after visiting a team in the field, you will verify the number of blood samples collected in each completed cluster that you are carrying back with you. For each completed cluster, count and record the total number of blood samples stored in the large zip-loc bag labeled with that cluster number in Column (3). Note any discrepancies in Column (7). Sign your name in Column (5) and the date in Column (6). Refold and store this transmittal sheet in the large zip-loc bag.

**General Statistical Office Receiver:** For each large zip-loc bag arriving from the field, you will verify the number of blood samples received. Count and record the total number of blood samples stored in the large zip-loc bag labeled with the cluster number in Column (3). Note any discrepancies in Column (7). Sign your name in Column (5) and the date in Column (6). Photocopy both sides of this transmittal sheet and file the photocopies (as instructed) in a designated, locking file cabinet. Refold and store the original transmittal sheet in the large zip-loc bag. Follow the AIS protocol for sending blood samples to the laboratory for testing.

**Laboratory Receiver:** Upon receiving blood samples from the General Statistical Office, verify that the unique bar code (identification) number on each blood sample (filter paper card) collected and stored in the large zip-loc bag labeled with the cluster number corresponds to a bar code number pasted to the back of this transmittal sheet and vice-versa. Note any discrepancies in Column (7). Count and record the total number of blood samples in Column (3). Sign your name in Column (4) and the date in Column (6). Follow the AIS protocol for storing and processing blood samples.

**Note:** this form will be destroyed under the direction of the Lab Director after all blood samples have been completely processed and a Final HIV Test Result has been determined for each usable sample.



Figure 3.5 Sample Sheet of Bar Code Labels



## SECTION 4: PROCEDURES FOR COLLECTING CAPILLARY BLOOD DROP SAMPLES

### 4.1 Steps in Obtaining Capillary Blood from the Finger

Capillary blood can be obtained from the palm side of the end of a finger. The following describes the steps that are involved in obtaining a capillary blood drop sample from the finger.

#### Step 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 should the respondent feel faint and need 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.

- Take out a **plastic bag** and spread it over a flat surface where you will lay out your supplies.
- Confirm the number of eligible respondents from the household questionnaire for whom blood samples will be collected. After you have established the number of respondents to test, take out the appropriate equipment and supplies (Table 4.1). You will want to have all materials in easy reach when you begin collecting blood samples from the respondents.
- When and where possible, wash and dry your hands. **Put on gloves** before beginning the collection of the blood sample from the first respondent.

**Table 4.1**  
**Equipment and Supplies Needed**

#### For the Finger Prick

Retractable lancets for adults  
Latex gloves  
Alcohol swabs  
Sterile gauze pads  
Band-aids

#### For the HIV Testing

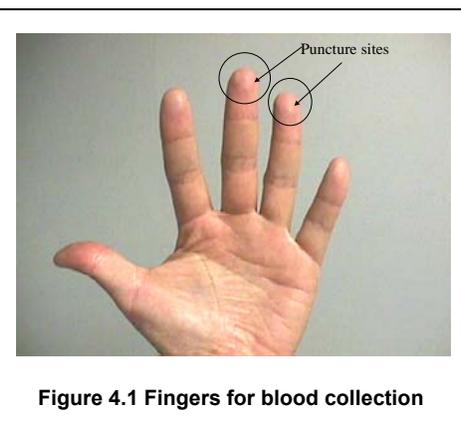
Filter paper cards  
Bar code labels  
Drying box

#### Other supplies/equipment:

Plastic bags for waste

#### Step 2: Select and Prepare the Puncture Site

- It is usually easier if you **sit on the side of the respondent 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 respondent.
- Use the third or fourth finger** for collecting the blood (Figure 4.1). Do not use a finger with a scar, a wound or cut, an infection, swelling, a deformity, or a rash. Also, do not use a finger on which the respondent is wearing a ring, because the ring may disrupt the free flow of blood to the tip of the finger.
- If the hand is cold, warm the skin** over the puncture site by rubbing it. This will increase blood flow by reducing tissue fluid and will improve the ease with which a sample can be obtained.



**Figure 4.1** Fingers for blood collection

- d. **With an alcohol swab, clean the skin of the finger** thoroughly (Figure 4.2). If the skin is very dirty, use a second swab. Allow the alcohol to air dry. Do not blow on the area to dry the alcohol. Blowing may allow bacteria to contaminate the site.
- e. **Ensure that the lancet is easily accessible.** For adults, you will use adult lancets which are blue in color and have a 2.4 mm blade.
- f. Remove the white blade slot cover by first twisting it 360° and then pulling it out. **Do not pull out the needle slot cover without twisting it first** as this may cause the needle not to pierce the skin.

### **Step 3: Prick the Finger**

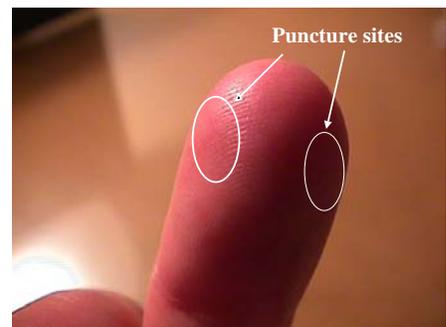
- a. **Make sure that the finger is below the level of the respondent's heart** to increase the flow of blood to the finger. Using a rolling movement of your thumb, **lightly press the finger from the top knuckle toward the tip.** That action will stimulate a flow of blood to the sample area.
- b. When your thumb reaches the fingertip, **maintain a gentle pressure. Place the lancet perpendicular to the surface of the end portion of the finger**, either centered or slightly off center. Avoid the very tip of the finger or the sides, because of the risk of piercing the underlying bone (Figure 4.3).
- c. Use the lancet to **puncture the skin** by placing the blade-slot surface against the area and pressing the trigger (Figure 4.4). The tip of the blade ejects through the blade slot, producing a micro-incision in the skin, and immediately retracts into the device. After puncturing the skin, turn the finger slightly to prevent blood from running into the grooves of the fingerprints.
- d. After blood collection is completed, the lancet should be placed in the plastic bag provided for biohazardous wastes, along with the other disposable materials used for the blood sample collection.

### **Step 4: Collect the blood drops**

- a. When the blood appears, use a sterile gauze pad to wipe away the first drop of blood (Figure 4.5). The second, third, and fourth and fifth drops of blood will be collected on a labeled filter paper card for HIV testing. Collect a sixth drop if possible.



**Figure 4.2 Clean the finger**



**Figure 4.3 Puncture sites on the finger**



**Figure 4.4 Using lancet to prick finger**



**Figure 4.5 Wiping away first blood drop**

- b. If the blood stops flowing before a sufficient amount has been collected, the skin puncture procedure may be repeated with the respondent's (parent's/responsible adult's) consent on a different finger. **Do not reuse any of the supplies used for the first puncture.**

**Step 5: Collect biohazardous wastes**

Place all biohazardous wastes (e.g., lancets, alcohol swabs, gauze, and gloves) into a plastic bag provided for field disposal of these items. At the end of the day, follow the procedures described in Section 7 for the proper disposal of these waste materials.

## SECTION 5: HIV TESTING FOR ADULTS AND YOUTHS

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This section of the manual focuses on the various steps involved in collecting the blood spot samples for HIV laboratory testing.

### 5.1 Collecting Blood for HIV Testing

The principal tasks involved in HIV testing for adults and youths include:

- Obtain voluntary consent of the respondent;
- Place the bar code labels on a filter paper card, the HIV Testing Page of the questionnaire, and the Blood Sample Transmittal Sheet;
- Prick the respondent's finger;
- Collect the second, third, fourth, and fifth blood drops from a finger prick on a labeled filter paper card;
- Stop the bleeding at the prick site;
- Place the filter paper card from the respondent in the drying box;
- Provide all respondents with an informational brochure on HIV/AIDS, coupons for free VCT services, and a list of participating VCT centers;
- Collect biohazardous waste.

Procedures for pricking the respondent's finger were detailed in Section 4. The following describes the steps that should be followed when collecting blood samples for HIV .

#### **Step 1: Placement of the bar code labels**

- a. Wearing a pair of latex gloves, carefully remove a new filter paper card from the plastic zip-loc bag in which you have stored the cards. Make sure to handle the card in such a way that you do not touch the areas within the preprinted circle. *Never handle a card with your bare hands as you may transfer sweat, dirt or other contaminants on to the card.*
- b. Put the card with the preprinted circles face-up on the clean absorbent paper sheet that you have spread out on a flat surface. Discard the card if it drops on the floor or ground or if it becomes dirty in any other manner.
- c. Take the **first bar code label** from the next full row on the sheet of labels and paste it in **Question 705** of the Blood Spot Collection Page for HIV testing.
- d. Take the **second bar code label** from the same row on the sheet of labels and place it at the bottom of the filter paper card where it says 'NAME'. Do not cover up any part of the preprinted circles. *Remember not to touch the circles.*
- e. Take the **third bar code label** from the same row on the sheet of labels. Paste it on the Blood Sample Transmittal Sheet for this cluster that will be sent together with the samples to the central statistical office and then to the laboratory.

- f. **DO THIS CAREFULLY.** The bar code label is the only means of identifying the blood sample and for eventually linking the final HIV antibody test results to the interview data. Mistakes will result in mismatches later on. **CHECK THAT ALL THREE LABELS FOR A RESPONDENT HAVE BEEN PLACED ON THE FILTER PAPER CARD, THE TEST PAGE, AND THE TRANSMITTAL FORM BEFORE YOU PROCEED TO COLLECT BLOOD DROPS FROM THE RESPONDENT.** If you have more than one eligible respondent from whom all necessary consents for HIV testing have been obtained, first finish all steps for one respondent before repeating all steps for the next respondent.

### **Step 2: Collecting Blood Spots on the Filter Paper Card**

- a. Follow the instructions in Section 4 for pricking the finger and wiping away the first blood drop. Be sure to hold the respondent's hand at a level lower than his/her heart to promote blood flow.
- b. While maintaining a firm grip on the finger, press gently on the side of the finger from which you are taking the blood sample to get a large second drop. Be careful to **avoid 'milking' or 'squeezing' the finger** as this could affect the test results. Wait until the drop is large enough to fill one of the preprinted circles on the card (Figure 5.1).

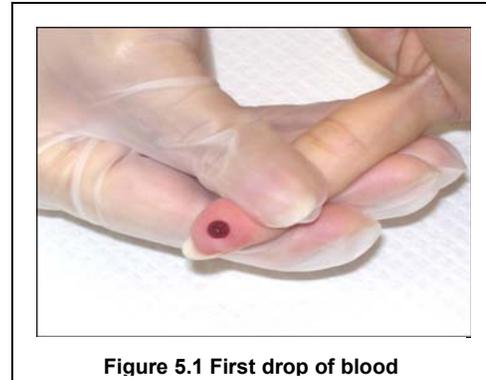


Figure 5.1 First drop of blood

- c. Move the card underneath the finger. Allow the drop to fall. If it does not, the filter paper can be touched gently against the LARGE blood drop (but not the skin) and, in one step, a sufficient quantity of blood should be allowed to soak through and completely fill the circle (Figure 5.2).



Figure 5.2 Filling circles on the filter paper card

The card must not be pressed against the puncture site on the finger. Make sure that the respondent's finger does not touch the card at any point when you are collecting the blood spots. **DO NOT 'layer' the sample** in an attempt to fill in the circle. Always use the pre-printed side of the card to collect the blood spots.

- d. To enhance blood flow, gently apply intermittent pressure to the area surrounding the puncture site to get a third and fourth drop. Allow sufficient time for a large blood drop to form before filling a second circle on the filter paper card. Again, avoid milking or squeezing the finger.
- e. There may be times when a drop of blood will not completely fill the circle. If a circle is not completely saturated, the next drop of blood may be used to saturate the circle if the drop is obtained immediately. If there is any interruption in getting the subsequent drop, you must begin filling another circle. Layering or application of successive drops of blood to the same printed circle causes

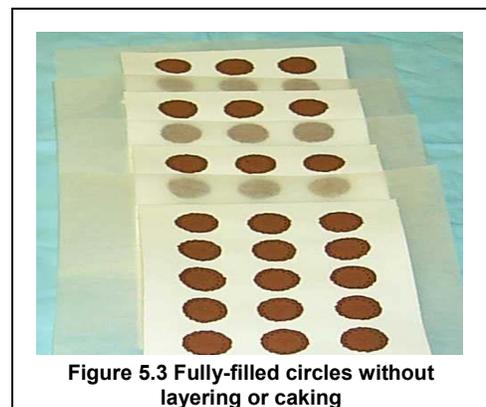


Figure 5.3 Fully-filled circles without layering or caking

caking and/or non-uniform concentrations of blood.

- f. You must continue to collect drops of blood until you have fully saturated at least four circles on the filter paper card (Figure 5.3) If the blood flow stops or decreases before you fully saturate the four circles, you will need to do another finger prick. Whenever this is necessary, you should explain to the respondent that you were unable to obtain an adequate sample and ask permission to obtain blood from another finger. Use fresh supplies and a **different finger** for the second finger prick.
- g. Place the card with the blood spots away from other items. Be careful not to drop the card.

### **Step 3: Stopping bleeding at puncture site**

- a. After the blood drop collection, wipe any remaining blood from the puncture site with a sterile gauze pad.
- b. After making sure that the blood flow has completely stopped, take an adhesive bandage from its wrapper and apply it to the puncture site.

### **Step 4: Placing the filter paper card in the drying box**

- a. The drying box should always be placed vertically on a flat surface before opening. Open the drying box. Carefully pick up the filter paper card with the blood spots and place it in a horizontal position in one of the slots in the drying rack in the box. *Avoid touching or smearing the blood spots on other cards in the box when you are storing a new card. Never put more than one filter paper card in a single slot in the drying rack.* As described in Section 2, blood spots must dry overnight at ambient temperature (15°C to 22°C). *Never set any materials on top of the open box as they might contaminate the filter paper cards that are stored there.*
- b. If the collection process in a household is interrupted for any reason, you should close the box to prevent any possible contamination.
- c. After all blood spot samples for the household have been collected, carefully close the box and return it to a vertical position. The drying box should be kept in a vertical position at all times so that the filter paper cards remain in a horizontal position in their slots inside the box.

### **Step 5: Collecting biohazardous waste**

Place all biohazardous waste (e.g., lancets, alcohol swabs, gauze, and gloves) into a plastic bag provided for field disposal of these items. At the end of the day, follow the procedures described in Section 7 for the proper disposal of these waste materials.

## **5.2 Providing Referrals for HIV VCT Material**

All eligible respondents and their spouse/partner, regardless of their participation or non-participation in the HIV testing, as well as anyone requesting such information should be provided with voucher(s) for free HIV VCT services, a list of participating VCT centers, and an informational brochure about HIV/AIDS.

## **5.3 Storing and Transferring the Dried Blood Spots**

The dried blood spot (DBS) samples must be carefully maintained until they are picked up and taken to the laboratory. They should never be exposed to sunlight during storage, and it is important to regularly

monitor the levels of humidity in the storage box(es). The following describes the steps that should be followed in storing and transferring the DBS samples.

### **Step 1: Storing the DBS Samples**

Each morning, before you go to the field, you must remove the filter paper cards with the blood spots that you collected on the previous day from the drying box and prepare them for storage. The following describes the procedures in this step.

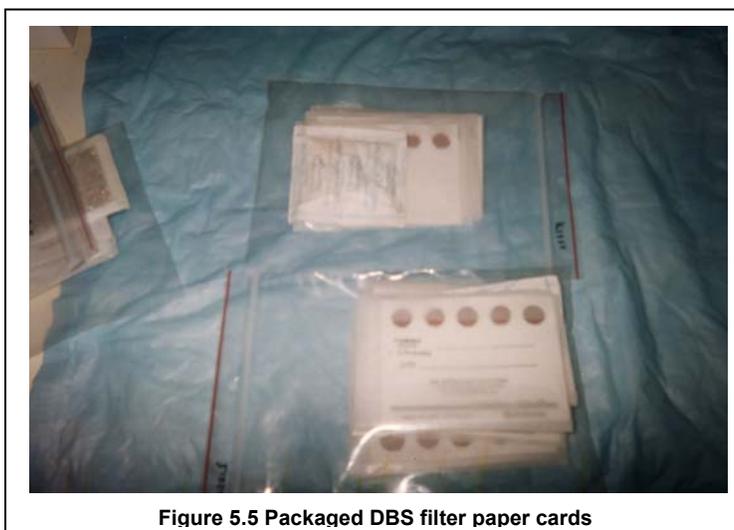
- a. Put on a pair of latex gloves and carefully open the drying box. Check that the blood spots on each filter paper are completely dried (chocolate brown).
- b. Separately remove each filter paper card on which the spots have dried from the drying box. Be careful not to touch the blood spots.



**Figure 5.4 Supplies for storing the filter paper cards**

- c. Gently fold a piece of glassine paper over the blood spots and put the filter paper card into a small (low gas-permeable) zip-loc bag (Figure 5.4). Put one desiccant packet and a humidity indicator card behind the filter paper card, facing out so that the humidity level is visible. It is important that the desiccant packet and the humidity indicator card do not touch the blood spots. Close the zipper, gently pushing out any excess air in the bag as you are zipping it, being careful not to press on the blood spots. **DBS samples should not be allowed to come into contact with other DBS samples during handling, shipment or storage.**
- d. Continue to package each of the filter paper cards from the previous day which have dried overnight, putting each one into a bag with a desiccant packet and a humidity indicator card. When you have packaged all of the filter paper cards, put them into the large zip-loc bag that has been labeled for the cluster in which the samples were collected. Note that the Cluster Sample (large zip-loc) Bag itself should also contain a few loose desiccant packets and a humidity indicator card.
- e. Every couple of days, before adding newly packaged DBS samples to an existing Cluster Sample Bag, check the humidity indicator cards for the individually packaged DBS samples that you have previously placed in the Cluster bag. **The buildup of humidity can damage the quality of the sample.**

A bottom circle that is pink (30% humidity) indicates a warning of increasing humidity. If the middle circle (40%) or top circle (50%) is pink, gently open the small zip-loc bag, remove the desiccant packet and replace it with a fresh desiccant packet. If any of the circles on the humidity indicator card have merged together so that they are not completely separated, remove the indicator card and replace it with a fresh indicator card. Close the zipper, gently



**Figure 5.5 Packaged DBS filter paper cards**

pushing out any excess air in the bag as you are zipping it (Figure 5.5).

Before closing the zipper on the Cluster Sample Bag, check the condition of the packets and humidity indicator card that are kept loose inside the bag and replace as needed.

If you have additional Cluster Sample Bags for completed clusters that have not yet been collected by a Field Supervisor, examine all of the samples in those bags in the same manner every couple of days, as long as they are with your team in the field.

## **Step 2: Transferring the DBS Samples**

The purpose of the Blood Sample Transmittal Form is to account for the samples at each step of the way. Fold the Blood Sample Transmittal Form along the dotted lines (so that the bar-code labels are not folded), and keep it in the Sample Cluster Bag along with the DBS samples for that cluster.

When you have completed the cluster, remove the packaged DBS samples from the Cluster Sample Bag (do not open the small zip-loc bags). One by one, check the bar codes on the labels on the filter paper cards against the bar codes affixed to the back side of the Blood Sample Transmittal Sheet. For each DBS sample, put a check mark in the column labeled TECHNICIAN for each corresponding bar code found on the transmittal sheet. Count the number of DBS samples and record in the boxes provided in Column (3) on the front side of the Transmittal Form in the row labeled TEAM LEADER. If there are any discrepancies, you must attempt to account for them. Use Column (7) to explain. Sign your name in Column (4) and the date in Column (6).

The team's field supervisor will follow behind you, verify the samples, and sign his name in the same row.

Periodically, a Field Coordinator or other staff member the central office will visit the teams to collect the completed questionnaires and the DBS samples for the completed clusters. When he/she collects the DBS samples, he/she will recount the DBS samples for each of the completed clusters and sign the Blood Sample Transmittal Form. The samples and transmittal sheet will be transported to the central survey office for logging before being transferred to the laboratory for processing.

## SECTION 6: PRECAUTIONS TO OBSERVE WHEN COLLECTING BLOOD SAMPLES

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This section of the manual reviews some of the major precautions that you must observe in collecting samples, both to protect themselves yourself and the subjects from which they you are collecting samples from injury or infection and to prevent contamination of the samples.

### 6.1 General Precautions when Collecting Blood

This section describes the universal (general) precautions to be followed during blood collection for HIV testing. The person responsible for collecting blood for HIV testing must take precautions to prevent parenteral, skin, and mucous-membrane exposures 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<sup>1</sup>.

- **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 subject is collected and all waste materials produced during the collection are disposed of. At that point, the gloves used with the subject should be treated as biohazardous waste. A new pair of gloves should be used with each subject. **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. Self-retractable lancet devices reduce the risk of penetrating injuries.

Lancets should not be used for purposes other than a single finger prick to collect blood for HIV testing. The lancets should not be broken or destroyed for curiosity or other purposes. Immediately after the testing is completed, the devices should to 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.

- **Never eat or drink during the testing.** Since eating, drinking, and applying cosmetics may distract you from performing the procedure, they are not permitted during HIV blood collection.
- **Properly dispose of all biohazardous materials.** All materials coming in contact with blood must be placed in a biohazardous waste container after use and disposed of according to the survey's organization's policy on infectious waste disposal (see Section 7). Take precaution when storing and transporting the waste container during the fieldwork.

### 6.2 Rules for Collecting Specimens for HIV Testing<sup>2</sup>

There are a number of specific rules or precautions that the you must observe when collecting the samples for HIV testing. These include:

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<sup>1</sup> For the universal precautions regarding blood-borne 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 blood-borne pathogen.

<sup>2</sup> Adapted from NCCLS 1997

- **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 may result in erroneous test results. Instead, the tester 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.** Alcohol, which is used to clean the puncture site, can mix with the blood and cause errors in the testing results. To avoid this problem, the finger must be wiped dry 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.
- **Do not layer.** Do not layer multiple drops of blood on the target spot (pre-printed circle on the filter paper card). If blood flow diminishes to incompletely fill circles, request consent from the subject (parent/guardian) to **repeat** the sample collection again using another finger.
- **Never touch circles on the filter card.** Avoid touching the area within the preprinted circles on the filter paper card before collection. Never touch the blood spots after collection.
- **Protect the filter card from contamination.** Do not allow water or other contaminants to come into contact with the specimen card before or after use.
- **Do not replace the specimens in the transport container until thoroughly dry** (chocolate brown). Insufficient drying adversely affects test results.

## **SECTION 7: BIOHAZARDOUS WASTE DISPOSAL**

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Any material coming in contact with blood or serum (lancets, alcohol swabs, gauze, and gloves) is considered to be biohazardous, i.e., 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. Biohazardous waste has to be collected in a special container during the blood collection and testing, securely stored and transported, and safely disposed at the end of each day of fieldwork.

If possible, commercially available biohazardous waste disposal containers should be used for waste disposal. These types of containers are red and have a special logo warning about biohazardous content. They can be securely closed for safe storage and transportation during the fieldwork. Whenever possible, these containers should be taken to health facilities, which employ standard procedures for biohazardous waste disposal.