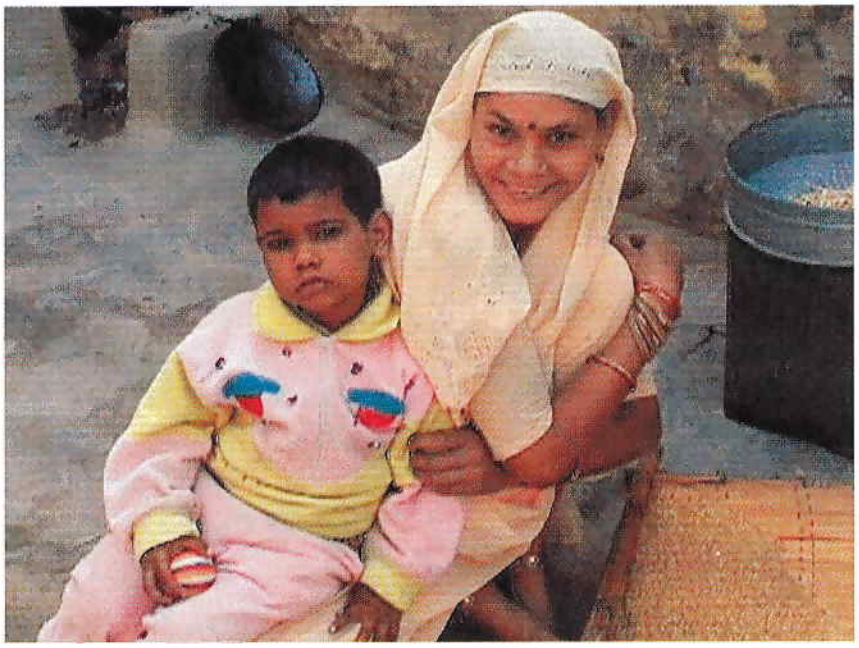
ANEMIA TESTING MANUAL
FOR POPULATION-BASED SURVEYS

Almaz Sharmanov, M.D., Ph.D.



MEASURE *DHS+*
Macro International Inc.

This manual was prepared under the auspices of the MEASURE *DHS*+ project. The MEASURE *DHS*+ project assists developing countries in the collection and use of data to monitor and evaluate trends in population, health, and nutrition. Funded by the U.S. Agency for International Development (USAID), MEASURE *DHS*+ is administered by Macro International Inc. in Calverton, Maryland.

The main objectives of the MEASURE *DHS*+ project are: 1) to provide decision-makers in survey countries with information useful for informed policy choices, 2) to expand the international population and health database, 3) to advance survey methodology, and 4) to develop in participating countries the skills and resources necessary to conduct high-quality demographic and health surveys.

Information about the MEASURE *DHS*+ project can be obtained by contacting:
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Calverton, Maryland, USA

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CONTENTS

FOREWORD

Anemia is recognized as a major public health problem throughout the world, affecting about half of the women and young children in developing countries. Early detection of anemia could help prevent some problems of child development and many severe complications of pregnancy and delivery. Population-based surveys such as those implemented through the MEASURE *DHS+* program provide an excellent opportunity to study the prevalence of anemia by including anemia testing. It is then possible to identify some of the socioeconomic, residential, and demographic differentials in the prevalence of anemia. These data provide important background information for public health policy decisions necessary for the development of national and community-based anemia-prevention programs.

This manual summarizes the experiences of the MEASURE *DHS+* program in anemia testing training. It is written primarily for health investigators who are involved in the field of anemia testing. The manual lays out a standardized approach for hemoglobin testing using the HemoCue system. Particular attention is paid to biohazardous waste disposal and safety precautions when taking blood. Descriptions of the protocols for hemoglobin testing and biohazardous waste disposal are illustrated with color photos of the procedures.

Following the standardized protocols and guidelines presented in this manual could help in collecting accurate population-based data on anemia prevalence, as well as ensuring protection of human subjects from blood-borne infections and other biohazards.

I would like to thank Dr. Ann Way, Dr. Ciro Franco, Dr. Fred Arnold, Ms. Anne Cross, and Ms. Sri Poedjastoeti of Macro International Inc. for critical reviews of the manuscript. Special thanks are also due to Dr. Sidney Moore of Macro International Inc. for editing the manuscript; and to Dr. Umesh Kapil of the All India Institute of Medical Sciences and Mr. Ibrahim Parvanta of the U.S. Centers for Disease Control and Prevention, for sharing their ideas on anemia training.

The photographs were taken by the author in New Delhi and Mumbai, India during the training of health investigators for the second National Family Health Survey in India in November 1998 and February 1999.

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CHAPTER 1

CAUSES AND CONSEQUENCES OF ANEMIA

Anemia is a condition characterized by a reduction in the volume of red blood cells and, consequently, a decrease in the concentration of hemoglobin in the blood. Human blood is composed of plasma and formed elements: red blood cells, white blood cells, and platelets. The red cells contain hemoglobin, which binds oxygen. As blood circulates throughout the body, oxygen is transported from the lungs to the tissues by the hemoglobin in the red blood cells. A reduction in the volume of red blood cells in the blood decreases the amount of oxygen reaching the tissues and organs of the body. This oxygen reduction is responsible for many of the symptoms experienced by anemic persons.

An anemic person often appears pale and weak and may feel breathless or faint. He/she may be aware of a pounding heart. An anemic person may have insomnia, decreased appetite, or a general feeling of malaise. Other symptoms are problems with movement or balance, tingling in the extremities, confusion, depression, memory loss, and an irregular heartbeat.

These symptoms may arise from a number of underlying conditions, including nutritional deficiency of iron, folate, vitamin B_{12}, or other nutrients. Although other causes of anemia, such as hemorrhage, infection, genetic disorders, and chronic disease have been identified, nutritional deficiency, due primarily to a lack of bioavailable dietary iron, accounts for the majority of anemia cases.

Anemia is known to have detrimental effects, especially on the health of women and children, and may become an underlying cause of maternal death, and infant loss. Anemia among children can be associated with impairment of cognitive performance, motor development, coordination, language development, and scholastic achievement. Anemia is also associated with increased morbidity from infectious diseases. Early detection of anemia among pregnant women could help to prevent some problems of child development and many severe complications of pregnancy and delivery. Although some forms of anemia require supervised medical care, those caused by improper nutrition can typically be treated at home once the condition has been diagnosed.

Hemoglobin testing is the primary method of anemia diagnosis. The testing can be done using various methods, including the HemoCue system. This system consists of a battery-operated photometer and a disposable microcuvette[1], coated with a dried reagent that serves as the blood-collection device. The test is performed using a drop of blood taken from a person's fingertip. Levels of anemia can be classified as severe, moderate, or mild, based on the hemoglobin concentration in the blood and according to criteria developed by the World Health Organization. Severe anemia is diagnosed when the hemoglobin concentration is less than 7.0 g/dl; moderate anemia when the hemoglobin concentration is 7.0 to 9.9 g/dl; and mild anemia when the hemoglobin concentration is 10.0 to 11.9 g/dl (10 to 10.9 for pregnant women and children; 10.0 to 12.9 for adult men).

[1] A microcuvette is a small, transparent laboratory vessel.

The procedures that should be followed in hemoglobin testing are described in detail in the following sections. Although the hemoglobin-testing technique looks straightforward, a lot of practice is required to perform it properly and in a standard fashion. The technique cannot be learned quickly. It is an art that must be developed by study, observation, and practice on adults, children, and infants, until the health investigator has the necessary skills and self-confidence.

A general suggestion is to perform as many hemoglobin tests as possible under the supervision of skilled personnel, prior to embarking on a study. Insufficient skills can give false individual results. It is the personal responsibility of the health investigator to put as much effort as possible into developing the appropriate skills for hemoglobin testing and into performing the testing accurately.

CHAPTER 2

GENERAL PRECAUTIONS WHEN COLLECTING BLOOD

This section describes the general precautions to be followed during blood collection for hemoglobin testing. 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 pathogens, excerpts from which are in Appendix A.

Personnel responsible for collecting blood for hemoglobin measurement must take precautions to prevent parenteral, skin, and mucous-membrane exposures to bloodborne infections, such as hepatitis B, or human immunodeficiency virus (HIV). Under general precautions the following rules should be followed to ensure protection from acquiring bloodborne infections.

(1) WEAR GLOVES. Gloves help to prevent skin and mucous-membrane exposure to blood. Gloves should be worn during blood collection and hemoglobin measurement until all specimens and materials are disposed of. Gloves must be disposed of as biohazardous wastes (see Chapter 7: Disposal of Biohazardous Wastes). Gloves must never be reused!

(2) AVOID PENETRATING INJURIES. Although gloves can prevent blood contamination of intact and nonintact skin surfaces, they cannot prevent penetrating injuries caused by the instruments used for finger or heel pricks. Generally, self-retractable lancet devices such as Tenderlett™ lancets, are recommended to reduce the risk of penetrating injuries. If other devices are employed for testing, care should be taken to develop procedures to prevent such injuries. Whatever the type of lancet, it should not be used for purposes other than a single finger or heel prick to collect blood for the anemia 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 container for further disposal.

(3) If an accident occurs, any skin surfaces or mucous membranes that become contaminated with blood should be immediately and thoroughly washed.

(4) Since eating, drinking, applying cosmetics, and handling contact lenses may distract from the procedure, they are not permitted during blood collection and hemoglobin measurement.

(5) PROPERLY DISPOSE OF ALL BIOHAZARDOUS MATERIALS. All materials coming in contact with blood must be placed in biohazardous waste containers after use and disposed of according to the survey organization's policy on infectious waste disposal (see Chapter 7: Disposal of Biohazardous Wastes).

(6) The biohazardous waste containers should be labeled "biohazard." Take precaution when storing and transporting the waste containers during the fieldwork, and establish procedures to ensure proper disposal of all waste products (see Chapter 7: Disposal of Biohazardous Wastes).

CHAPTER 3

PREPARATIONS FOR HEMOGLOBIN TESTING

3.1 Personnel Required for Hemoglobin Testing in the Field

Two trained people are required to collect a blood sample and measure and record hemoglobinlevel data from the HemoCue: a health investigator (measurer), and an assistant. The health investigator performs the skin puncture, collects the blood sample into the HemoCue capillary microcuvette, and takes the measurements. The assistant helps the mother to hold the child, if necessary, and records the results on the questionnaire. If the assistant is not available, then the health investigator should record the measurements on the questionnaire.

3.2 Approaching the Respondent Prior to Hemoglobin Testing

It is important for the health investigator performing the blood collection on women to show the respondent respect and kindness, since this may be the first time she has had a blood test. The respondent may be anxious and fearful of the blood-testing procedure. Before taking the blood sample, the health investigator should give a careful explanation of what will be done in a light and positive voice. Nonverbal communication is an important consideration. For example, proper eye contact, a firm handshake, and appropriate humor can reduce anxiety.

When the health investigator is dealing with a child, his or her approach is doubly important. It is necessary to gain the child's confidence before proceeding with blood collection. If the child is crying excessively, the mother should be asked to calm him or her down by giving him or her a toy or candy (when appropriate). The health investigator can compare a fingerprick to a mosquito sting or describe it as a mother pricking her finger with a needle or pin while sewing.

Never perform blood testing on a child if the mother refuses or if the child is sick or distressed.

3.3 Informed Consent

Prior to the test, the woman must be asked whether she agrees to participate in the study and, if so, to sign a form giving permission for the collection of a blood droplet from herself and her children. The form explains that the hemoglobin-testing procedure is part of a national research program to determine the rate of anemia among women and children.

In addition to information on anemia, its complications, and the threat to women's and children's health, the consent form explains the general procedures for anemia testing using the HemoCue system, including the use of sterile disposable devices. The woman is assured that the results obtained from the testing will be kept confidential (see Appendix B for the Informed Consent form used in MEASURE *DHS*+ surveys. Filling in the anemia section of the MEASURE *DHS*+ Household Questionnaire is discussed in Section 5).

If a woman or a child is diagnosed as having severe anemia – with Hb levels less than 7 g/dl (less than 9 g/dl for pregnant women) – the health investigator should ask the woman or responsible adult to sign a second consent form giving the study team permission to inform a doctor[2] about her condition (see Appendix B for an example of text for form requesting referral).

[2] In countries with low literacy levels, the health investigator signs the informed consent form.

CHAPTER 4

MATERIALS AND EQUIPMENT

Materials and equipment necessary for hemoglobin testing using the HemoCue B-Hemoglobin system include the following (see Illustration 1):

1) sterile, dry gauze pads;
2) alcohol preps (pads);
3) latex gloves;[3]
4) disposable lancets (such as Tenderlett[tm]);
5) HemoCue B-Hemoglobin photometer for detecting hemoglobin levels;
6) HemoCue B-Hemoglobin microcuvettes;
7) adhesive bandages.

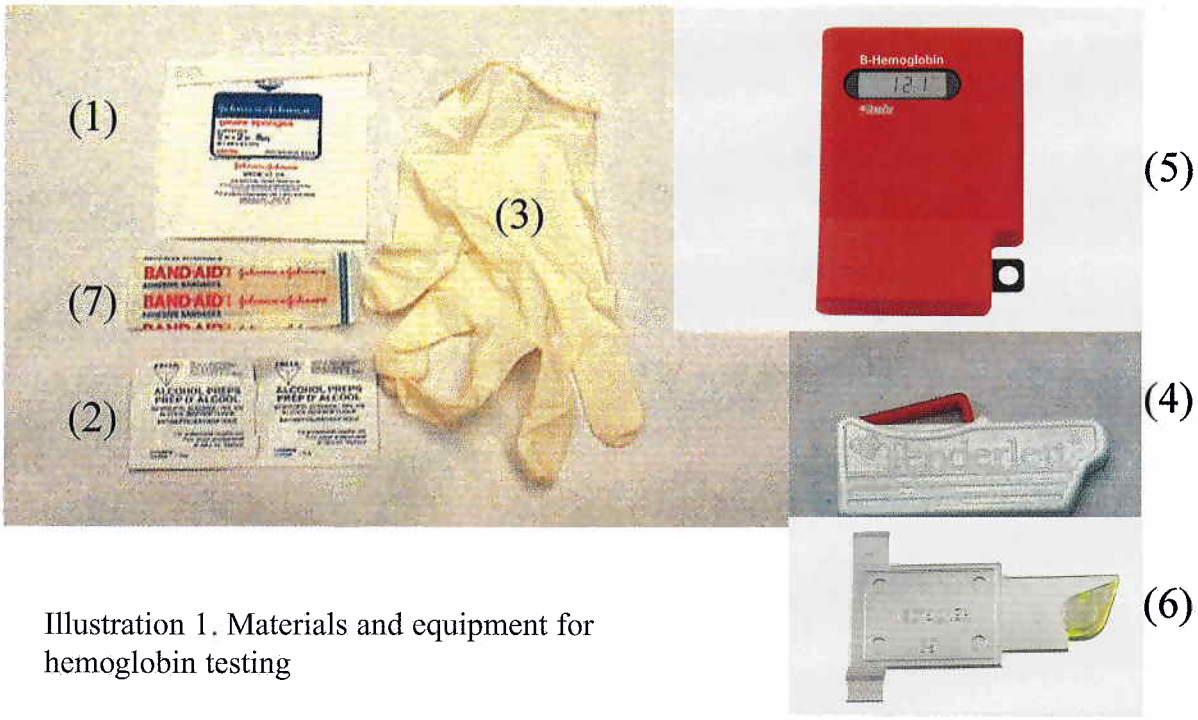

Illustration 1. Materials and equipment for hemoglobin testing

It is important that each team has ample sterile supplies such as dry gauze pads and adhesive bandages. *Sterile gauze pads* are used to wipe away the first drops of blood to stimulate a spontaneous capillary blood flow. In the absence of gauze pads, sterile cotton balls

[3] If the survey includes lead testing, use of powder-free latex gloves is required

can be used for that purpose. After the blood collection, an adhesive bandage has to be applied on the puncture site, especially if a heel prick is done.

Alcohol pads are used for cleaning the skin at the puncture site. If commercial alcohol pads are not available, cotton balls soaked in 75% aqueous solution of isopropanol may be used.

Latex gloves are used by the health technician to prevent infection.

Although the HemoCue system (photometer and microcuvettes) has proven to be durable and reliable under field conditions, there are some technical limitations. In addition to a thorough understanding of correct blood-sampling techniques, it is important to know the requirements for handling the HemoCue photometer and storing the microcuvettes. Below are the technical requirements for appropriate use of the HemoCue system.

The *HemoCue B-Hemoglobin* photometer measures light absorption and presents the results on a display. The photometer can be safely operated between 15 and 40 degrees centigrade (59 to 104 degrees Fahrenheit). Allow the instrument to come to the ambient temperature and protect it from direct sunlight.

The function of the photometer has to be checked on a daily basis by measuring the control microcuvette. Values obtained should not deviate from the assigned value on the control microcuvette card by more than ± 0.3 g/dl.

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 daily using a cotton swab soaked in soapy water or alcohol. The microcuvette holder should be completely dry prior to reinserting it in the photometer.

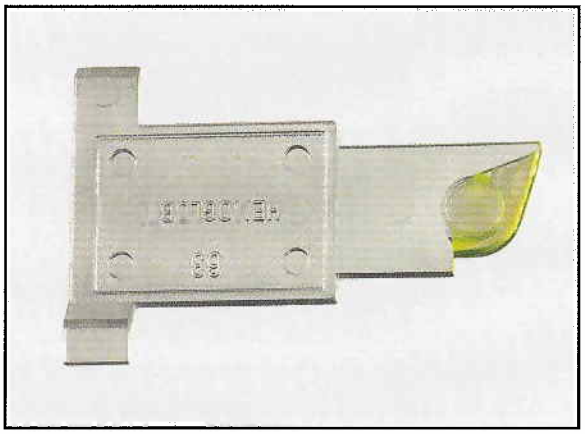

Illustration 2. HemoCue microcuvette

The *HemoCue B-Hemoglobin* microcuvette is a plastic disposable unit that serves as both a reagent vessel and a measuring device. It contains a reagent (sodium azide) in dry form. The reagent is yellow and covers the tip portion of the microcuvette (see Illustration 2). The microcuvette is designed to draw up the exact amount of blood needed for the test. It is important to ensure that the entire portion of the microcuvette covered by the reagent (including both circle and the tip), is filled with capillary blood.

Microcuvettes are sensitive to humidity. Immediately after taking out a microcuvette, reseal and close the container. Staff should observe the following requirements for the proper handling and storage of hemoglobin microcuvettes:

- Record on the container the date on which it is first opened;
- Remove from the container only those microcuvettes required for immediate testing;
- Always keep the microcuvette container lid snapped on;
- 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 (60 days) after opening. Sealed and unopened containers can be stored up to the expiration date on the container.

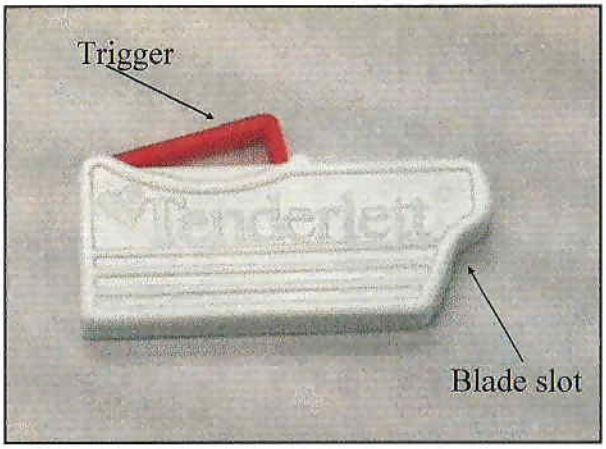

Illustration 3a. Tenderlett™ lancet

Illustration 3b. HemoCue Safety Lancet

A disposable lancet such as the Tenderlett™ (manufactured by Technidyne Corp.) is an automated, disposable incision device used to obtain blood samples from the fingertip or heel. The device is specially shaped to fit easily on the skin surface, thus minimizing skin indentation. When the trigger is pressed, a surgical blade quickly protrudes from the device and then automatically retracts. The angle of the blade is set for maximum blood flow (see Illustration 3a) and the action of the blade is so fast it cannot be seen. There are other disposable and self-retractable lancet devices, such as the HemoCue Safety Lancets (see Illustration 3b), available on the market. These are similar to the Tenderlett™ and are also recommended for capillary blood collection.

CHAPTER 5

MEASURING HEMOGLOBIN

5.1 General Preparations

It is preferable that the blood-collection procedure should be done indoors. Make sure there is adequate light. A couch, bed, or mat should be easily accessible if the respondent feels faint during the testing and needs to lie down.

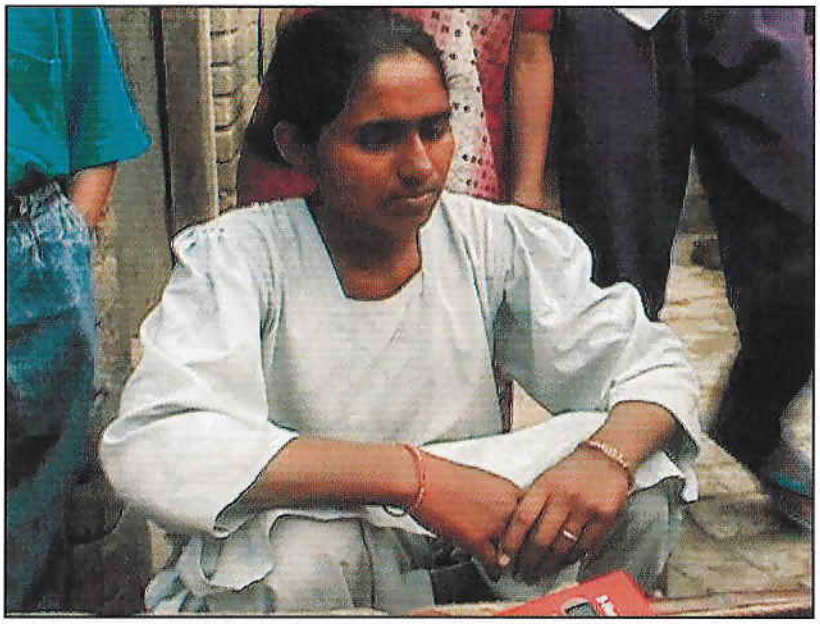

Illustration 4. Respondent sitting in relaxed position

Before blood collection, the respondent should be relaxed and sitting comfortably (see Illustration 4).

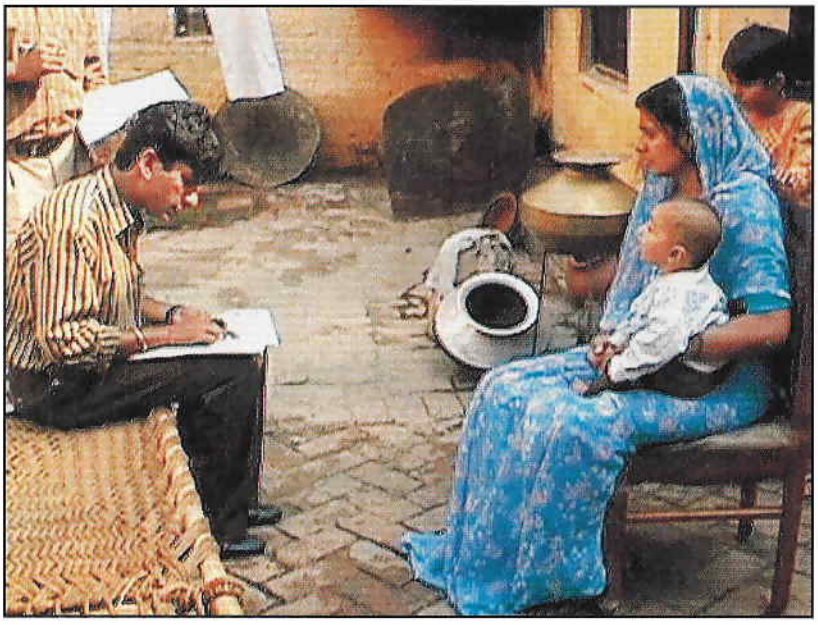

Illustration 5. Child sitting on mother's lap

The testing of infants and children may be done with the child sitting on the mother's lap (see Illustration 5).

All supplies required for hemoglobin testing, including gloves, alcohol preps, lancet (e.g., Tenderlett™ device), sterile gauzes, and a HemoCue microcuvette, need to be removed from their wrappers or containers and placed in front of the health investigator prior to the skin puncture and blood collection. Be careful when removing the lancet not to touch the blade-slot end or to allow it to come in contact with any non-sterile surface.

5.2 Selecting a Skin-Puncture Site

Skin-puncture blood can be obtained from the palm side of the end of a finger or from a heel. For adults and children six months of age and older, a finger should be used. For children under six months of age, use the heel. However, if a child is undernourished and skinny, the underlying tissue can be very thin and a lancet is likely to pierce the bone. For such children, heel puncture is recommended until they are one year old. The selected puncture site must be warm and free of infection, swelling, or damage to the integrity of the skin.

5.3 Collecting Blood from Adults

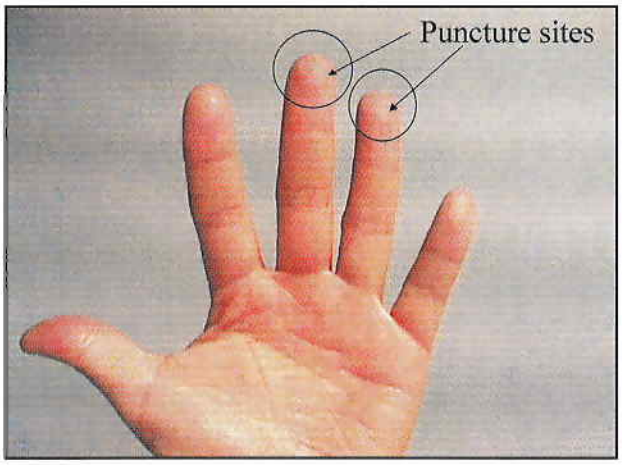

Illustration 6. Fingers used for blood collection

The *finger* is the puncture site for most adults and children. Use the third or fourth finger for collecting blood (see Illustration 6). Do not use a finger on which the subject is wearing a ring.

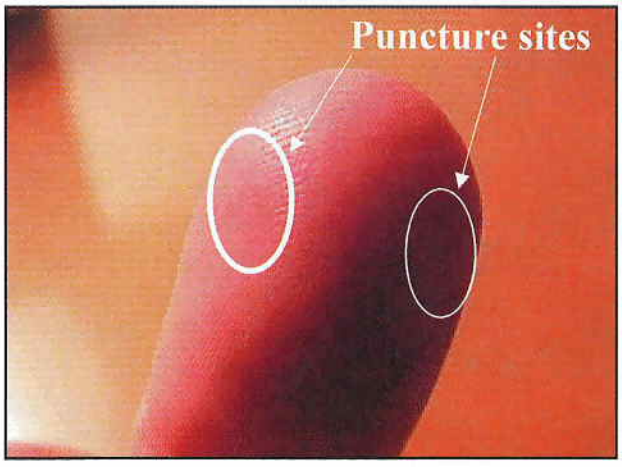

Illustration 7. Puncture sites on the finger

The puncture should be made perpendicular to the fingerprint, on the palmar surface of the end portion of the finger, slightly off-center (see Illustration 7). Care should be taken to avoid puncturing the tip or sides of the finger because of the danger of piercing the underlying bone.

The finger should be straight but relaxed to avoid the stasis effect[4], which occurs when the fingers are bent. If the extremity is cold, warm the skin over the puncture site by rubbing it. This will increase blood flow by reducing the proportion of tissue fluid, and will improve the ease with which a sample can be obtained.

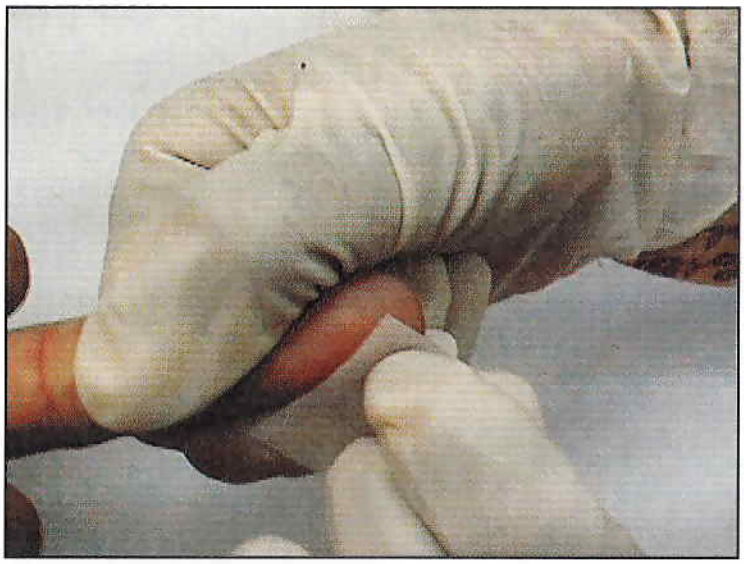

Illustration 8. Preparation of skin for finger prick

Step 1: Clean the skin thoroughly with the alcohol prep or isopropanol solution (see Illustration 8). The skin must be completely dry before being punctured, since any residual alcohol will cause hemolysis[5] in the specimen. Wipe away any excess alcohol with a sterile gauze pad. Do not blow on the puncture site to dry the alcohol. Blowing may cause bacteria to invade the puncture site.

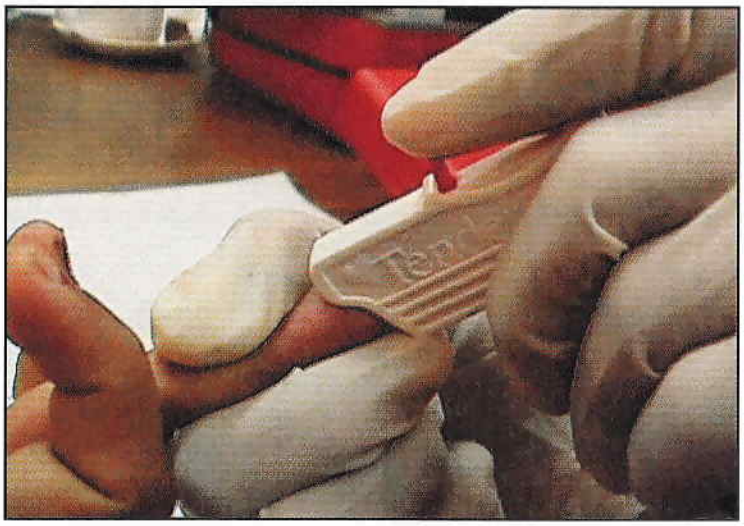

Illustration 9. Finger prick using a lancet (adult)

Step 2: Using a rolling movement of your thumb, lightly press the finger from the top knuckle towards the tip. This stimulates the flow of blood toward the sampling area. When your thumb reaches the fingertip, maintain a gentle pressure and use the lancet to puncture the skin by placing the blade-slot surface against the area and pressing the trigger. (see Illustration 9). The tip of the blade ejects through the blade slot, producing a microincision in the skin, and immediately retracts into the device. When puncturing the skin, cut across the site to allow the blood to bubble and prevent it from running into the grooves of the fingerprints.

[4] Slowing of the current of circulating blood.

[5] Hemolysis is lysis (dissolution) of red blood cells with liberation of hemoglobin.

The lancet should be put aside while blood collection and hemoglobin measurement proceed. After the testing is completed, the lancet should be placed in the biohazardous waste container, along with the other materials used for the blood collection.

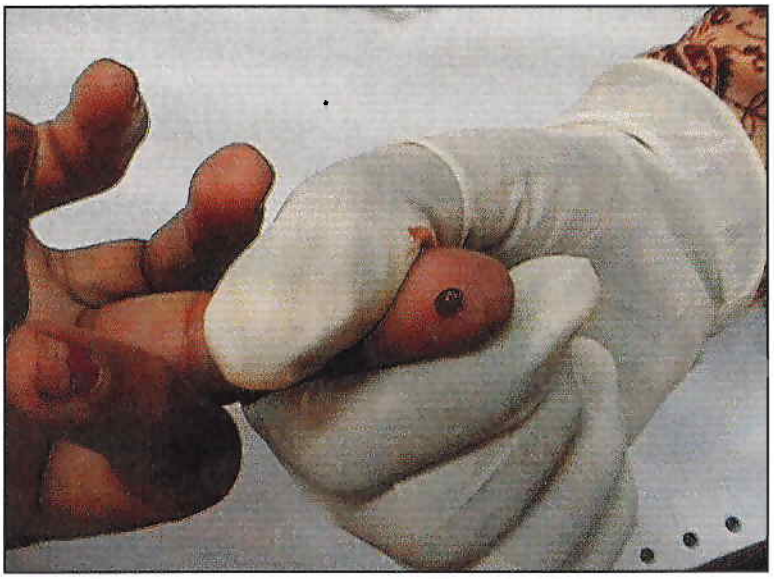

Illustration 10. First drop of blood

Step 3: When the blood appears, use a sterile gauze to wipe away the first two drops of blood to stimulate a spontaneous blood flow. If necessary, press gently again until another drop of blood appears. Avoid "milking" the site. Ensure that the drop of blood collected is big enough to fill the HemoCue microcuvette completely (see Illustration 10).

If the blood stops flowing before a sufficient amount has been collected, the skin puncture procedure may be repeated. In such cases, the sample should be taken from a *different* finger, using the procedures described in steps 1 to 3. **Do not reuse any of the supplies used in the first test.**

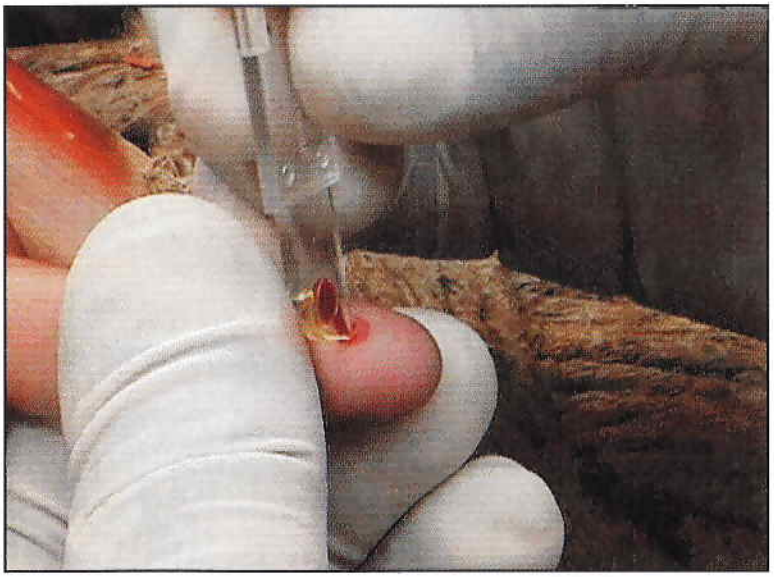

Illustration 11. Collecting blood using a microcuvette

Step 4: Apply the HemoCue microcuvette to the middle of the blood drop. The microcuvette will fill itself automatically by capillary action. **The microcuvette needs to be filled completely** (see Illustration 11). Never top off the microcuvette after the first filling. Wipe any surplus blood off both sides of the microcuvette "like butter from a knife," using the clean end of a sterile gauze. Ensure that no blood is sucked out of the microcuvette.

After filling, the microcuvette needs to be visually inspected for air bubbles. Since air bubbles may influence the results of hemoglobin testing, microcuvettes containing them should be discarded. In such cases, the testing should be repeated using a different finger. Again, you must use new supplies and follow all of the steps described above in obtaining the new sample.

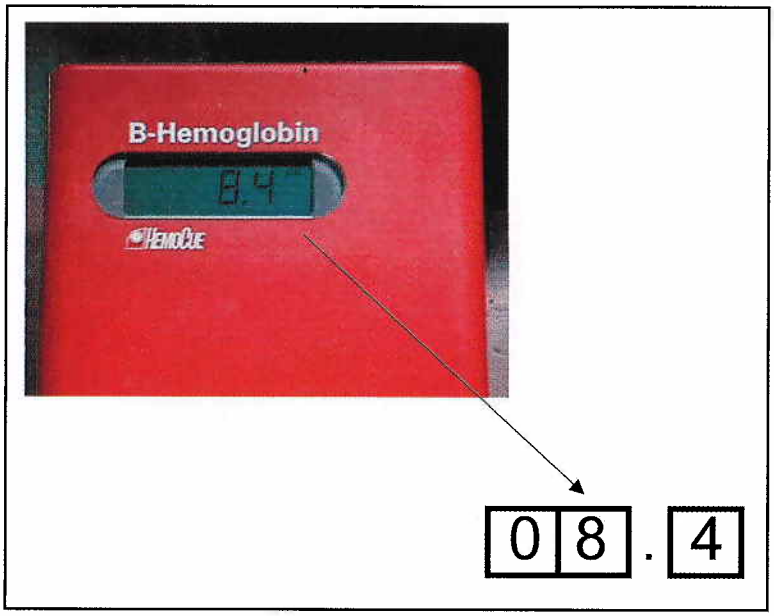

Illustration 12. Format for recording test results

Step 5: Place the microcuvette in its holder and gently push the holder into the photometer. The microcuvette should be analyzed immediately, no later than ten minutes after being filled. Blood hemoglobin results are displayed after 15 to 45 seconds. Record the hemoglobin level shown on the photometer in the appropriate boxes in the questionnaire (see Illustration 12). (See Section 5.6: Recording hemoglobin test results.)

Step 6: Remove another sterile gauze from the package and wipe the blood from the puncture site. While keeping the gauze on the puncture site, ask the respondent to put pressure on it with her thumb for 3 to 5 minutes. Remove the gauze and make sure that the blood flow has completely stopped. Then take the adhesive bandage from its wrapper and apply it to the puncture site.

After completing the testing, carefully follow the procedures for disposal of all materials used for the sampling (see Chapter 7, Steps 1 to 12 of the Procedure for Field Biohazardous Wastes).

5.4 Collecting Blood from Children

For children 6 months of age and older, the finger prick should be performed in the same way as for adults (see Section 5.3, Steps 1 to 6). It may be helpful if the mother assists the health investigator by holding the child's hand.

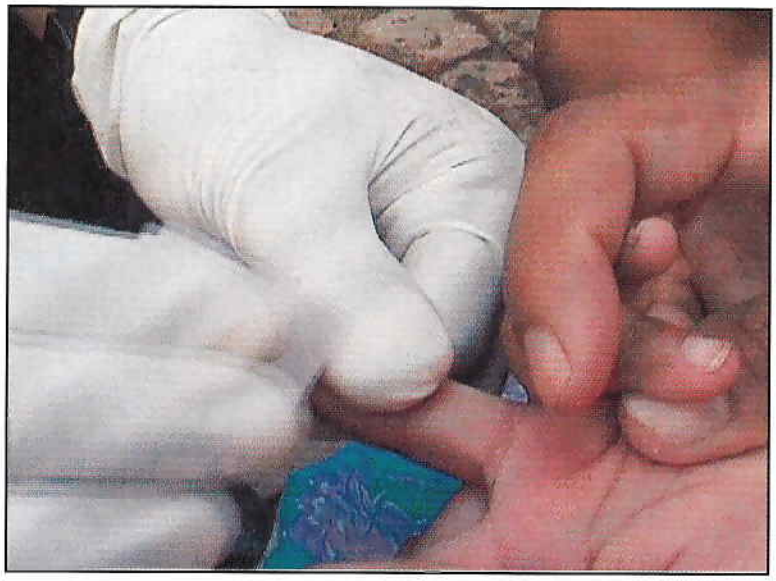

Illustration 13. Cleaning the puncture site

Step 1: Clean the site with an alcohol prep (see Illustration 13). Make sure the site is dry before puncturing the skin with the lancet (see Illustration 14).

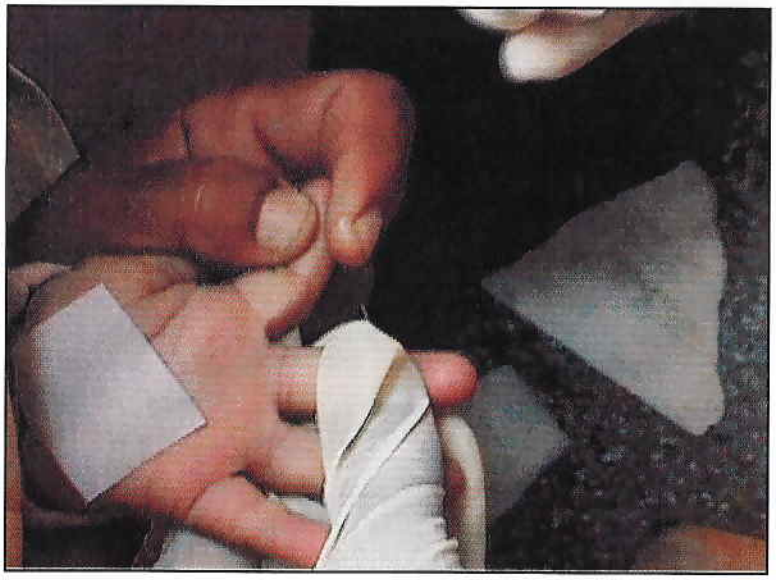

Illustration 14. Making sure the puncture site is dry

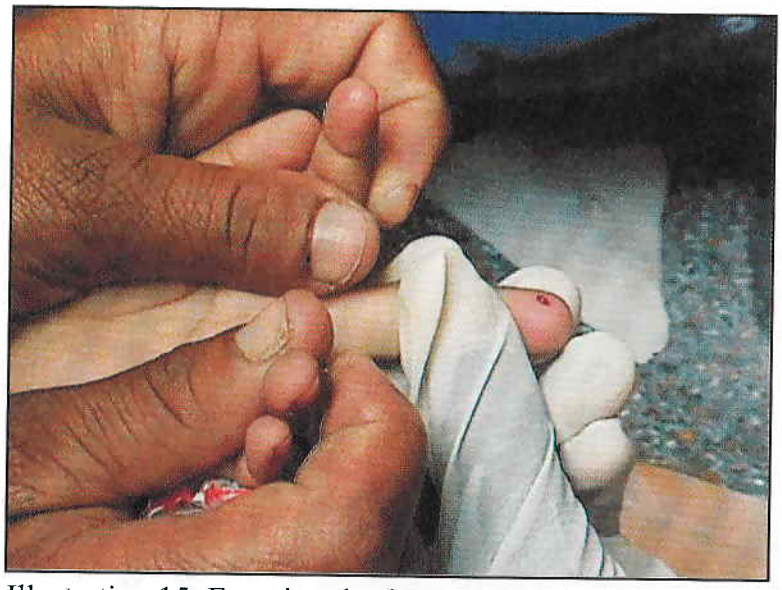

Illustration 15. Ensuring the free flow of blood

Step 2: 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 (see Illustration 15) but do not "milk" the site.

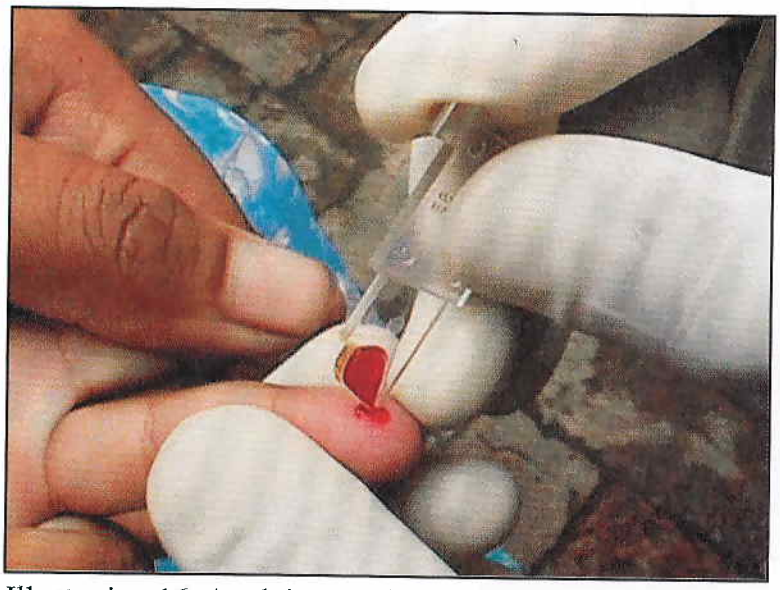

Illustration 16. Applying a microcuvette to collect blood

Step 3: Wipe the first two drops of blood away, using a sterile gauze. Make sure that the blood drop is large enough to completely fill the HemoCue microcuvette (see Illustration 16). Collect the next drop in the microcuvette, wipe off the excess blood from the microcuvette, and check for air bubbles.

Step 4: Place the microcuvette in its holder, and insert into the photometer. Measure the hemoglobin level and take care to record the level accurately.

Step 5: After the blood collection, remove another sterile gauze from the package and wipe the blood from the puncture site. Keep the gauze on the puncture site until the blood flow is completely stopped. Remove the gauze, take an adhesive bandage from its wrapper, and apply it on the puncture site.

5.5 Collecting Blood from Infants

The *heel* is the puncture site for children less than 6 months of age.

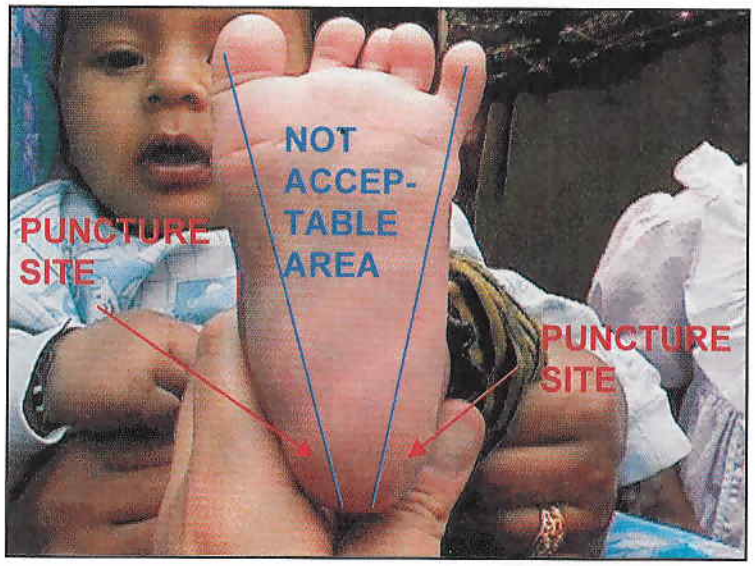

Illustration 17. Puncture sites on the heel

The puncture 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 (see Illustration 17). 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).

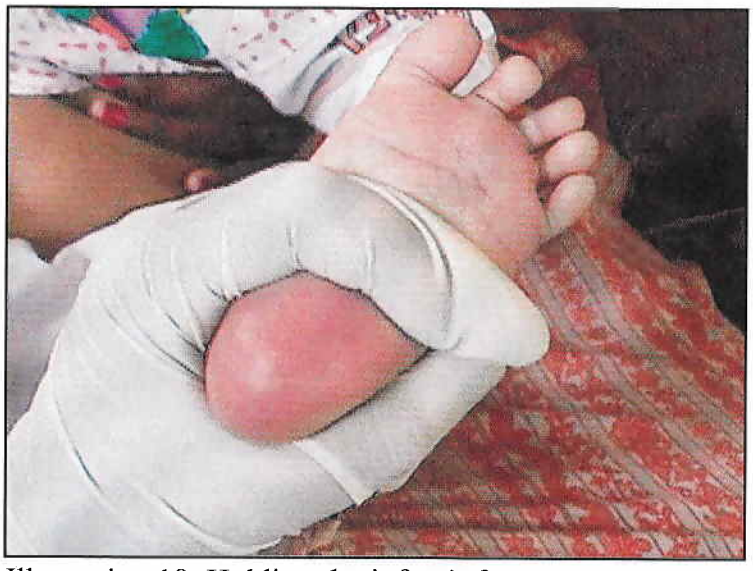

Illustration 18. Holding the infant's foot

Hold the heel firmly. Apply moderate pressure near the puncture site. This could be done by wrapping the heel using your thumb and second finger (see Illustration 18).

Step 1: Clean the site with an alcohol prep. 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.

Step 2: 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.

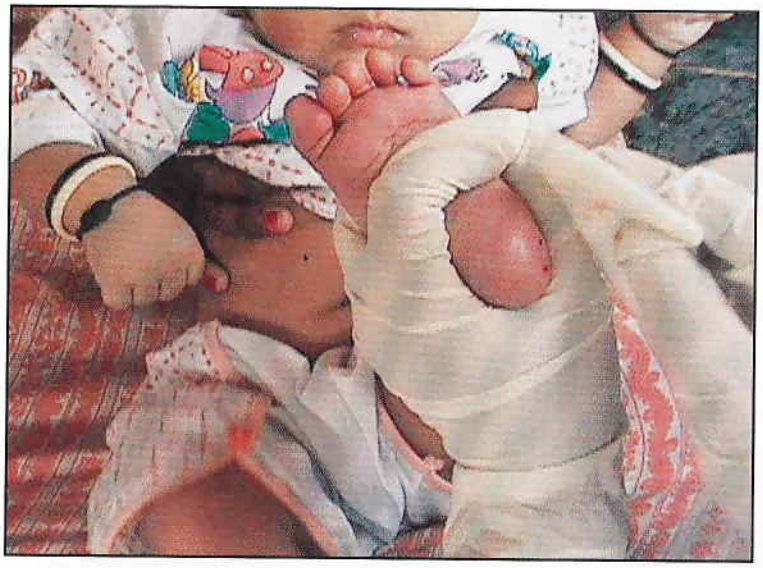

Illustration 19. Wiping away the drops of blood

Step 3: Wipe away the first two drops of blood by using a sterile gauze (see Illustration 19).

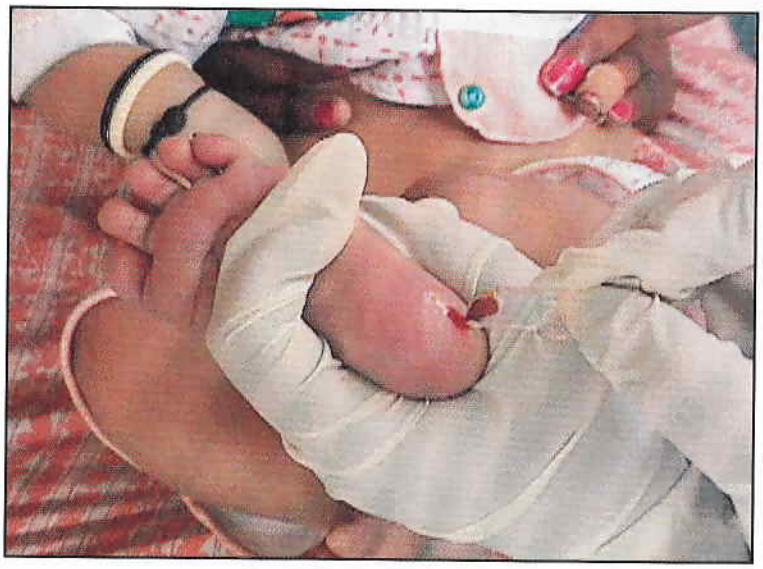

Illustration 20. Collecting infant blood with a microcuvette

Collect the next drop in the microcuvette (see Illustration 20). Make sure that the microcuvette is completely filled with the blood.

Step 4: Wipe off any excess blood from the microcuvette and check it for the presence of air bubbles. Place the microcuvette in its holder, insert it into the photometer, measuring the hemoglobin level. Record the level in the appropriate boxes in the questionnaire.

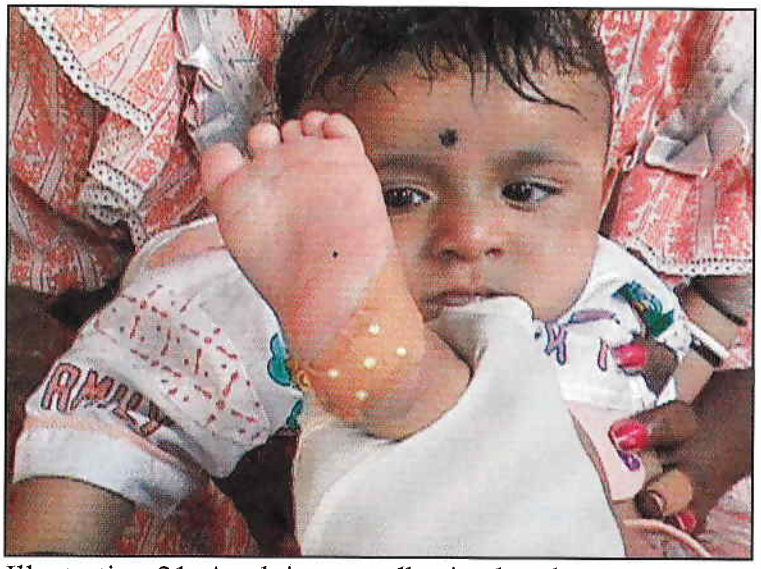

Illustration 21. Applying an adhesive bandage

Step 5: After the blood collection, take another sterile gauze from the package and wipe the blood from the puncture site. After making sure that the blood flow has completely stopped, take an adhesive bandage from its wrapper and apply it to the puncture site (see Illustration 21).

5.6 Recording Hemoglobin Test Results

In MEASURE *DHS+* surveys, all women between the ages of 15 and 49, as well as children under age 6, are tested for hemoglobin level in the blood. The data are recorded in the anemia section of the Household Questionnaire, which has two parts (see Appendix B for the anemia section of the MEASURE *DHS+* Household Questionnaire). The top part is for recording the measurements of women 15 to 49, and the bottom part is for children less than 6 years old. Below are instructions on how to properly fill in the anemia section of the MEASURE *DHS+* Household Questionnaire.

Check the age of the woman in column 38. If she is less than 18, circle "1" in column 44, and if 18 or older, circle "2." For women between the ages of 15 to 17, the name of the parent or responsible adult needs to be identified, and his or her line number from the Household Schedule needs to be recorded in column 45. For women 18 years or older, proceed by reading the consent statement at the bottom of the page.

In column 47 record the hemoglobin level. If the level is less than 10, be sure that a zero ("0") is recorded in the first box (see Illustration 12). In column 48, record the result of the measurement.

CHAPTER 6

PROBLEMS OF HEMOGLOBIN TESTING IN THE FIELD

"Milking" 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 in yielding low levels of hemoglobin concentration in the blood. 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.

Mixing alcohol with the blood. Alcohol, which is used to clean the puncture site, can mix with the blood and cause errors in the hemoglobin reading. Any residual alcohol will cause hemolysis and specimen dilution, as well as excessive platelet clumping, red blood cell aggregation, and sedimentation at the skin-puncture site. To avoid this problem, the finger or heel must be wiped dry completely before being punctured.

Removing 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.

Using the first or second drop of blood. Only the third or fourth drop of blood should be used for hemoglobin testing. This ensures the free flow of blood and allows for the collection of blood with a representative concentration of red blood cells.

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

Inadequate filling of the microcuvette. The compartment of the HemoCue 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. An inadequately filled microcuvette that contains air bubbles should be discarded.

Inadequate placement of the microcuvette. The microcuvette has to be carefully placed on HemoCue's microcuvette holder and pushed slowly inside the photometer into position for reading. Avoid "slamming" the microcuvette holder and spraying the blood into the HemoCue's optic system. This action can damage the photometer.

Improperly stored microcuvettes should not be used for testing. Microcuvettes should not be kept in unsealed containers for longer than 3 months. The containers must be kept closed when not in use to avoid exposure to moisture, which can destroy the reagents.

CHAPTER 7

DISPOSAL OF BIOHAZARDOUS WASTES

Any material coming in contact with blood or serum (lancets, HemoCue microcuvettes, alcohol swabs, gauzes, and gloves) is considered to be biohazardous, i.e., hazardous to other human subjects. Safe disposal of such materials is very important to prevent the transmission and spread of various bloodborne diseases, such as Hepatitis B and HIV, among survey personnel and within the study community. Biohazardous wastes have to be collected in a special container during the anemia 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 during the hemoglobin testing. 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. The containers are made from flammable materials, so they can easily be burnt in the field. Whenever possible, these containers can also be taken to health facilities, which employ standard procedures for biohazardous waste disposal.

Unfortunately, in most areas where the population-based surveys are implemented, it will not be possible to arrange to use health facilities for proper biohazardous waste disposal. In addition, commercial biohazardous waste containers may not be available. In these situations, the following procedures should be observed to ensure proper disposal of biohazardous materials in the field.

7.1 Materials and Supplies

The following items are required in the field for disposal of biohazardous materials after hemoglobin testing:

Kerosene
Four percent sodium hypochlorite solution[6]
Matches
Spade or other tool for digging a small pit
Ziplock-type polyethylene bags
Forceps
Sharps container labeled "Biohazard"[7]
Scissors

[6] Four percent hypochlorite solution could be purchased as a commercially available product. It could also be prepared in the field by substituting a hypochlorite powder using water. The liquid solutions (sodium hypochlorite solution and kerosene) should be stored in leakproof and airtight containers.

[7]

7.2 Procedures for Field Disposal of Biohazardous Wastes

At the end of each blood collection and hemoglobin measurement, all materials used during the testing (gloves, HemoCue microcuvettes, lancets, alcohol swabs, and gauze pads have to be placed in sharps container (a wide-mouth plastic jar) and kept there until the end of the working day. The following are the steps that should be followed in disposing of biohazardous materials.

First, a health investigator needs to determine a place where the waste disposal will be destroyed. Open field areas with loose soil is preferable, since the materials need to be burnt and buried. Because of risk of fire, drought areas, as well as proximity to flammable materials, should be avoided.

Illustration 22. Adding sodium hypochlorite

Step 1: At the end of each working day, bring the sharps container (plastic jar) with biohazardous materials to the area selected for the waste disposal. Add a half liter of 4 percent sodium hypochlorite solution into the sharps container (plastic jar) with the biohazardous materials (see Illustration 22). After adding, close the container (jar) so it is airtight. Keep the jar in an upright position for five minutes. After that, invert the plastic jar and keep in that position for an additional five minutes.

This step is necessary to ensure that all of the materials in the sharps container (plastic jar) are disinfected by complete immersion in the 4 percent sodium hypochlorite solution.

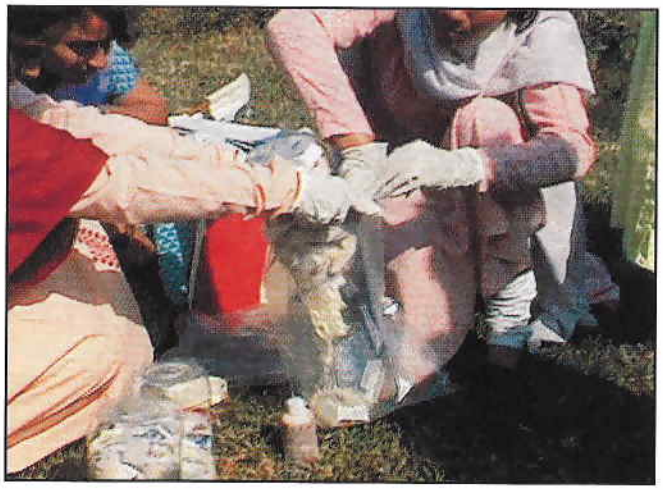

Illustration 23. Transferring contaminated materials

Step 2: Transfer the contents of the plastic jar, including the sodium hypochlorite solution to a thick polyethylene bag (see Illustration 23).

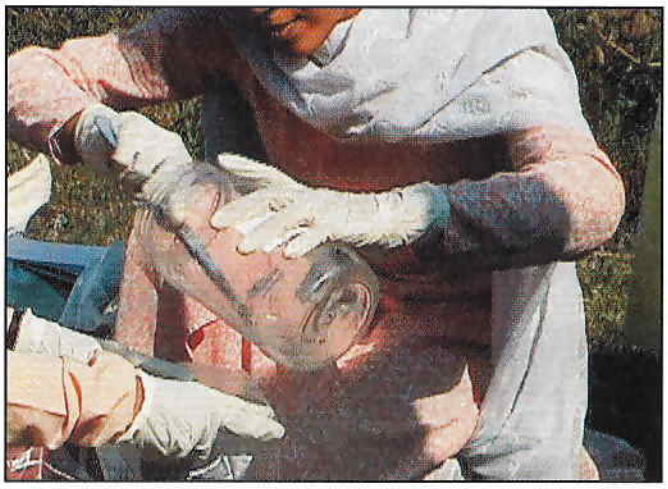

Illustration 24. Removing remaining materials

Step 3: A forceps can be used if any material adheres or sticks to the walls of the plastic jar to transfer it to the polyethylene bag (see Illustration 24).

Illustration 25. Making a hole in the bag

Step 4: Use a scissors to make a hole at the bottom of the polyethylene bag (see Illustration 25).

Illustration 26. Draining off hypochlorite solution

Step 5: Drain off the hypochlorite solution from the polyethylene bag (see Illustration 26).

Illustration 27. Digging a pit for bag

Step 6: Dig a small hole with a spade, and put the polyethylene bag containing the biohazardous materials in the pit (see Illustration 27).

Illustration 28. Putting waste paper on top of bag

Step 7: Put waste paper on the polyethylene bag containing biohazardous materials (Illustration 28).

Illustration 29. Pouring kerosene on bag

Step 8: Pour some kerosene on the bag (see Illustration 29).

Illustration 30. Burning Contaminated Materials

Step 9: Burn the polyethylene bag containing the biohazardous materials in the pit (see Illustration 30).

Illustration 31. Ascertaining that contaminated materials are completely burned

Step 10: Wait until all of the contents are burned (Illustration 31).

Illustration 32. Covering the pit with soil

Step 11: Cover the pit with soil (see Illustration 32).

It is the health investigator's responsibility to ensure proper disposal of biohazardous waste. It is unacceptable that the materials used during the testing in one fieldwork cluster are carried by the team to the next cluster. Biohazardous materials must be destroyed at the end of each working day and should not be kept until the next day.

APPENDIX A

UNIVERSAL PRECAUTIONS IN COLLECTION OF BLOOD

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UNIVERSAL PRECAUTIONS IN COLLECTION OF BLOOD

Universal precautions, as defined by the U.S. Centers for Disease Control and Prevention (CDC), are a set of precautions designed to prevent transmission of the human immuno-deficiency virus (HIV), the hepatitis B virus (HBV), and other bloodborne pathogens when providing first aid or health care.

Below are excerpts from two documents on universal precautions: 1) CDC's "Perspectives in Disease Prevention and Health Promotion Update: Universal Precautions for Prevention of Transmission of Human Immunodeficiency Virus, Hepatitis B Virus, and Other Bloodborne Pathogens in Health-Care Settings,"[8] and 2) OSHA's "Occupational Exposure to Bloodborne PathogensóPrecautions for Emergency Responders."[9]

Universal Blood Precautions (U. S. Centers for Disease Control and Prevention (CDC)

In 1983, CDC published a document entitled Guideline for Isolation Precautions in Hospitals (1) that contained a section entitled Blood and Body Fluid Precautions. The recommendations in this section called for blood and body fluid precautions when a patient was known or suspected to be infected with bloodborne pathogens. In August 1987, CDC published a document entitled Recommendations for Prevention of HIV Transmission in Health-Care Settings (2). In contrast to the 1983 document, the 1987 document recommended that blood and body fluid precautions be consistently used for all patients, regardless of their bloodborne-infection status. This extension of blood and body fluid precautions to all patients is referred to as Universal Blood and Body Fluid Precautions or Universal Precautions. Under universal precautions, blood and certain body fluids of all patients are considered potentially infectious for human immunodeficiency virus (HIV), hepatitis B virus (HBV), and other bloodborne pathogens.

Universal precautions are intended to prevent parenteral, mucous membrane, and nonintact-skin exposures of healthcare workers to bloodborne pathogens. In addition, immuni-zation with the HBV vaccine is recommended as an important adjunct to universal precautions for health-care workers who have exposure to blood (3,4).

Since the recommendations for universal precautions were published in August 1987, CDC and the Food and Drug Administration (FDA) have received requests for clarification of the following issues: 1) body fluids to which universal precautions apply, 2) use of protective barriers, 3) use of gloves for phlebotomy, 4) selection of gloves for use while observing

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[8] Morbidity and Mortality Weekly Report 1988; 37(24):377-388.

[9] U.S. Department of Labor, Occupational Safety and Health Administration. 1998 (Revised) OSHA 3106.

universal precautions, and 5) need for making changes in waste management programs as a result of adopting universal precautions.

Universal precautions apply to blood and to other body fluids containing visible blood. Occupational transmission of HIV and HBV to health-care workers by blood is documented (4,5). Blood is the single most abundant source of HIV, HBV, and other bloodborne pathogens in the occupational setting. Infection-control efforts for HIV, HBV, and other bloodborne pathogens must focus on preventing exposure to blood and delivering HBV immunization.

Protective barriers reduce the risk of exposure of the health-care worker's skin or mucous membranes to potentially infective materials. For universal precautions, protective barriers reduce the risk of exposure to blood, body fluids containing visible blood, and other fluids to which universal precautions apply. Examples of protective barriers include gloves, gowns, masks, and protective eyewear. Gloves should reduce the incidence of contamination of hands, but they cannot prevent penetrating injuries due to needles or other sharp instruments. Masks and protective eyewear or face shields should reduce the incidence of contamination of mucous membranes of the mouth, nose, and eyes.

Universal precautions are intended to supplement, rather than replace, recommendations for routine infection control, such as handwashing and using gloves to prevent gross microbial contamination of hands (6). Because specifying the types of barriers needed for every possible clinical situation is impractical, some judgment must be exercised.

The risk of nosocomial transmission of HIV, HBV, and other bloodborne pathogens can be minimized if health-care workers use the following general guidelines:

1. Take care to prevent injuries when using needles, scalpels, and other sharp instruments or devices; when handling sharp instruments after procedures; when cleaning used instruments; and when disposing of used needles. Do not recap used needles by hand; do not remove used needles from disposable syringes by hand; and do not bend, break, or otherwise manipulate used needles by hand. Place used disposable syringes and needles, scalpel blades, and other sharp items in puncture-resistant containers for disposal. Place puncture-resistant containers as close to the use area as is practical.

1. Use protective barriers to prevent exposure to blood, body fluids containing visible blood, and other fluids to which universal precautions apply. The type of protective barrier(s) should be appropriate for the procedure being performed and the type of exposure anticipated.

3. Immediately and thoroughly wash hands and other skin surfaces that are contaminated with blood, body fluids containing visible blood, or other body fluids to which universal precautions apply.

Gloves should reduce the incidence of blood contamination of hands during phlebotomy (drawing blood samples), but they cannot prevent penetrating injuries caused by needles or other sharp instruments. The likelihood of hand contamination with blood containing HIV, HBV, or other bloodborne pathogens during phlebotomy depends on several factors: 1) the skill and

technique of the health-care worker, 2) the frequency with which the health-care worker performs the procedure (other factors being equal, the cumulative risk of blood exposure is higher for a health-care worker who performs more procedures), 3) whether the procedure occurs in a routine or emergency situation (where blood contact may be more likely), and 4) the prevalence of infection with bloodborne pathogens in the patient population. The likelihood of infection after skin exposure to blood containing HIV or HBV will depend on the concentration of virus (viral concentration is much higher for hepatitis B than for HIV), the duration of contact, the presence of skin lesions on the hands of the health-care worker and, for HBV, the immune status of the health-care worker. Although not accurately quantified, the risk of HIV infection following intact-skin contact with infective blood is certainly much less than the 0.5% risk following percutaneous needlestick exposures (5). In universal precautions, all blood is assumed to be potentially infective for bloodborne pathogens, but in certain settings (e.g., volunteer blood-donation centers) the prevalence of infection with some bloodborne pathogens (e.g., HIV, HBV) is known to be very low. Some institutions have relaxed recommendations for using gloves for phlebotomy procedures by skilled phlebotomists in settings where the prevalence of bloodborne pathogens is known to be very low.

Institutions that judge that routine gloving for all phlebotomies is not necessary should periodically reevaluate their policy. Gloves should always be available to health-care workers who wish to use them for phlebotomy. In addition, the following general guidelines apply:

1. Use gloves for performing phlebotomy when the health-care worker has cuts, scratches, or other breaks in his or her skin.

2. Use gloves in situations where the health-care worker judges that hand contamination with blood may occur; for example, when performing phlebotomy on an uncooperative patient.

3. Use gloves for performing finger or heel sticks on infants and children.

4. Use gloves when persons are receiving training in phlebotomy.

Selection of Gloves. The Center for Devices and Radiological Health, FDA, has the responsibility of regulating the medical-glove industry. Medical gloves include those marketed as sterile surgical gloves or nonsterile examination gloves made of vinyl or latex. General-purpose utility ("rubber") gloves are also used in the health-care setting, but they are not regulated by the FDA since they are not promoted for medical use. There are no reported differences in barrier effectiveness between intact latex and intact vinyl, which are used to manufacture gloves. Thus, the type of gloves selected should be appropriate for the task being performed. The following general guidelines are recommended:

1. Use sterile gloves for procedures involving contact with normally sterile areas of the body.

2. Use examination gloves for procedures involving contact with mucous membranes, unless otherwise indicated, and for other patient care or diagnostic procedures that do not require the use of sterile gloves.

3. Change gloves between patient contacts.

4. Do not wash or disinfect surgical or examination gloves for reuse. Washing with surfactants may cause wicking, i.e., the enhanced penetration of liquids through undetected holes in the glove. Disinfecting agents may cause deterioration.

5. Use general-purpose utility gloves (e.g., rubber household gloves) for house-keeping chores involving potential blood contact and for instrument cleaning and decontamination procedures. Utility gloves may be decontaminated and reused but should be discarded if they are peeling, cracked, or discolored, or if they have punctures, tears, or other evidence of deterioration.

References

1. Garner, J.S. and B.P. Simmons. Guideline for isolation precautions in hospitals. Infect Control 1983; 4:245-325.
2. Centers for Disease Control. Recommendations for prevention of HIV transmission in health-care settings. MMWR 1987;36 (suppl no. 2S).
3. Immunization Practices Advisory Committee. Recommendations for protection against viral hepatitis. MMWR 1985;34:313-24,329-35.
4. Department of Labor, Department of Health and Human Services. Joint advisory notice: Protection against occupational exposure to hepatitis B virus (HBV) and human immunodeficiency virus (HIV). Washington, D.C: U.S. Department of Labor, U.S. Department of Health and Human Services, 1987.
5. Centers for Disease Control. Update: Acquired immunodeficiency syndrome and human immunodeficiency virus infection among health-care workers. MMWR 1988;37:229-34,239.
6. Garner, J.S. and M.S. Favero. Guideline for handwashing and hospital environmental control, 1985. Atlanta: U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control, 1985; HHS publication no. 99-1117.

STANDARD PRECAUTIONS AGAINST BLOODBORNE PATHOGENS (U. S. OCCUPATIONAL SAFETY AND HEALTH ADMINISTRATION (OSHA))

Universal precautions are designed to protect health-care workers and patients from exposure to bloodborne pathogens and other potentially infectious body substances. They are mandated by the United States Occupational Safety and Health Administration (OSHA) and include the following.

(1) Wear personal protective equipment (aprons, gowns, gloves, goggles, face shields, masks, and CPR devices) when exposure to blood, blood droplets, and other body fluids is anticipated; these precautions are always mandated during invasive procedures.

(2) Wear gloves when doing patient care if skin is cut, abraded, or chapped; when collecting or handling specimens or body fluids; cleaning specimen containers; or decontaminating. If you anticipate contact with mucous membranes, nonintact skin, GI or GU tract, or active bleeding wounds; or anticipate invasive procedures such as venipuncture or vascular access procedures, then universal precautions must be observed.

(3) Gowns, aprons, scrubs, or lab coats must cover exposed skin areas when there is a potential for splashing blood or body fluids on clothing; however, this protection is not required for routine-care situations in which blood or body substances are not likely to be present. Perform all procedures in such a way such that splashing, spattering, or droplet formation is minimized.

(4) Keep mouth-to-mouth emergency resuscitation equipment in strategic locations; make personal mouthpieces available for healthcare workers, since saliva is considered to be potentially infectious (even though it has not been implicated in HIV transmission).

(5) Prevent injuries that can be caused by needles, scalpels, and other "sharps." Dispose of all these in puncture-resistant containers. Do not recap, bend, break by hand, or remove needles from disposable syringes. Tape "piggyback" needle devices in place to prevent accidental dislodging.

(6) Remove torn or punctured gloves promptly. Thoroughly wash hands and other skin surfaces immediately if contaminated with blood or other body fluids.

(7) Place and transport specimens in leak-proof receptacles properly sealed. Decontaminate or label as "biohazard" those fluids and tissues that present a potential problem. Warning labels and tags should contain a "signal word" or symbol (such as "biohazard," "biochemical material") and should be identified as such.

(8) Eating, drinking, applying cosmetics or lip balm, and handling contact lenses are not permitted in work areas where there is a reasonable likelihood of exposure to blood or other body substances.

(9) Health-care workers should protect and always take care of themselves first. Presume that all patients have hepatitis B or HIV. In cases of suspected HIV or hepatitis B (HBV) exposure, identify, obtain consent, and test for exposure if the patient consents to testing. If the patient refuses consent or outcome is positive, the health-care worker must receive HIV-antibody testing immediately. Advise HIV-negative person who has been exposed to seek medical evaluation of any acute febrile illness within 12 weeks of exposure to HIV and to retest in 6 to 12 weeks and 6 months after exposure. Some institutions offer prophylactic drug therapy or hepatitis B vaccinations to their employees. If exposed or injured, the healthcare worker must make the decision to accept drug therapy within a few hours of the incident.

(10) The following are definitions of infection-control terminology:

Bloodborne pathogens: Organisms that can be transmitted from one person to another by exposure to the infected person's blood. The major pathogens include hepatitis B virus, hepatitis C virus, human immunodeficiency virus (HIV; the AIDS virus), and syphilis.

Body substances: Any fluids or solids that come out of or off of the human body. Examples include saliva, sputum, urine, feces, wound drainage, and all the fluids referred to as "other potentially infectious materials" (see later).

Exposure incident: The contact of blood or other body substances with an employee's mucous membranes (eyes, mouth), nonintact skin (skin with cuts, abrasions, dermatitis, or other); or contact by piercing or puncturing mucous membranes or skin with a contaminated item.

Regulated (infectious) waste: items caked or saturated with blood or other potentially infectious materials; contaminated sharps; pathologic and microbiologic waste.

Other potentially infectious materials (OPIM): Body substances specifically designated by the CDC and OSHA that may transmit bloodborne pathogens include semen, vaginal secretions, cerebrospinal fluid, synovial fluid, pleural fluid, amniotic fluid, saliva in dental procedures, any body substance that is visibly contaminated with blood, and all body substances in situations for which it is difficult or impossible to determine whether blood is present.

APPENDIX B

ANEMIA SECTION OF THE MEASURE *DHS*+ HOUSEHOLD QUESTIONNAIRE WITH INFORMED CONSENT FORM

HEMOGLOBIN MEASUREMENT OF WOMEN 15-49

CHECK COLUMN (38):	LINE NO. OF PARENT/ RESPONSIBLE ADULT. RECORD '00' IF NOT LISTED IN HOUSEHOLD SCHEDULE	READ CONSENT STATEMENT TO WOMAN/PARENT/RESPONSIBLE ADULT* CIRCLE CODE (AND SIGN)	HEMOGLOBIN LEVEL (G/DL)	CURRENTLY PREGNANT	RESULT 1 MEASURED 2 NOT PRESENT 3 REFUSED 6 OTHER
(44)	(45)	(46)	(47)	(48)	(49)
AGE 15-17 AGE 18-49 1 2 GO TO 46		GRANTED REFUSED 1 2 SIGN _____ NEXT LINE	.	YES NO/DK 1 2	
1 2 GO TO 46		1 2 SIGN _____ NEXT LINE	.	1 2	
1 2 GO TO 46		1 2 SIGN _____ NEXT LINE	.	1 2	

HEMOGLOBIN MEASUREMENT OF CHILDREN BORN IN 19941 OR LATER

	LINE NO. OF PARENT/ RESPONSIBLE ADULT. RECORD '00' IF NOT LISTED IN HOUSEHOLD SCHEDULE	READ CONSENT STATEMENT TO PARENT/RESPONSIBLE ADULT* CIRCLE CODE (AND SIGN)	HEMOGLOBIN LEVEL (G/DL)		RESULT 1 MEASURED 2 NOT PRESENT 3 REFUSED 6 OTHER
		GRANTED REFUSED 1 2 SIGN _____ NEXT LINE	.		
		1 2 SIGN _____ NEXT LINE	.		
		1 2 SIGN _____ NEXT LINE	.		
		1 2 SIGN _____ NEXT LINE	.		
		1 2 SIGN _____ NEXT LINE	.		
		1 2 SIGN _____ NEXT LINE	.		

* CONSENT STATEMENT

As part of this survey, we are studying anemia among women and children. Anemia is a serious health problem which results from poor nutrition. This survey will assist the government to develop programs to prevent and treat anemia.

We request that you (and all children born in 1994[1] or later) participate in the anemia testing part of this survey and give a drop of blood from the finger. The test uses disposable sterile instruments that are clean and completely safe. The blood will be analyzed with new equipment and the results of the test will be given to you right after the blood is taken. The results will be kept confidential.

May I now ask that you (and NAME OF CHILD[REN]) participate in the anemia test. However, if you decide not to have the test done, it is your right and we will respect your decision. Now please tell me if you agree to have the test(s) done.

Note: In countries where some enumeration areas are higher than 1,000 meters, altitude information should be collected for each enumeration area higher than 1,000 meters.

1 For fieldwork beginning in 2000, 2001 or 2002, the year should be 1995, 1996 or 1997, respectively.

50	CHECK 5, 47 AND 48: NUMBER OF USUAL RESIDENTS WITH HEMOGLOBIN LEVEL BELOW THE CUTOFF POINT* ONE OR MORE ☐ → GIVE WOMAN/PARENT/RESPONSIBLE ADULT RESULT OF HEMOGLOBIN MEASUREMENT AND CONTINUE WITH 51 NONE ☐ → GIVE WOMAN/PARENT/RESPONSIBLE ADULT RESULT OF HEMOGLOBIN MEASUREMENT AND END HOUSEHOLD INTERVIEW.	
51	We detected a low level of hemoglobin in (your blood/the blood of NAME OF CHILD(REN)). This indicates that (you/NAME OF CHILD(REN)) have developed severe anemia, which is a serious health problem. We would like to inform the doctor at _____ about (your condition/the condition of NAME OF CHILD(REN)). This will assist you in obtaining appropriate treatment for the condition. Do you agree that the information about the level of hemoglobin in (your blood/the blood of NAME OF CHILD(REN) may be given to the doctor? AFTER READING THE ABOVE STATEMENT, I HAVE FOUND THAT _____ (NAME) AGREED TO REFERRAL FOR THE FOLLOWING PERSONS (NAME) AGREES TO REFERRAL 1 (NAME) DOES NOT AGREE TO REFERRAL 2	

* The cutoff point is 9g/dl for pregnant women and 7g/dl for children and women who are not pregnant (or who don't know if they are pregnant.)