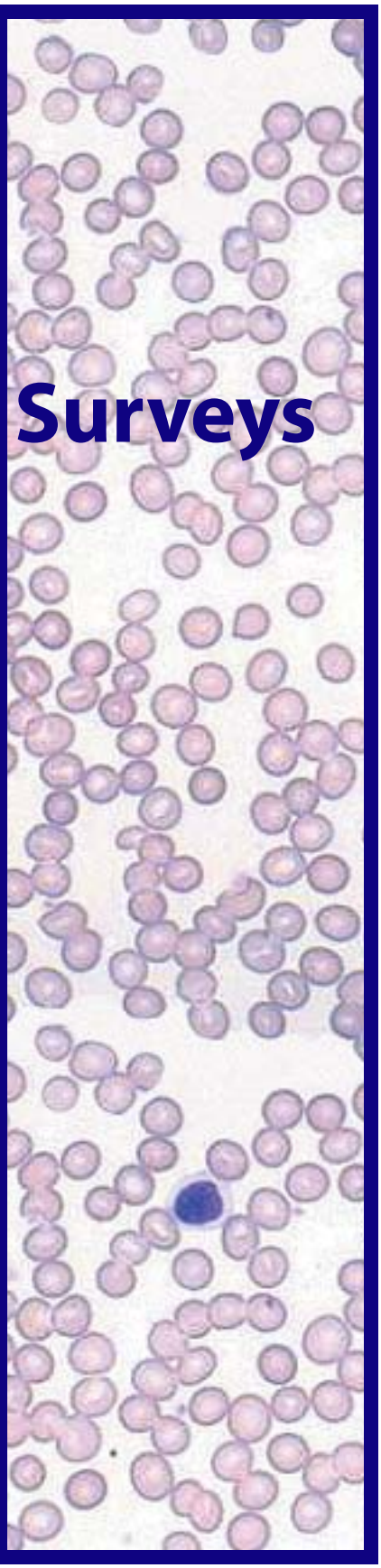
Anemia Testing in Population-Based Surveys



MEASURE *DHS+*

Anemia Testing in Population-Based Surveys

General Information and Guidelines for Country Monitors and Program Managers

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The main objectives of the MEASURE *DHS*+ project are as follows: 1) to provide decisionmakers in survey countries with information that is 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 ORC Macro, 11785 Beltsville Drive, Suite 300, Calverton, MD 20705 USA (Telephone: 301-572-0200; Fax: 301-572-0999; E-mail: reports@macroint.com; Internet: http://www.measuredhs.com).

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FOREWORD

Anemia testing has now become a standard part of many health and nutrition programs. The MEASURE *DHS*+ project has extensive experience in hemoglobin testing using the HemoCue system for anemia screening in a number of countries including Kazakhstan, Uzbekistan, Peru, Bolivia, Kyrgyz Republic, Madagascar, and India. The MEASURE *DHS*+ program also has experience with addressing ethical issues related to the protection of human subjects during biological sampling. The MEASURE *DHS*+ program follows standard requirements on the handling of biological (blood) specimens, as well as standard requirements on the proper disposal of biohazardous materials. Descriptions of the protocols for hemoglobin testing and biohazardous waste disposal used in the MEASURE *DHS*+ program can be found in a separate publication, *Anemia Testing Manual for Population-Based Surveys* (Sharmanov, 2000).

These guidelines are written for health coordinators and demographers who provide training for anemia testing, as well as for program managers, researchers, and other individuals who are involved in planning, managing, and coordinating anemia-prevention programs.

The first part of this document presents general information about anemia, particularly about its causes, clinical manifestations, prevalence, physiologic iron requirements, control, and prevention. It could be used as a source of information on many theoretical aspects of anemia.

The second part summarizes the MEASURE *DHS*+ program's experience in training for anemia testing. In addition to the training guidelines, this report presents a discussion of modern methods of anemia testing as well as methodological issues of population-based anemia testing. The discussion includes 1) the variability of the results of individual hemoglobin testing; 2) the importance of standardization of training protocols and anemia testing procedures (capillary versus venous blood sampling and methods used for hemoglobin testing); 3) the use of hemoglobin cut-off values; 4) the selection of adequate samples; 5) hemoglobin high-altitude adjustments; and 6) determination of iron-deficiency status. Special attention is paid to referral systems, field implementation of anemia surveys, data analysis, tabulation, and report writing.

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Part I

General Information on Anemia: Causes, Prevalence, Control and Prevention

DEFINITION, CONSEQUENCES, CLINICAL MANIFESTATIONS AND PREVALENCE OF ANEMIA 1

1.1 Definition and Consequences of Anemia

Definition of Anemia

Anemia is a condition characterized by a reduction in the red blood cell count or in the concentration of hemoglobin. Anemia is not a disease; it is a manifestation of various diseases and pathologic conditions.

Blood consists of cellular elements and plasma. The cellular elements include erythrocytes, or red blood cells; leucocytes, or white blood cells; and platelets. Red blood cells are the most numerous cells in the blood; approximately 20 billion of them circulate in the blood of an adult. They are required to transport oxygen to the tissues and organs of the body. Red blood cells contain hemoglobin, an iron-containing protein that acts in the transportation of oxygen to the tissues and carbon dioxide from the tissues. When the concentrations of hemoglobin or red blood cells in the blood are reduced to below normal, anemia is developed.

Anemia and Maternal Health

Anemia is known to have detrimental health implications, particularly for mothers and young children. Women with severe anemia can experience difficulty meeting oxygen-transport requirements near and at delivery, especially if significant hemorrhaging occurs. This may be an underlying cause of maternal death and prenatal and perinatal infant loss (Fleming, 1987; Thonneau et al., 1992; Omar et al., 1994; Allen, 1997). In fact, unfavorable pregnancy outcomes have been reported to be more common in anemic mothers than in nonanemic mothers (Scrimshaw, 1984; INACG, 1989; WHO, 1992; Allen, 1997).

The adverse effects of mild anemia are less well documented than the effects of severe anemia. However, in several studies, premature delivery, placental hypertrophy, and reduced excretion of estriol (maternal hormone) have been observed to be more common in mildly anemic mothers than in nonanemic mothers (Hercberg and Galan, 1992).

Effects of Anemia on Mental Performance

Iron-deficiency anemia among children has been demonstrated in many studies to be associated with impaired cognitive and intellectual performance, motor development, coordination, language development, and scholastic achievement (Scrimshaw, 1984; Lozoff et al., 1991; de Andraca et al., 1997; Pollitt, 1997). Other effects include irritability, apathy, lack of attention, reduced learning capacity, and low school performance scores. Some of these symptoms can be reversed after iron therapy.

Because of multifactorial determinism of these types of abnormalities, the contribution of iron deficiency to these symptoms is hard to evaluate. Iron deficiency may result in an imbalance of the brain hormones, particularly of the monoamine oxidase system, which plays an important role in the brain functions. This may lead to impaired cognitive performance and other types of dysfunctions of the central nervous system.

Anemia and Resistance to Infections

Anemia increases morbidity from infectious diseases because important immune mechanisms, especially cellular immunity, are adversely affected. Several epidemiological and clinical studies have shown that higher morbidity rates due to infections have been found in anemic subjects than in nonanemic subjects, and that iron supplementation has been shown to have a beneficial effect upon the incidence of infection (Scrimshaw, 1984; Hercberg and Galan, 1992).

The antibacterial affects of two iron-binding proteins, transferrin and lactoferrin, have been studied. These proteins prevent microorganisms from using iron and thereby limit the microorganisms' growth.

Effect on Work Capacity

Several studies have shown a direct relationship between hemoglobin concentration and the physical performance of agricultural workers: sugar cutters in Guatemala, latex tappers and weeders in Indonesia, tea harvesters in India. The deleterious effect of anemia on work capacity may be related to iron's role in the biochemical reactions which facilitate oxygen transport to the muscle cells. Many studies have shown that the work capacity of anemic persons can be increased by iron supplementation (Hercberg and Galan, 1992).

Impaired work performance in anemic individuals and the possibility of improving it by balancing their iron status may have far-reaching socioeconomic consequences. For this reason, anemia-control-and-prevention programs have been recognized by the World Bank to be among the most cost-effective interventions in the field of public health (Levin, 1986).

1.2 Clinical Manifestations of Anemia

Patients with anemia usually complain of decreased work tolerance, fatigue, shortness of breath, palpitations, and other signs of adjustments of the heart and lungs to anemia. When significant anemia is developed, the patient may notice a humming or whirring sound in the head, attributed to the rapid blood flow through blood vessels in the brain (Lee, 1999a).

It is important to note, however, that patients' complaints are often quite subjective and may not be related to anemia. On the other hand, in some cases the patient's adjustment to anemia may be so good that despite the presence of severe anemia, the patient may not experience enough symptoms to appreciate the situation and become motivated to seek medical attention.

Clues to a diagnosis of anemia and a determination of its causes may be found after physical examination, evaluation of the patient's history, laboratory investigation, and systematic analysis of the factors and mechanisms that may operate to produce anemia.

On physical examination of patients with anemia, many symptoms related to dysfunction of cardiovascular, pulmonary, neuromuscular, gastrointestinal and genitourinary systems may be evident. Skin pallor is perhaps the most evident sign of anemia, since there is a general relationship between skin and mucous membrane color and hemoglobin level. Skin pallor in patients with anemia is usually noticed by their friends and family. The pallor associated with anemia is detected most accurately in the eye conjunctivae, the lips, the nail beds, the palmar creases of the hand, and the mucous membranes of the mouth and pharynx.

However, it is important to note that many factors other than hemoglobin concentration may affect skin color. These factors include temperature, age, and kidney or endocrine disorders. Certain people

normally have pale skin. Many symptoms of anemia, such as shortness of breath, dizziness, and palpitation, are noticeable only after exertion or excitement.

For these reasons, laboratory examination is a far more definitive measure of anemia status than physical examination and the patient's history. Among laboratory diagnostic methods, three measures may be used to establish the presence of anemia: hemoglobin, hematocrit, and number of red blood cells. The blood hemoglobin concentration is recognized as the most informative, in part because of its relatively high accuracy and reproducibility, and in part because it is the value most indicative of the pathophysiologic consequences of anemia (Perkins, 1999). Hemoglobin measurement is also recommended for anemia testing in population-based surveys, which will be discussed in chapter 4.

1.3 Prevalence of Anemia in the World

Anemia due to iron deficiency is recognized as a major public health problem throughout the world. According to the epidemiological data collected from multiple countries by the World Health Organization (WHO), more than one-third of women and two-fifths of young children in the world are affected by anemia. In developing countries, about half of women and young children are anemic. Table 1.1 illustrates the prevalence of anemia among women in various regions of the world, based on estimation by the World Health Organization (WHO, 1992).

Table 1.1 Estimated prevalence of anemia in women

Region	Percent of pregnant women with Hb below normal	Percent of nonpregnant women with Hb below normal	Percent of all women with Hb below normal
World	51	35	37
Developing countries	56	43	44
Developed countries[1]	18	12	13
Africa	52	42	44
Eastern	47	41	42
Middle	54	43	45
Northern	53	43	45
Southern	35	30	30
Western	56	47	48
Asia	60	44	45
Eastern[1]	37	33	33
South-eastern	63	49	50
Southern	75	58	60
Western	50	36	38
Latin America	39	30	31
Caribbean	52	36	37
Central	42	39	39
South	37	25	26
Northern America	17	10	11
Europe	17	10	11
Oceania[1]	71	66	67

[1] Japan, Australia, and New Zealand have been excluded from the regional estimates, but are included in the total for developed countries. Figures may not add to totals due to rounding.
Source: WHO, 1992

The highest overall rates of anemia are reported in southern Asia and in certain regions of Africa (Florentino and Guirriec, 1984; DeMaeyer et al., 1989; WHO, 1992). In the United States and Europe, the prevalence of anemia fluctuates between 7 and 12 percent among women and children (Hallberg, 1981; Dallman et al., 1984; Yip, 1994; Lee, 1999a).

In demographic and health surveys conducted in Central Asia between 1995 and 1997, high rates of anemia were observed in environmentally affected areas of the Aral Sea, where more than 70 percent of women and children have some degree of anemia (Sharmanov, 1998). There are certain evidences to suggest that iron deficiency plays a key role among the causes of anemia in that region.

Since 1995, the Demographic and Health Surveys (MEASURE *DHS*+) program has been involved in testing women and children for anemia. As of February 2000, data collection had been completed in seven DHS countries, including Kazakhstan, Uzbekistan, Peru, Kyrgyz Republic, Bolivia, Madagascar, and India. Anemia data collection was scheduled to begin in Egypt, Haiti, Cambodia, and Turkmenistan by early 2000. Figure 1.1 and table 1.2 summarize information on anemia testing in these countries.

Figure 1.1 Countries with a DHS anemia testing component

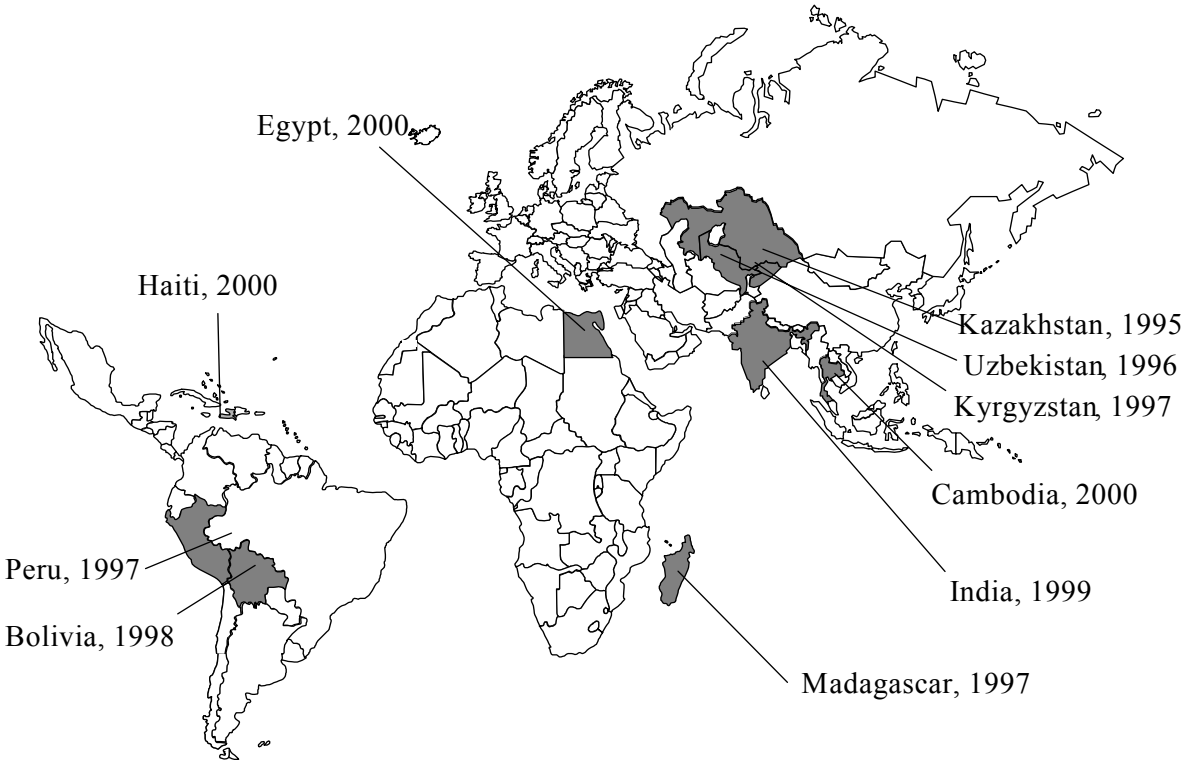


Table 1.2 Summary of anemia testing in the Demographic and Health Surveys program

Country	Year of data collection	Sample size for women	Sample size for children	Anemia prevalence for women (%)	Anemia prevalence for children (%)
Kazakhstan	1995	3,658	739	49	69
Uzbekistan	1996	4,274	1,018	60	61
Peru	1996	2,274	1,046	36	57
Madagascar	1997	3,529	2,680	42	67
Kyrgyz Republic	1997	3,760	980	38	50
Bolivia	1998	3,531	971	27	67

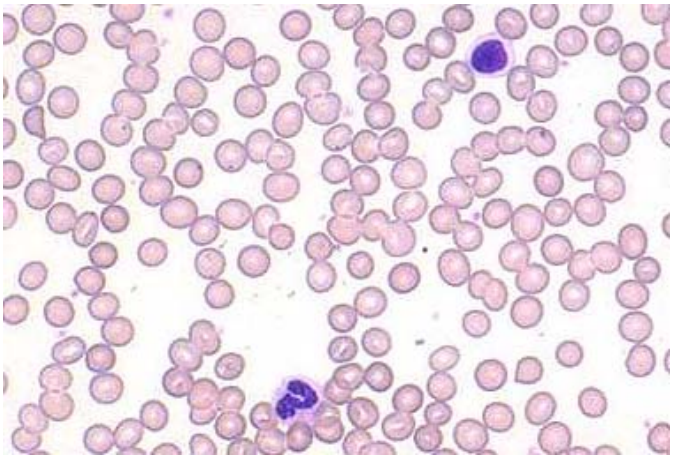
PHYSIOLOGY OF IRON REQUIREMENTS AND MAIN CAUSES OF ANEMIA

2

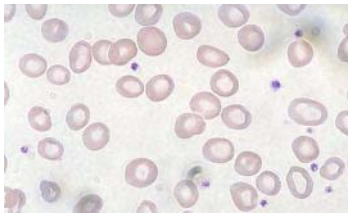
2.1 MAIN CAUSES OF ANEMIA

Commonly, anemia is the final outcome of nutritional deficiency of iron, folate, vitamin B_{12}, and some other nutrients (Lee and Herbert, 1999). Many other causes of anemia have also been identified. They include malaria, hemorrhage, infection, genetic disorders (hemoglobinopathies), parasite infestation (hookworm), chronic disease, and others. The blood images shown in figure 2.1 reflect significant morphologic differences among various clinical forms of anemia caused by iron deficiency, vitamin B_{12} deficiency, a genetic disorder called thalassemia, and malaria. A detailed classification of anemia is presented in appendix A.

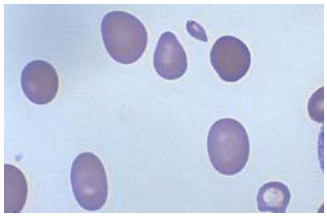
Figure 2.1
Microscopic blood images reflecting the morphologic diversity of various clinical forms of anemia



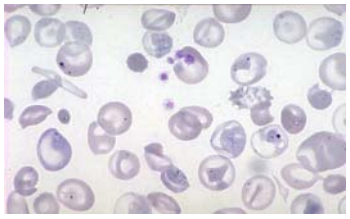
2.1.1 Normal blood



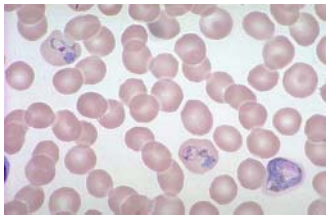
2.1.2 Iron deficiency anemia



2.1.3 Folic acid deficiency



2.1.4 Thalassemia



2.1.5 Malaria

A blood smear can provide important morphologic clues to diagnosing different kinds of anemia (figure 2.1):

2.1.1. Normal blood smear for comparison.

2.1.2. In iron deficiency anemia, the red blood cells are smaller than normal. They also look pale due to a decreased level of hemoglobin. Iron deficiency anemia may result from nutritional deficiency of iron, bleeding caused by parasites such as hookworm, or other causes.

2.1.3. In folic acid and vitamin B_{12} deficiency, the size of red blood cells is significantly increased, reflecting defects in their maturation. This type of anemia is called megaloblastic or macrocytic.

2.1.4. Variations in cell size and shape are observed in the blood of patients with thalassemia. Note the so-called sickle cells, which are characteristic of defects in hemoglobin synthesis.

2.1.5. In malaria, a parasite infiltrates the red blood cells and causes their destruction. This blood smear illustrates different stages of intracellular development of the malaria parasite.

Nutritional deficiency, due primarily to a lack of bioavailable dietary iron, accounts for the majority of anemia cases in the world (Yip and Dallman, 1988; INACG, 1979, 1989; Hercberg and Galan, 1992; Yip, 1994; Lee, 1999). The contribution of other causes of anemia depends on many factors, including level of economic development, climate, condition of health care, and the existence of anemia control and prevention programs.

In semideveloped and developed countries, iron deficiency is the main cause of anemia among women and children. In approximately 50 to 80 percent of anemia cases, iron deficiency is considered to be the main etiologic factor (R. Yip, 1999, personal communication).

In many countries, however, a number of other factors besides iron deficiency contribute to the burden of anemia. Of particular importance in developing countries are malaria and intestinal parasites, especially hookworm infestation. The level of contribution of these factors to the overall prevalence of anemia depends on the magnitude of malaria epidemics, the existence of iron supplementation and fortification programs, and other conditions in each particular country.

Recently, the role of HIV epidemics as an important factor contributing to anemia in countries of sub-Saharan Africa has been emphasized. It has been shown that HIV negatively affects the release of erythropoietin, which is a kidney hormone that stimulates production of red blood cells. Perhaps because of this mechanism, HIV-infected women, even without clinical symptoms of opportunistic infections, are more likely to become anemic than HIV-free women (R. Stoltzfus, 2000, personal communication).

2.2 Physiologic Iron Requirements

To better understand why iron deficiency anemia is more prevalent among children and women, especially pregnant women, it is important to discuss iron requirements during different periods of life.

Iron is essential for life. As a constituent of hemoglobin and other proteins, called enzymes, iron participates in many important processes that facilitate oxygen transport and supply to the tissues and organs. In a healthy, well-nourished individual, iron balance occurs when the quantity of iron absorbed from the diet is sufficient to compensate daily iron loss, and to maintain adequate body iron stores. The iron balance can be disturbed by a variety of factors, such as low dietary iron intake, increased iron losses and increased iron requirements.

The amount of iron in the human body is approximately 2,500 mg. More than half of this amount, approximately 1,700 mg, is present in the hemoglobin of red blood cells (INACG, 1989). A certain amount of iron is lost daily due to basal iron losses with epithelial cells from internal and external surfaces. About one-third is lost from the gastrointestinal tract. A tiny fraction of iron is lost in urine or sweat.

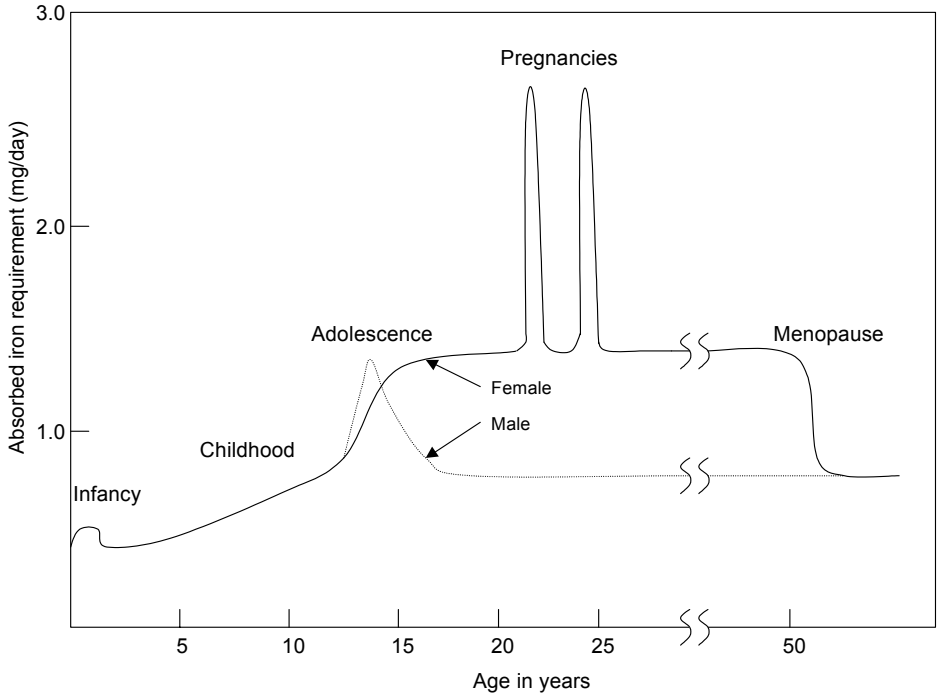
Significant iron loss occurs in women during menstruation. Median monthly loss of blood during menstruation has been estimated at 35 ml (the upper limit of normal is about 80 ml), which is equivalent to more than 12 mg of iron (Lee, 1999). Menstrual blood flow is increased by approximately 100 percent in women using intrauterine devices and reduced by about 50 percent in women using oral contraceptives (INACG, 1989). The DHS survey conducted in Kazakhstan in 1995 showed that among women who were using intrauterine devices, the rate of moderate-to-severe anemia was approximately 1.5 times higher than among those who were not using them (Sharmanov, 1996). This may be related to the observation that chronic use of intrauterine devices can lead to iron depletion and iron deficiency anemia (Palomo et al., 1993). Thus, taking into account their greater physiologic iron losses, menstruating women, especially those using intrauterine devices, are at a greater risk of iron deficiency than men.

Basal and menstrual blood losses can usually be replenished by consumption of foods rich in bioavailable iron, such as meat, and foods containing so-called promoters of iron absorption, e.g., ascorbic acid. An adequate iron balance can be maintained and iron deficiency prevented by means of an iron-rich diet.

Because of women's greater iron requirements due to menstruation, and also because they usually consume less food than men, women's daily iron intake tends to be marginal, and they are therefore more likely to develop iron deficiency anemia than men. This is especially true in many developing countries where the general food consumption is reduced because of poverty, unwise agricultural practices, and other reasons.

There are certain critical periods when iron requirements are significantly increased and the iron balance can be easily disturbed. Such situations include pregnancy and growth during infancy and early childhood (figure 2.2).

Figure 2.2 Iron requirements of males and females of various ages



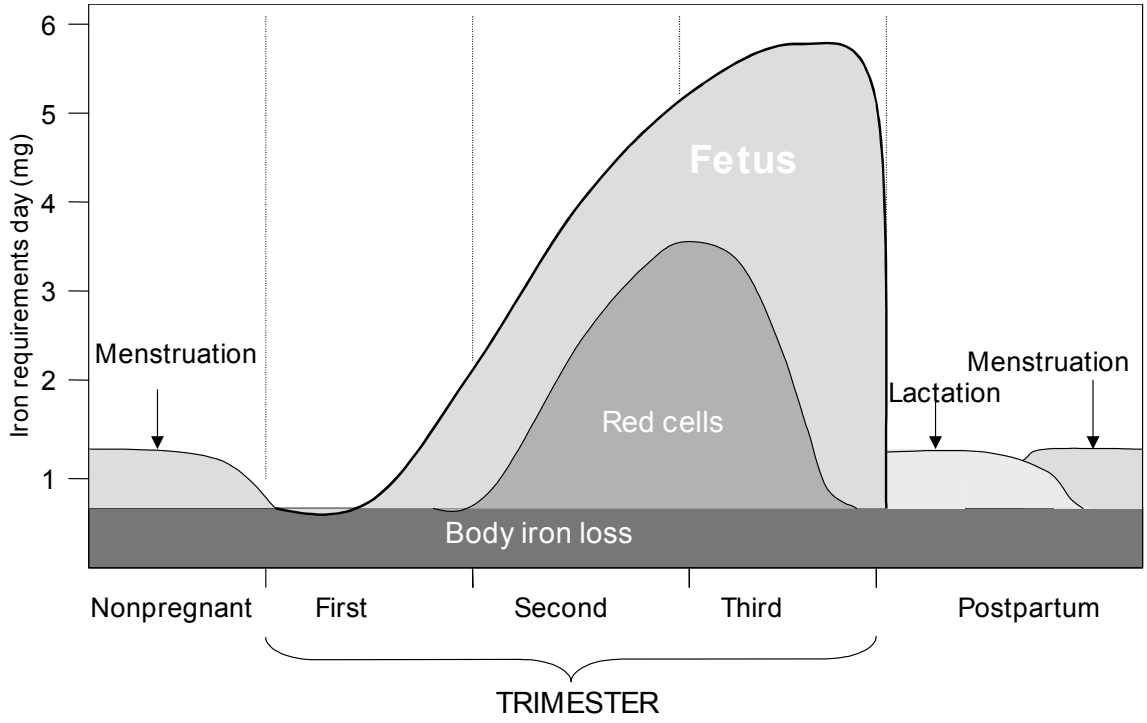
Source: Redrawn from Lee (1999)

As illustrated, great nutritional iron requirements occur during infancy and early childhood, or between 6 and 24 months of age. Fast growth during adolescence leads to a further increase in iron requirements, more so in the male than in the female. Thereafter, when menstruation starts, the iron requirements of the female exceed those of the male. A significant increase in iron requirements occurs during pregnancy. Iron supplementation is essential during periods of fast growth and pregnancy to prevent iron deficiency.

Iron Losses in Pregnancy and Lactation

The second and third trimesters of pregnancy constitute a major drain on the iron reserves of women of reproductive age (figure 2.3).

Figure 2.3 Daily iron requirements during pregnancy and postpartum



Note: The iron requirements are needed to meet the normal losses from the body and to provide iron for the enlarging cell mass, for the fetus, and for lactation after birth of the child.
Source: Redrawn from Bothwell and Finch (1962)

During these periods, the requirements of the growing fetus (about 270 mg), umbilical cord and placenta (90 mg) are especially great. There is also an expansion of maternal red-blood-cell mass (about 450 mg), which raises the iron requirements even more. Expressed in terms of need for iron, these changes along with blood loss during delivery (150 mg) and basal iron losses (170 mg) are equivalent to about an additional 1,130 mg of iron. This amount is greater than that which can be absorbed from diet; hence iron supplementation is necessary during pregnancy. After delivery, expanded red cell mass contracts and some iron returns to reserve sites. However, despite such return, the average cost of each pregnancy in terms of iron loss is still high, approximately 680 mg (Lee, 1999).

The size of iron stores at the beginning of pregnancy plays an important role. Iron requirements are higher in mothers who begin pregnancy with depleted or low iron stores. Such situation is common in developing countries. Low birth intervals may negatively affect initial iron stores and increase the chance of iron deficiency during pregnancy.

During lactation a woman loses about 0.5 to 1 mg of iron per day. However, because of amenorrhea, the iron requirements of lactating women are approximately the same as those of menstruating

women (figure 2.3). It is important to note that the content of human breast milk is not affected by maternal iron stores. In many developing countries, breastfeeding is often greatly prolonged, more than 6 months, which implies higher iron requirements after 6 months of lactation.

Iron Requirements in Infancy and Childhood

Because of the rapid growth of children and the increased needs of rapidly growing tissues, the requirements for iron in infancy and early childhood are great. During the first year of life, body weight and blood volume nearly triple, and the circulating hemoglobin mass approximately doubles. The requirements are even greater for premature infants since their weight and blood volume increase nearly sixfold (Table 2.1). For this reason, premature babies as well as babies with low birth weight are more likely to become anemic later in their life than those who were born at term and with normal weight. Anemia occurs more often among twins than among single births (Lee, 1999).

Table 2.1 Iron balance during the first year of life

	Full-term infant		Premature infant	
	Birth	One year	Birth	One year
Weight (kg)	3.3	10.5	1.5	9.5
Blood hemoglobin (g/dl)	20.0	12.3	20.0	12.3
Blood hemoglobin mass (g)	580.0	984.0	270.0	886.0
Blood volume (ml)	290.0	800.0	135.0	720.0
Total hemoglobin (g)	58.0	98.0	27.0	89.0
Hemoglobin iron (mg)	198.0	335.0	90.0	300.0
Storage and tissue iron (mg)	60.0	73.0	27.0	67.0
Total; body iron (mg)	258.0	408.0	117.0	367.0
Net positive iron balance (mg/year)		150.0		250.0
(mg/day)		0.4		0.7

Source: Modified from Lee (1999)

Maternal iron status has little or no effect on the body iron stores of the newborn child. Due to relatively rich iron stores at birth, iron deficiency is rarely observed during the first few months of infancy unless the infants were born with very low birth weight. However, by 2 to 6 months, infants' iron stores are depleted and they become increasingly dependent on an external iron supply.

Important sources of iron supply for infants are breast milk and cow's milk. Both of them contain relatively small amounts of iron; about 0.8 mg/L in breast milk and 0.6 mg/L in cow's milk. However, the bioavailability of iron in human breast milk is five times as high as in cow's milk (UNICEF/WHO, 1999). Therefore, the most critical period in terms of adequacy of iron stores starts with weaning (usually 6 months of age) and lasts throughout the period of rapid growth (usually up to 24 months of age).

Similar to infancy, the adolescent growth spurt (between 11 and 14 years of age) also leads to a further increase in iron requirements, more so in the male than in the female (Fig. 2.2). The latter is probably related to the fact that the maximum yearly weight gain is somewhat greater for boys than for girls. With the onset of menstruation in girls, their iron requirements become equal to those of adult women.

2.3 Dietary Iron Content and Bioavailability of Iron

There are at least four dietary factors that determine iron status in humans: (1) food iron content, (2) levels of iron absorption (the so-called "bioavailability" of iron), (3) the presence of nutritional factors that promote iron absorption, and (4) the presence of nutritional factors that inhibit iron absorption.

The iron contents of different foods are presented in table 2.2. According to food balance sheets, in developing countries the total iron in diet per capita usually varies between 13 and 21 mg/day, and most of this iron is provided by vegetables.

While the amount of dietary iron is important in maintaining iron balance, perhaps the more important factor is the level of iron absorption, which determines the bioavailability of iron. In humans, iron is absorbed in the upper intestine in two forms: heme iron and nonheme iron.

Heme iron is present in foods from animal sources (meat or fish). It is easily absorbed (15 to 20 percent), and its absorption is unaffected by gastric acidity and dietary composition. Heme iron is of great nutritional significance and is called "bioavailable."

Nonheme iron comes from plant sources. Only 2 to 5 percent of it is absorbed. Absorption of nonheme iron requires three major factors: (a) an adequate gastric acidity; (b) the presence of dietary components that promote iron absorption, such as ascorbic acid or meat; and (c) the absence of factors that inhibit iron absorption, such as phytates and tannates (contained in tea, coffee, and cereals), phosphates, egg yolk, and isolated soy protein.

Table 2.2 Iron contents of different foods

Food	Iron content (mg/100g of food)	Food	Iron content (mg/100g of food)	Food	Iron content (mg/100g of food)
Maize		Taro	1.2	Antelope	2.1
White	3.6	Plantain	1.3	Lamb	2.0
Yellow	4.9	Bean	1.4	Chicken	1.1
Sorghum		Soybean	6.1	Beef	2.9
Red	15.6	Lentil	7.0	Pork	2.0
White	5.8	Chickpea	11.1	Caterpillars	0.5
Yellow	5.0	Cowpea	7.6	Termites	52.0
Millet	39.0	Peanut	3.8	Grasshoppers	11.0
Teff		Pumpkin	1.4	Cake of lake flies	65.6
Red	75.5	Amaranth	8.9	Iguana	3.7
White	20.9	Baobab leaves	24.0	Snail	41.0
Rice		Shea butterseed	3.0	Hen eggs	2.6
Brown	2.0	Tomato	0.6	Milk	0.2
White	1.7	Pineapple	0.4	Eel	2.4
Acha	8.5	Monkey bread	7.4	Prawn	1.6
Wheat	6.5	Soursop	2.0	Tilapia	3.2
Cassava	1.9	Datte	6.0	Snapper	2.2
Yam	0.8	Guava	1.3	Sardine	1.3
Gari	1.6	Mango	1.2	Carp	1.1
Potato	1.1	Orange	0.1	Mackerel	1.2
Sweet Potato	2.0	Papaya	0.6		

Source: Hercberg and Galan, 1992

The overall absorption of iron may be significantly increased if fruits and vegetables with high concentrations of ascorbic acid (oranges, lemons, grapefruits, guavas, papayas, and green leafy vegetables) are present in the diet. On the other hand, tea, widely consumed in many countries, is a powerful inhibitor of nonheme iron absorption because of its tannate content.

The inequality in the levels of iron absorption probably originated from the evolution of human dietary patterns. Initially, humans were characterized by hunter-gatherer food patterns and diets rich in meat. Based on these patterns, original standards in terms of iron absorption and metabolism were set. In later stages of human evolution, these patterns have been replaced by agricultural development and con-

sumption of grains and vegetables. Adaptation to such a new environment required that humans had to consume sufficient amounts of foods from animal sources or foods enriched with promoters of iron absorption in order to meet the originally set standards.

Today, the typical diet of most inhabitants of developing countries is based on cereals or roots and tubers, with little or no meat, fish, and foods rich in ascorbic acid, and with high concentrations of iron absorption inhibitors. It has been estimated that the average iron absorption of this type of diet is between 1 and 5 percent (Galan et al., 1990). Such a dietary pattern is a major reason for the high prevalence of iron deficiency anemia in economically deprived and developing countries.

2.4 Anemia Caused by Deficiency of Folic Acid

Folic acid deficiency causes so-called megaloblastic anemia, which is a disorder characterized by impaired DNA synthesis. Megaloblastic anemia may also be caused by vitamin B_{12} deficiency. The cells primarily affected by deficiencies in these two vitamins are those having relatively rapid turnover, especially precursors of the red blood cells and gastrointestinal epithelial cells. Cell division is sluggish, but cytoplasmic development progresses normally, so red blood cells tend to be large and are therefore megaloblastic (see figure 2.1.3).

Folic acid is synthesized by many different plants and bacteria. Fruits and vegetables constitute the primary dietary source of the vitamin. Some forms of dietary folic acid are labile and may be destroyed by cooking. The minimum daily requirement of folic acid is normally about 50μg, but this amount may be increased severalfold during periods of enhanced metabolic demand such as pregnancy. Normal individuals have about 5 to 20 mg folic acid in various body stores, half in the liver. In light of the minimum daily requirement, it is not surprising that a deficiency will occur within months if dietary intake or intestinal absorption is curtailed (Babior and Bunn, 2000).

Folic acid deficiency can generally be attributed to one or more of the following factors: 1) increased demand, 2) inadequate intake, and 3) impaired absorption in the gastrointestinal tract.

Increased Demand

Folic acid deficiency may occur during the growth spurts of infancy and adolescence because of increased demand. A pregnant woman may become deficient in folic acid because of the high demand of the developing fetus. Deficiency in pregnancy can cause neural tube defects in newborns and fetal malformation. During pregnancy, folic acid deficiency may also be associated with an increased prevalence of a variety of obstetric conditions such as abruptio placentae, spontaneous abortion, neonatal deaths, low birth weight, prematurity, toxemia, and postpartum hemorrhage (Baker and DeMaeyer, 1979). Thus, pregnant women should receive oral folic acid supplementation.

Inadequate Intake

Folic acid deficiency frequently occurs in alcoholics because their main source of caloric intake is alcoholic beverages. Distilled spirits are virtually devoid of folic acid, while beer and wine do not contain enough of the vitamin to satisfy the daily requirement. Alcohol also may interfere with folic acid metabolism. Narcotic addicts are also prone to become folic acid deficient because of malnutrition. Many indigent and elderly individuals who subsist primarily on canned foods or tea and toast, and some teenagers whose diets consist of junk food, develop folic acid deficiency.

Impaired Absorption

Folic acid deficiency commonly accompanies the intestinal disease *tropical sprue*. Both the gastrointestinal symptoms and malabsorption are improved by the administration of either folic acid or antibiotics by mouth. Similarly, folic acid deficiency in alcoholics may be due in part to impaired absorption.

Patients with folic acid deficiency are often malnourished and are likely to appear wasted. Diarrhea is often present, as well as visible defects of the tongue and mucosal surfaces of the mouth. In contrast to vitamin B_{12} deficiency, which has similar blood characteristics but also affects the nervous system, neurologic abnormalities do not occur in patients deficient in folic acid. In acutely developed folic acid deficiency, symptoms such as sleeplessness, irritability, and forgetfulness are common (Hercberg and Galan, 1992; Babior and Bunn, 2000).

2.5 Parasite Infestations and Anemia

Parasites are considered to be among the important causes of anemia because they cause bleeding from the gut, bladder, and other internal organs. At the population level, parasite infestations contribute to the prevalence of anemia if they are endemic in certain areas. Parasites also tend to have the greatest effect on the prevalence of moderate and severe anemia, as opposed to nutritional anemias, which are characterized by a broader spectrum of deficiencies and clinical manifestations. Three families of parasites are considered to be of great public health concern because they cause bleeding and iron deficiency anemia. They are hookworms, whipworms, and schistosomes.

Hookworm Infestation

Hookworm parasites are common in tropical and subtropical areas and their frequency is promoted by the hot and humid climate common in many populated areas of the developing world. Approximately one-fourth of the world's population is infected by hookworm, especially by two of its forms: *Necator americanus* and *Ankylostoma duodenale*.

Parasite transmission occurs year-round, but transmission rates are highest after the rainy season. Parasites are transmitted by contact between the skin and soil polluted by the parasite's eggs. At particular risk are people who live and work barefoot. In most areas, older children have the greatest incidence and intensity of hookworm infection. In rural areas where fields are fertilized with night soil, older working adults also may be heavily affected (Liu and Weller, 2000).

Hookworm parasites cause bleeding by attaching themselves to the gut's mucosa and sucking blood from the submucosal vessels. The intestinal blood loss caused by hookworm infestation may vary from 2.4 ml to 4.5 ml of blood per day, which is the equivalent of 1 to 2 mg of daily iron loss (Fleming, 1981; Hercberg and Galan, 1992).

Whipworm Infestation

Another parasite causing intestinal bleeding is whipworm, or *Trichuris trichiura*. Like the other soil-transmitted parasites, whipworm is distributed globally in the tropics and subtropics and is most common among poor children. The average iron loss caused by the whipworm may be as high as 1 mg of iron per day (Liu and Weller, 2000).

Schistosomiasis

Among schistosomes, three primary species—*Schistosoma mansoni*, *Schistosoma haematobium*, and *Schistosoma japonicum*—and a number of less prevalent species all infect humans. Schistosomiasis is

usually most prevalent and severe among children, older boys, and men, who are all more likely to swim, since the parasite is transmitted when people swim or wade in bodies of water that are habitats for infected snails. Schistosomes cause bleeding by discharging eggs into the veins draining the large bowel or bladder. Iron losses caused by schistosomes may be considerable, corresponding to about 1 to 6 mg of iron per day (Hercberg and Galan, 1992; Nash, 2000).

Worldwide, as many as 200 million people may be infected with schistosomes, and infection of entire communities is common. The areas where the intestinal species (*S. mansoni* and *S. japonicum*) are widespread include parts of South America (Brazil, Venezuela, and Surinam), some Caribbean islands, Africa, the Near East, and the Far East, mostly China and the Philippines. Infestations of *S. haematobium* that cause bleeding from the ureters and bladder mostly occur in Africa and the Near East (Nash, 2000).

2.6 Anemia Caused by Malaria

Malaria is a disease transmitted by the bite of infected *Anopheles* mosquitoes. It is the most important of the parasitic diseases in humans. Malaria affects more than 500 million people and causes between 1 and 3 million deaths each year. Most of these deaths are among young African children (Reisberg, 1997; White and Breman, 2000).

Epidemiology of Malaria

Four species of the genus *Plasmodium* cause nearly all malaria infections in humans. These are *Plasmodium vivax*, *Plasmodium ovale*, *Plasmodium malariae*, and *Plasmodium falciparum*. Almost all malaria-related deaths are caused by falciparum malaria.

Malaria occurs throughout most of the tropical regions of the world. *P. falciparum* predominates in Africa, New Guinea, and Haiti; *P. vivax* is more common in Central America and the Indian subcontinent. The prevalence of these two species is approximately equal in South America, eastern Asia, and Oceania. *P. malariae* is found in most endemic areas, especially throughout sub-Saharan Africa, but is much less common. *P. ovale* is relatively unusual outside Africa.

Malaria behaves like an epidemic disease in some areas, such as in northern India, Sri Lanka, Southeast Asia, Ethiopia, southern Africa, and Madagascar. An epidemic can develop when there are changes in environmental, economic, or social conditions, such as heavy rains following drought, or migrations (usually of refugees or workers) from a nonmalarious region to an area of high transmission. This situation usually results in considerable mortality among all age groups.

Pathogenesis of Malarial Anemia

After invading a red blood cell, the growing malaria parasite progressively consumes and degrades intracellular proteins, principally hemoglobin, causing the red cell to become more irregular in shape, and leading to its eventual destruction (see figure 2.1.5).

The malaria parasite inserts new parasite-derived proteins, which mediate attachment of the red blood cells to the walls of blood vessels—an event termed *cytoadherence*. This cytoadherence causes accumulation of infected red blood cells inside the small blood vessels. At the same stage, these malaria-infected red cells may also adhere to uninfected red cells to form *rosettes*, which make accumulation of the red blood cells inside the blood vessels even worse. The processes of cytoadherence and rosetting are central to the pathogenesis of malaria. They result in the accumulation of the parasite-infected red cells in vital organs (particularly the brain, heart, and lungs), where they interfere with organ function by affecting blood circulation.

Pregnant women are especially prone to malaria, which can cause severe anemia, hypoglycemia, and acute pulmonary edema. Fetal distress, premature labor, and stillbirth or low birth weight are common results. Congenital malaria occurs in fewer than 5 percent of newborns whose mothers are infected and is related directly to the parasitic density in maternal blood and in the placenta.

Malaria in children is characterized by convulsions, coma, and other clinical manifestations. Severe anemia is relatively common among children with severe malaria. In general, children tolerate the antimalarial drugs well and respond rapidly to treatment.

Despite enormous control efforts, malaria has resurged in many parts of the tropical and subtropical countries. Added to this resurgence are the increasing problems of the parasite developing drug resistance and mosquitoes developing insecticide resistance. Malaria remains today, as it has been for centuries, a heavy burden on tropical communities and a danger to travelers.

ANEMIA CONTROL AND PREVENTION 3

3.1 MAGNITUDE OF THE PROBLEM AND IMPORTANCE OF ANEMIA CONTROL AND PREVENTION

Global Burden of Anemia and Effectiveness of Anemia Control and Prevention

The WHO/World Bank-supported analysis of the Global Burden of Disease ranked iron deficiency anemia as the third leading cause of loss of disability-adjusted life years (DALYs) for females age 15 to 44 across the globe. This factor was more important globally than war-related death and disability, and nearly as important as the global scourge of tuberculosis (UNICEF/WHO, 1999). The World Bank recognized anemia control and prevention as among the most cost-effective programs (Levin, 1986; UNICEF/WHO, 1999).

Using different but equally compelling criteria, USAID produced a 1994 analysis estimating that in south Asia, a two-thirds reduction in anemia would result in a US $3.2 billion increase in agricultural production over a 7-year period (1994-2000).

Concerning the effect of anemia on education, the WHO/World Bank analysis noted that control of iron deficiency anemia improves attitude, capacity to concentrate, and school attendance (UNICEF/WHO, 1999).

A recent document prepared by the Micronutrient Initiative on the Economic Consequences of Iron Deficiency analyzed relationships between anemia and several economically quantifiable factors, including

- lower future productivity of children
- lower current productivity of adults
- costs for care of low birth weight and premature infants
- cost of maternal mortality
- other consequences on growth
- decreases in immunity and increased absenteeism due to infectious disease
- increases in morbidity and mortality
- greater susceptibility to heavy metal toxicity

Economic analysis demonstrates the importance of anemia control and prevention programs to policymakers in agencies, to ministerial and parliamentary leaders who deal with resource allocations, and to the leaders of agencies and private-sector firms that give financial support. Information on the cost-effectiveness of anemia control and prevention programs, as well as the data on the health and developmental impact of anemia, reinforces the moral and legal obligations of governments to address this issue, based on human rights (UNICEF/WHO, 1999).

Integrated Anemia Control and Prevention Strategy

In developing countries, an integrated strategy for anemia control and prevention might include the following major components:

- dietary improvement through education to encourage selection of iron-rich foods to improve iron content and bioavailability

- fortification (adding iron to common foods) and fermentation (reducing inhibitors of iron absorption)
- iron supplementation and deworming; distributing pills by means of the health system
- malaria control
- linking intervention strategies to related health and nutrition programs

3.2 Dietary Improvement

As described in chapter 2, bioavailable iron-containing foods are essentially all animal flesh products (meat or fish). With the exception of societies where a vegetarian diet predominates, most individuals would consume more meat if it were affordable. However, in most developing countries, as well as in countries with economies in a state of transition, cost is a limiting factor in obtaining meat and fish products, which are often beyond the reach of impoverished populations.

Although many nonanimal products contain high levels of iron (table 2.2), the nonheme form of this iron means that it is not bioavailable and that it is susceptible to the action of a variety of absorption inhibitors. However, dietary balance can be improved by using education aimed at 1) including items known as enhancers of iron absorption (such as ascorbic acid) and 2) avoiding inhibitors of iron absorption, such as tannates, which are present in tea (Yip, 1994).

Despite the obvious advantage and effectiveness of such measures, there is a lack of successful examples showing that nutrition education programs improved iron nutrition based on dietary changes. This absence of evidence is largely due to the fact that dietary improvement usually follows advances in the economic status of a population, which is a rare situation in many developing countries.

3.3 Iron Supplementation

The priority among target groups for iron supplementation is pregnant and postpartum women, and children age 6 to 24 months. This priority is due to the very high iron intake required during infancy and pregnancy. As discussed in chapter 2, a pregnant woman has great need for iron supplementation. She needs 4.5 mg of iron per day, or a total of 1,200 mg of iron for the entire pregnancy. A good diet can provide 1.5-2.0 mg/day, or less than half of the requirement. Thus, routine iron supplementation is justified based on this gap between intake and requirement.

In many developing countries, where anemia is highly prevalent, supplementation would also benefit all women of reproductive age, adolescents, preschool-age and school-age children.

Iron Supplementation in Pregnancy

The protocols for iron supplementation in pregnant women, children age 6 to 24 months, and other population groups are presented in tables 3.1-3.5. Routine supplementation of iron during pregnancy is a common practice in both developed and developing countries. The recommended dosage of iron supplementation for pregnant women is currently 60 mg per day. This dosage may be increased to 120 mg if the duration of supplementation is short. Also, where the prevalence of anemia in pregnant women is more than 40 percent, supplementation should continue into the postpartum period (table 3.1).

Table 3.1 Guidelines for oral iron and folic acid supplementation for pregnant women

Prevalence of anemia in pregnancy	Dose	Duration
< 40 percent	60 mg iron + 400 μg folic acid daily	6 months in pregnancy
> 40 percent	60 mg iron + 400 μg folic acid daily	6 months in pregnancy and continuing 3 months postpartum

Note: If 6 months' duration cannot be achieved in pregnancy, continue to supplement during the postpartum period for 6 months or increase the dose to 120 mg of iron in pregnancy. Where iron supplements containing 400 μg of folic acid are not available, an iron supplement with less folic acid may be used. Supplementation with less folic acid should be used only if supplements containing 400 μg are not available.
Source: Stoltzfus and Dreyfuss (1998)

In addition to iron supplementation, supplementation of 400 μg of folic acid around the time of conception not only prevents megaloblastic anemia, but also significantly reduces the incidence of neural tube defects, which are severe birth defects. In areas where parasite infestations (hookworm or malaria) are prevalent, complementary parasite-control measures should be implemented (Table 3.2).

Iron supplementation fits well within the health care delivery system and requires a well-functioning primary health care system. Success in iron supplementation programs depends on several factors, including

1) commitment of central and local governments, which have to develop effective policies and procedures for iron supplementation;
2) procurement of supplies, and distribution to the primary health care level (obviously, the latter requires that the primary health care system should function well and that health care workers be involved in iron pill distribution);
3) receipt of supplements by women who understand their indication.

Table 3.2 Complementary parasite control measures in pregnancy

Where hookworms are endemic (prevalence of 20 to 30 percent or more), give antihelminthic treatments once in the second trimester of pregnancy: If hookworms are highly endemic (prevalence of more than 50 percent), repeat antihelminthic therapy in the third trimester of pregnancy. The following antihelminthic treatments are effective and safe outside of the first trimester of pregnancy:

Albendazole 400 mg single dose
Mebendazole 500 mg single dose or 100 mg twice daily for 3 days
Levamisole 2.5 mg/kg single dose, best if second dose is repeated on next two consecutive days
Pyrantel 10 mg/kg single dose, best if dose is repeated on next two consecutive days

Where P. falciparum malaria is endemic and transmission of infection is high, women in their first or second pregnancies should be given curative antimalarial treatments at the first prenatal visit, followed by antimalarial prophylaxis according to local recommendations.

Source: Stoltzfus and Dreyfuss (1998)

Iron Supplementation During Infancy and Early Childhood

Because of high iron requirements due to fast growth, iron supplementation is essential for children age 6 to 24 months. Supplementation is especially important in developing countries where breast milk is the predominant diet during the first year of life, supplementary foods are relatively low in iron, and iron-fortified complementary foods are not widely available. The recommended protocol for iron supplementation for children 6 to 24 months of age is presented in table 3.3.

Table 3.3 Guidelines for iron supplementation for children 6 to 24 months of age

Anemia prevalence in children 6 to 24 months	Dosage	Duration
< 40 percent	12.5 mg iron + 50 μg folic acid daily	6 to 24 months of age
≥40 percent	12.5 mg iron + 50 μg folic acid daily	6 to 24 months of age

Note: If a child was born with low birth weight (< 2500 g) the duration of iron supplementation is 2 to 24 months. If the prevalence of anemia in children 6 to 24 months of age is not known, assume it is similar to the prevalence of anemia in pregnant women in the same population. Iron dosage is based on 2 mg iron/kg body weight/day.
Source: Stoltzfus and Dreyfuss (1998)

Iron Supplementation to Other Population Groups

Iron supplementation during pregnancy may not be sufficient to prevent detrimental effects of iron deficiency. For this reason, it is important to also cover other population groups, especially pre-school-age and school-age children and adolescents. The recommended protocols for iron supplementation and complementary parasite control measures for these age groups, as well as for adults, are presented in tables 3.4 and 3.5.

Table 3.4 Guidelines for iron supplementation for selected population groups

Population group	Dosage
Children 2-5 years	20-30 mg of iron
Children 6-11 years	30-60 mg of iron
Adolescents and adults	60 mg of iron

Note: For children 2 to5 years of age, iron dosage is based on 2 mg/kg of body weight/day. If the population group includes girls or women of reproductive age, 400 μg folic acid should be included with the iron supplementation to prevent of birth defects.
Source: Stoltzfus and Dreyfuss (1998)

Table 3.5 Complementary parasite control measures for selected population groups

Where hookworms are endemic (prevalence of 20 to 30 percent or more), it will be most effective to combine iron supplementation with antihelminthic treatment for adults and children above the age of 5 years. Universal antihelminthic treatment, irrespective of infection status, is recommended at least annually. High-risk groups, women and children, should be treated more intensively (2 to 3 times per year). The following single-dose treatments are recommended:

Albendazole 400 mg single dose
Mebendazole 500 mg single dose
Levamisole 2.5 mg/kg single dose
Pyrantel 10 mg/kg single dose

Antihelminthic therapy can be given to pregnant and lactating women. However, as a general rule, no drug should be given in the first trimester of pregnancy.

Where urinary schistosomiasis is endemic, provide annual treatment for urinary schistosomiasis to school-age children who report having blood in their urine. Give the following treatment:
Praziquantel 40 mg/kg, single dose

Source: Stoltzfus and Dreyfuss (1998)

Research is ongoing to determine the most cost-effective dosing regimen for iron supplementation to these age groups in different contexts. The efficacy of once- or twice-weekly supplementation in these groups appears promising, and the operational efficiency of intermittent dosing regimens is being evaluated. While policy recommendations are being formulated, program planners should adopt the dosing regimen believed to be most feasible and sustainable in their communities (Stoltzfus and Dreyfuss, 1998; UNICEF/WHO, 1999).

Limitations of Iron Supplementation Programs

Despite the obvious effectiveness and efficacy of iron supplementation, there are certain limitations. The main limitation is the lack of compliance, especially when long-term daily administration is required. General factors affecting compliance with iron supplementation are as follows (UNICEF/WHO, 1999):

- general constraints on supplementation success
- lack of knowledge and concern about anemia
- women who do not perceive themselves to be ill
- forgetfulness or lack of motivation to take a supplement frequently (daily)
- dose-related gastrointestinal side effects (nausea, diarrhea, constipation)
- unacceptable color, taste, or other characteristic of the supplement
- fear that the supplement is a contraceptive
- lack of supportive education and counseling
- lack of compliance by functionaries to their work protocol
- poor distribution and/or supply of supplements to delivery outlets

These limitations could be approached by providing adequate education to both health workers and pregnant women about the benefits and side effects of iron pills, reducing the iron dosage, altering the dosing of iron pills (provide weekly supplementation instead of daily), or using better iron compounds.

Another potential limitation of the iron supplementation program is its cost. Iron supplementation is commonly perceived to be a costly program. Current costs for a 1-year supply based on a prevention-oriented dosage of 60 mg of iron and 400 μg folic acid per week range from US \$0.12 to US \$0.52. Supplies needed for a pregnant woman using a dosage of 60 mg of iron per day and 400 μg of folic acid per day for 48 weeks range from US \$0.74 to US \$3.30 (UNICEF/WHO, 1999).

In a broad public health perspective, coverage of a large proportion of the population could become expensive. Recent experience from Bolivia has shown that policies and mechanisms can be developed so that families and individuals would have to pay for the supplements (Rolando Figueroa, 2000, personal communication). This approach may become the most feasible and self-sustainable.

3.5 IRON FORTIFICATION

Fortification of suitable food vehicles with absorbable forms of iron is a well-established practice with approximately 50 years of history. It is a desirable and highly efficient approach. If consumed by many people at risk of iron deficiency anemia, fortified food can provide broad coverage and anemia control to a large proportion of the population.

Iron fortification can be implemented at relatively low cost and it usually does not require special effort to promote or educate consumers. Supplements other than iron can be added to a fortified food vehicle.

A fortified food is usually a staple food that is consumed in significant quantities by a large proportion of the population. Fortification of wheat flour is probably the most popular. It has

Table 3.6 Examples of countries fortifying flour with iron and corresponding fortification rates

Country	Iron (mg/kg)
Canada	29-43
Chile	30
Costa Rica	28.7-36.4
Dominican Republic	29.29
Ecuador	58.65
El Salvador	28.7
Guatemala	55.65
Honduras	28.70
Nigeria	28.9-36.7
Panama	28.8
Saudi Arabia	>36.30
United Kingdom	>16.5
United States	44.1
Venezuela	20

Source: UNICEF/WHO (1999), Micronutrient initiative

been successfully implemented in several countries of the world (table 3.6).

In addition to wheat flour, other widely consumed food products could be fortified, including fish sauce, salt, sugar, or dried and liquid milk. Fortification of infant foods is especially important.

Despite the attractiveness of iron fortification, there are some limitations. One is that any activity requires multiple partners beyond the health and nutrition sector. Also, suitable vehicles may not be available, or a single vehicle may not reach all segments of the population.

Overall Approach to Anemia Control and Prevention

Since a single approach can not eliminate the problem, all approaches, including dietary modification, iron supplementation, and iron fortification, are needed. Fortification can help to improve the baseline iron status for the population. Supplementation is needed for high-risk subpopulations, such as pregnant women and infants. Dietary improvement will follow advances in economic status.

Greater attention is being paid to iron supplementation and maternal anemia. Iron fortification is not widely used, but is starting to increase in developing countries. There are some limited efforts and experience in dietary diversification and education.

Anemia control activities can benefit other health and nutrition programs. For example, home distribution of iron drops for infants can be combined with immunization activities or with distribution of vitamin A capsules.

When implementing an anemia control and prevention program, it is important to establish links with other existing efforts, such as Safe Motherhood, programs aimed at improving breastfeeding and complementary feeding practices for infants, and Integrated Management of Childhood Illnesses.

Important contributing factors that reduce the prevalence of anemia include preventing adolescent pregnancies, increasing birth intervals, and limiting the number of pregnancies and births. Exclusive breastfeeding for about 6 months followed by breastfeeding with complementary feeding into the second year of life could also benefit the iron status of women. Dealing with obstetric and gynecologic complications are also important since they may cause bleeding and consequent iron loss.

3.6 Management and Treatment of Patients with Anemia

When diagnosed as anemic, women can be referred to a local health facility for treatment. This section discusses details of management and treatment protocols for patients identified as severely anemic. Details of the referral system for patients with severe anemia, which is implemented in anemia surveys, are presented in section 7.4 of this document.

Management of Patients with Anemia

The management of a patient with anemia is governed by the anemia's etiology and severity. While it is important to perform a careful history, physical examination, and order additional laboratory tests, it is equally important to quickly initiate any indicated treatment. The therapeutic options for the treatment of various anemias has expanded dramatically during the past two decades. Blood components are readily available and extremely safe. Effective therapies are now well established for nutritional and other types of anemias (Hillman and Finch, 1996; Hillman, 2000).

When anemia is so severe that it threatens the patient's survival, immediate steps must be taken to guarantee oxygen delivery to tissues. This may involve the appropriate infusion of special solutions to

restore the proper amount of body fluids, or red-blood-cell transfusions to guarantee oxygen delivery to the tissues.

If the anemia is less severe, red-cell transfusions and vitamin or mineral therapy should be withheld until the diagnosis is certain. "Shotgun" therapy, where several vitamins and iron are administered simultaneously, is never appropriate. The selection of the right therapy should be firmly based on the documented cause or causes of the anemia. Often, more than one etiologic component must be addressed in management (Hillman, 2000).

Treatment of iron deficiency secondary to blood loss or other conditions requires an accurate diagnosis and effective control of the underlying cause of anemia. Continued blood loss can easily exceed the capacity of iron therapy to replenish iron supplies. When anemia is caused by diseases of the gastrointestinal tract, oral iron therapy may not be effective because of low iron absorption. As it will be discussed below, in some situations the patient's ability to tolerate oral iron preparations will also be a factor, since higher dosages of oral iron result in significant gastrointestinal side effects. These side effects can interfere with patient compliance and prevent a full and rapid recovery (Hillman and Finch, 1996).

Protocols for Drug Therapy of Patients with Severe Anemia

Situations of negative iron balance secondary to increased physiologic needs are readily corrected by dietary supplementation. At least 3 months of iron supplementation are required in a normal adult to rebuild iron stores.

It is recommended that the treatment of patients with severe anemia should be performed in a hospital if the patient is a pregnant woman beyond 36 weeks of gestation (i.e., in the last month of pregnancy), or if signs of respiratory distress or cardiac abnormalities (e.g., labored breathing at rest or edema) are present (Stoltzfus and Dreyfuss, 1998). Other individuals may be treated at an ambulatory setting, as indicated in table 3.7.

Standard oral preparations of iron in tablet and elixir form are listed in table 3.8. While the various preparations contain different amounts of ferrous iron, they are all readily absorbed and therefore quite effective in the treatment of iron deficiency anemia. A number of other compoundings of iron are sold on the market. Some contain "absorption-enhancing" substances, such as amino acids and ascorbic acid. Others are advertised as delayed-release formulations aimed at prolonging iron absorption over several hours. All of these preparations tend to be more expensive. Moreover, attempts to enhance absorption can increase the incidence of gastrointestinal side effects.

Table 3.7 Guidelines for oral iron and folic acid therapy to treat severe anemia

Age group	Dose	Duration
< 2 years	25 mg iron + 100 to 400 μg folic acid daily	3 months
2 to 12 years	60 mg iron + 400 μg folic acid daily	3 months
Adolescents and adults, including pregnant women	120 mg iron + 400 μg folic acid daily	3 months

Note: After completing 3 months of therapeutic supplementation, pregnant women and infants should continue a preventive supplementation regimen. Children with kwashiorkor or marasmus should be assumed to be severely anemic. However, oral iron supplementation should be delayed until the child regains appetite and starts gaining weight, usually after 14 days.
Source: Stoltzfus and Dreyfuss (1998)

To maximize the response to iron in an adult patient with moderate to severe iron deficiency anemia, a standard oral iron preparation such as ferrous sulfate should be given in tablet or elixir form. The recommended dosage is one tablet (325 mg) or 5 ml (300 mg) to be taken three to four times a day between meals. Such an amount should provide 200 to 250 mg of elemental iron per day, which is 2 to 3

mg/kg in the average-sized adult. From this dosage, the iron deficient patient will absorb 40 to 60 mg of iron. This iron level will support a red blood cell production level of up to three times higher than what is observed in normal individuals. However, as the hemoglobin level rises, iron absorption declines and the rate of red blood cell production falls, regardless of the oral iron intake. Therefore, the dosage can be reduced as the hemoglobin rises to levels above 11 to 12 g/dl. This reduced dosage will help guarantee patient compliance for a therapy that must continue for several months (Hillman, 2000).

Table 3.8 Oral iron preparations

Generic name	Tablets (Iron content) mg	Elixir
Ferrous sulfate	325 (65)	300 (60)
	195 (39)	90 (18)
Extended release	525 (105)	
Ferrous fumarate	325 (107)	
	195 (64)	100 (33)
Ferrous gluconate	325 (39)	300 (35)
Polysaccharide iron	150 (150)	100 (100)
	50 (50)	

Source: Hillman (2000)

More than 25 percent of people taking iron supplements experience gastrointestinal distress, either abdominal pain, nausea, vomiting, constipation, or diarrhea, with the full treatment dose of three to four iron tablets per day. Side effects improve with smaller doses, although some patients have difficulty tolerating even one or two tablets a day. This intolerance can be a significant barrier in management, especially in reestablishing adequate iron stores. In patients who are unable to tolerate oral iron or who suffer from gastrointestinal diseases, iron can be administered intravenously. Several types of iron formulations are available for intravenous administration (Hillman, 2000).

In areas with a high prevalence of parasite infestation, treatment of severe anemia cases should include deworming (antiparasite treatment) (table 3.9).

Table 3.9 Complementary parasite treatment for individuals with severe anemia

Where hookworms are endemic (prevalence of 20 to 30 percent or more), if the affected person is older than 2 years, give one of the following antihelminthic treatments:

Albendazole 400 mg single dose
Mebendazole 500 mg single dose or 100 mg twice daily for 3 days
Levamisole 2.5 mg/kg single dose, best if second dose is given after 7 days
Pyrantel 10 mg/kg single dose, best if dose is repeated on next 2 consecutive days

If the affected person is a woman who might be in the first trimester of pregnancy, delay antihelminthic treatment until pregnancy can be ruled out (e.g. menstruation resumes) or until the second trimester of pregnancy (e.g., until the uterus can be easily palpated).

Where urinary schistosomiasis is endemic, if the affected person is older than 5 years of age, check for visual hematuria. If present, give the following treatment:

Praziquantel 40 mg/kg, single dose

Where P. falciparum malaria is endemic, if the affected person is a child younger than 5 years, give antimalarial treatment according to local recommendations. If the affected person is a pregnant woman, give curative antimalarial treatment at the first prenatal visit, followed by antimalarial prophylaxis according to local recommendations. For other affected individuals, examine blood film for malarial infection and treat if the film is positive. If a blood film cannot be made, give presumptive treatment.

Source: Stoltzfus and Dreyfuss (1998)

Part II

Methodology, Training Guidelines, and Field Implementation of Anemia Surveys

METHODS OF ANEMIA TESTING 4

The main purpose of population-based anemia testing is to estimate a country's or region's socio-economic, residential, and demographic differentials in the prevalence of anemia. These data can provide important background information for public health policy decisions that are necessary for the development of national and community-based anemia prevention programs. Because anemia testing results have a strong impact on public health programs, it is important to ensure that the data collection is done with the proper methodology. One of the important aspects is the selection of appropriate methods for anemia testing.

4.1 MODERN METHODS OF ANEMIA TESTING

As mentioned previously, among the laboratory diagnostic methods, hemoglobin, hematocrit, and red blood cell count are the most important in establishing the presence of anemia. Among these three methods, hemoglobin measurement is recognized as a primary method for individual anemia screening in the clinical setting, for studying anemia prevalence in population, and for monitoring anemia prevalence (surveillance).

Modern Methods of Anemia Testing

The modern accurate and reliable techniques of hemoglobin measurement are based on the conversion of hemoglobin to one of its compounds, such as cyanmethemoglobin, which can be detected by measuring absorption in a spectrophotometer. Other simple field test procedures for hemoglobin testing are available. For example, use of the WHO hemoglobin color scale is simple and inexpensive; it has been recommended for use in screening for anemia in antenatal clinics in settings where resources are limited (Münster et al., 1997; Lewis et al., 1998; Van den Broek et al., 1999). Clinical pallor is a valuable indicator for severe anemia, especially in primary health care settings (Stoltzfus et al., 1999). It could be used for referral purposes in areas where malaria or parasite infestations are prevalent (Stoltzfus and Dreyfuss, 1998). A method of copper sulfate densitometry can be used for anemia screening in pregnancy (Pistorius et al., 1996).

A new test kit for red-cell protoporphyrin level assessment has recently been developed and adapted for field anemia testing (R. Stoltzfus, 2000, personal communication). Protoporphyrin is the molecule within the red blood cells to which iron is added to form hemoglobin. Higher-than-normal levels of protoporphyrin indicate an inadequate iron supply to support the formation of hemoglobin. For this reason, the level of red-cell protoporphyrin can serve as a sensitive indicator of iron deficiency anemia. The greatest advantage of this assay is that it can be performed on very small amounts of blood with no relation to the aliquots of capillary blood samples taken for analysis. This alleviates the problem of individual blood sampling variability, which is a significant problem with some other hemoglobin testing assays, including the HemoCue system, which is discussed below.

In the population-based anemia studies conducted over the past two or three decade, various methods have been used to determine anemia status. Despite the importance of several comparative studies conducted mostly under the aegis of WHO that provided world and regional estimates of anemia, the credibility of some of these comparisons is doubtful because of differences in methodology (DeMaeyer and Adiels-Tegman, 1985; WHO, 1992). Obviously, the results of recent surveys that used the standard cyanmethemoglobin photometric method cannot accurately be compared with the results derived from earlier surveys that used the old Salhi method or hematocrit estimation.

When publishing anemia testing results, one must always indicate what methodological approach was used. Generally speaking, the differences among methods of blood sampling and hemoglobin measurements should not restrict their selection, which is largely determined by the level of funding available and the scale of the anemia survey. However, for comparability purposes, it is important to make uniform decisions on the methodology to be used for anemia testing.

HemoCue System

In the MEASURE *DHS+* project, a single method is used in all surveys, which is hemoglobin measurement using the HemoCue system. This approach of selecting a single method has the advantage of increasing the comparability of anemia data collected in different DHS surveys. It also allows for comparisons of DHS anemia data with the results of other large-scale surveys, including those conducted by the U.S. Centers for Disease Control and Prevention during the past decade.

The HemoCue system is based on the conversion of hemoglobin to cyanmethemoglobin and its detection by measuring absorption in a spectrophotometer[1]. The system consists of a portable photometer and a one-step blood collection device (microcuvette) that is covered with dry hemoglobin conversion reagents. The fact that this system does not use wet reagents and that it allows for measuring hemoglobin levels within a minute makes it uniquely suited for rapid and conventional population-based surveys. The HemoCue system is a simple and relatively inexpensive technique that has been accepted as a standard method for hemoglobin measurement by the International Committee for Standardization in Hematology.

The system has proven to be efficient and accurate in clinical settings, especially when venous blood is used (McNulty et al., 1995). In capillary sampling, individual variability in test results is highly dependent on the tester's skills. In a large-scale population-based survey, individual errors in capillary sampling are less critical than in clinical settings. However, systematic errors resulting from insufficient training of field personnel in the use of the HemoCue system may cause significant biases in survey-based estimates of the prevalence of anemia. For this reason, it is very important to ensure adequate and uniform training of field personnel in the use of the HemoCue system.

4.2 Hemoglobin Cutoff Points

To diagnose anemia, i.e., to identify an abnormally low hemoglobin value, it is necessary to establish a mean normal value and the limits of normal values. Such values can be established based on observation of hemoglobin distributions in a sample of "normal" individuals. The outer limits of a "normal" hemoglobin distribution curve representing two standard deviations (2 SD) from the mean are referred to as the "normal range" (95 percent range).

A decade ago, extensive data from a large, diverse, and carefully selected sample of "normal" individuals became available from the second National Health and Nutrition Examination Survey in the United States (NHANES II, 1976-1980). When the hemoglobin values of 11,547 such subjects were used to calculate a 95 percent reference range, the cutoff points were estimated at 13.2 g/dl for men, 11.7 for nonpregnant women, and 10.7 for children 1 to 2 years of age (Dallman et al., 1984). These cutoff points are quite similar to the normal values originally recommended by the World Health Organization, which are presented in table 4.1.

[1] The principle of HemoCue testing is based on conversion of blood hemoglobin to methemoglobin by sodium nitrite, and production of azidemethemoglobin after reaction with sodium azide. The absorption of azidemethemoglobin can be measured at two wavelengths using the HemoCue photometer, which displays hemoglobin levels in grams per deciliter of blood. The HemoCue microcuvette serves both as the blood collection device and as the reaction site with the dried reagent (sodium deoxycholate, sodium nitrite, sodium azide, and non-reactive ingredients).

Table 4.1 WHO anemia cut-off points for population groups

Population group	Hemoglobin below (g/dl)	Hematocrit below (%)
Children 6 - 60 months	11.0	33
Children 5 - 11 years	11.5	34
Children 12 - 15 years	12.0	36
Nonpregnant women	12.0	36
Pregnant women	11.0	33
Men	13.0	39

Source: WHO (1968); UNICEF/WHO (1999)

Some attempts were recently made to change the WHO hemoglobin cutoff points. However, it is important to keep in mind that changes in cutoff points may have a tremendous effect on the prevalence of anemia presented. As shown previously, by setting the cutoff level for the moderate-to-severe anemia at 9.0 g/dl instead of 10.0 g/dl, the difference in the prevalence of anemia between the populations may increase from 1.8-fold to 7.7-fold (Stoltzfus, 1997). Since there has been no widely accepted alternative to the original WHO hemoglobin cutoff points, most health and nutrition surveys are still using these values (Khusun et al., 1999). Particularly, they are used in the MEASURE *DHS+* surveys to determine the severity of anemia.

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 WHO (WHO, 1968; DeMaeyer et al., 1989). Severe anemia is diagnosed when 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 hemoglobin concentration is between 10.0 g/dl and cutoff points (Table 4.1).

From the point of view of developing public health intervention programs, the study of anemia should be focused on the prevalence of severe and moderate forms (hemoglobin levels less than 10 g/dl). This focus is because the health risk and mortality associated with severe and moderate anemia is significantly greater than with mild anemia. Focusing on moderate-to-severe anemia could help better target usually scarce public health resources to prevent and control anemia in populations.

As seen in Figure 4.1 a significant proportion of women in Uzbekistan have hemoglobin levels within limits for moderate-to-severe anemia (1996 Uzbekistan DHS survey). Women in Peru have far fewer cases of moderate-to-severe anemia (1997 Peru DHS survey). Almost no women among the healthy U.S. reference population have hemoglobin levels within those limits (data from NHANES II provided by R. Yip, personal communication, 1996).

While focusing on moderate-to-severe anemia is justified for population-based studies, on the individual level in a clinical setting, the importance of mild anemia should not be ignored, since it may represent an underlying cause of many serious conditions, including hemorrhages, chronic diseases, and even tumor.

Besides statistical meaning, there is a physiologic rationale behind selecting different cutoff points for various age and sex groups. The rationale is related primarily to the differences in iron requirements for children, nonpregnant and pregnant women, and men, which were discussed in section 2.2 of the previous chapter. There are some other physiologic factors, which are important to mention here. For example, in children, high levels of phosphates in the body may lower hemoglobin values. In men, the stimulatory effect of androgens on the production of red blood cells may result in higher hemoglobin values. In pregnant women, the hemoglobin decline of about 1 g/dl may result from the effects of placental hormones (Hillman and Finch, 1996).

Figure 4.1 Percent distribution of women in Uzbekistan and Peru by hemoglobin levels, DHS data compared with a U.S. reference population

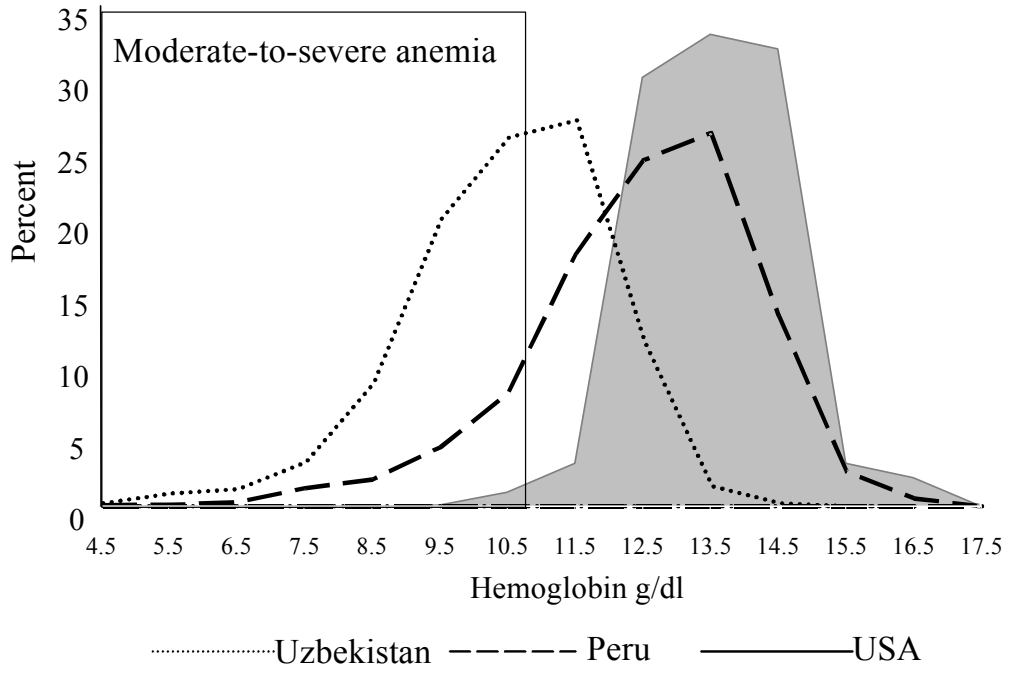
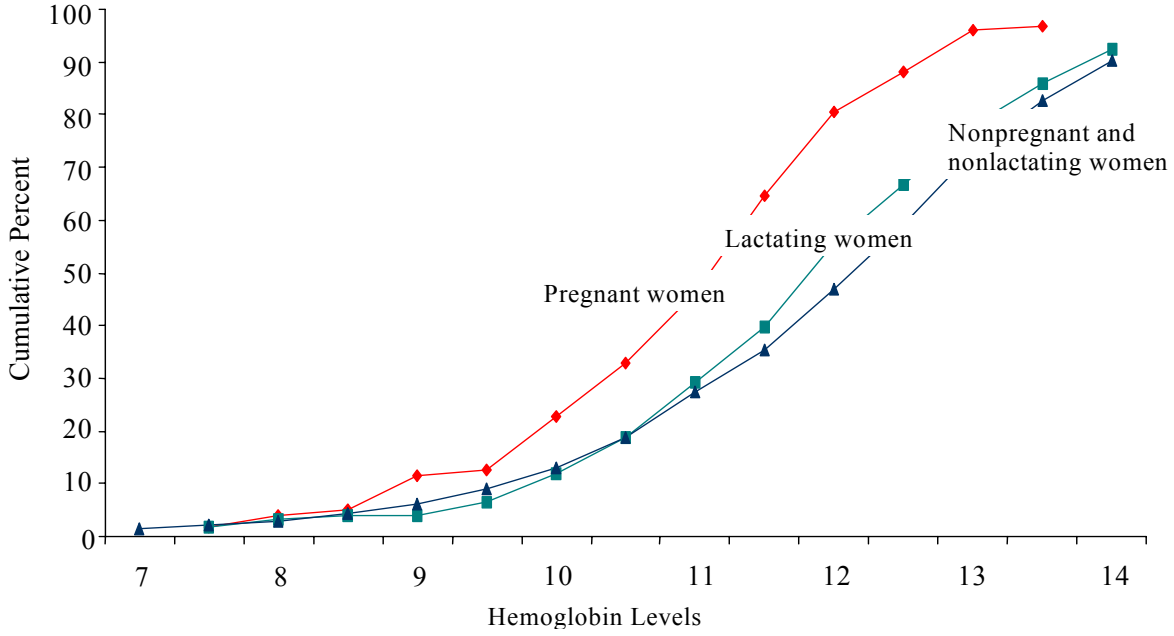


Figure 4.2 Probability plots of cumulative percent distributions for hemoglobin concentrations in pregnant, lactating, and nonpregnant/ nonlactating women, 1999 Kazakhstan DHS survey



In addition to the abovementioned factors affecting hemoglobin concentration during pregnancy, wide swings in extracellular fluid volume, especially during the second and third trimesters of pregnancy, can make the decline of hemoglobin levels even more pronounced. The lower hemoglobin values observed during pregnancy are well illustrated in figure 4.2.

As illustrated, the hemoglobin distribution curve for pregnant women shifts significantly downward (toward lower concentrations of hemoglobin) compared to nonpregnant women. Even when the lower hemoglobin cutoff point is selected for pregnant women (less than 11 g/dl compared with 12 g/dl for nonpregnant women), in many population-based studies, the percentage of pregnant women who are anemic is 1.5 to 2 times higher than nonpregnant women (Sharmanov, 1998). This fact may serve as a good checkpoint for internal consistency of anemia data collected during MEASURE *DHS*+ and other population-based studies.

In addition to age and sex, there are several environmental factors, particularly those affecting oxygen supply, which can influence the levels of hemoglobin in the blood. Among others, these factors include high altitude and smoking. They need to be considered in setting hemoglobin cutoff points for individual hemoglobin testing as well as for population-based anemia studies. Adjustments of hemoglobin values for high altitude and smoking are discussed in appendix C.

METHODOLOGICAL ISSUES OF POPULATION-BASED ANEMIA TESTING 5

There are some methodological issues that need to be considered in selecting and performing a technique for blood sampling and in interpreting and presenting the results of the anemia study. Some of these issues are discussed in this chapter. Individual variability of anemia testing results remains a serious problem in data collection, and it may dramatically affect the results of the anemia study. To minimize individual variability, health technicians must be universally and intensively trained and closely supervised (guidelines for anemia training and field supervision are presented in chapters 6 and 7).

In addition to individual variability, this chapter addresses the issues of analysis and interpretation of anemia testing results. These issues include differences between venous and capillary blood samples and options for determining iron status in population-based settings.

5.1 INDIVIDUAL VARIABILITY OF CAPILLARY HEMOGLOBIN TESTING

While the precision and accuracy of the HemoCue system is very high (concordance coefficients of 0.99 and 0.98 and covariance [CV] less than 1 percent), high within-subject variability (unreliability) of capillary blood samples (only one of the methods tested by the HemoCue system) has been reported in several studies (Marks et al., 1989; Boulton et al., 1994; Morris et al., 1999). For example, high within-subject variability was identified when capillary blood from the left hand was compared with that from the right hand (CV was 6.3 percent), and when measurements were taken on four consecutive days (CV was 7 percent). Reliability in this study was only 69 and 50 percent, respectively (Morris et al., 1999).

Obviously, such within-subject fluctuations in hemoglobin concentrations can lead to misclassification of anemia in individuals, and, if there are systematic biases, they may lead to overestimation or underestimation of anemia prevalence in populations. In order to deal with such variations it is important to understand their nature.

Physiologic Variation of Capillary Blood Sampling

The physiologic sources of variations are in the mechanisms that regulate blood distribution and in the body's reaction to stress. For example, if the blood is collected when the person is in an upright position, the hemoglobin level is about 0.7 g/dL higher than when he or she is in a supine position. This is due to a physiological reaction called the orthostatic blood distribution effect.

Undue anxiety or excessive pain associated with the blood sampling procedure can cause an increase in hemoglobin concentration by as much as 1 g/dl. Stress induces a discharge of a hormone called catecholamine. This discharge normally results in immediate constriction of the blood vessels, reduction in plasma volume, and increase in concentration of cellular components of the blood, including red blood cells and hemoglobin. Some other physiological changes in the body's fluid balance may also affect plasma volume and, thereby, hemoglobin concentration (Hillman and Finch, 1996).

Although it is difficult to control such physiological reactions, standardization of the blood collection procedure may significantly reduce individual physiological variability. For example, as illustrated on page 14 of the *Anemia Testing Manual for Population-Based Surveys* (Sharmanov, 2000), the standard procedure has the respondent in a sitting position (neither upright nor supine) during the capillary sampling. The respondent's fingers should be relaxed and warmed up prior to testing. Following these stan-

dard requirements will reduce the variability of physiological reactions. Using standard devices for painless skin puncture, such as Tenderlett[tm] or HemoCue Safety lancets, can also improve the results.

Variability Caused by Improper Capillary Sampling

More important than physiological reasons are subjective factors causing variability in the test results. Such variability usually occurs when an insufficiently trained person performs the hemoglobin testing. The simplicity of the HemoCue technique may result in a tendency to pay less attention to the training for blood sampling and hemoglobin measurement. In such situations, untrained or inadequately trained persons may perform excessive finger squeezing in order to get a sufficient amount of blood ("milking" the finger), or may fill only a portion of the HemoCue microcuvette. In studies showing significant within-subject variability, improper capillary sampling techniques may have contributed significantly to such variability.

Using replicate sampling to reduce the influence of unreliability of capillary blood sampling has previously been recommended (Morris et al., 1999). Such an approach might be feasible and well justified in an individual clinical setting. However, in large-scale surveys, replicate sampling would inevitably have cost implications, since disposable lancets and microcuvettes are the most expensive parts of the HemoCue system.

Our experience has shown that by using intensive standardized training under the close supervision of a skilled phlebotomist, it is possible to significantly improve the capillary sampling technique and reduce within-subject variability. The training protocol, quality assurance procedures, and other recommendations for anemia training and field supervision are presented in chapters 6 and 7 of this publication.

5.2 Capillary Blood Testing Versus Venous Blood Testing

Important physiological discrepancies are related to the differences in hemoglobin concentration between capillary blood and venous blood. It has been shown in several studies that a capillary sample gives an approximately 3 percent (0.4 g/dl) higher hemoglobin estimate than a venous sample (Daae et al., 1988). The physiological sources of such differences are related to blood composition and blood flow in the capillaries versus the veins.

Compared with venous blood, capillary blood contains a greater proportion of oxygenated arterial blood. Thus, capillary blood creates a higher demand for hemoglobin, which is responsible for oxygen transport. Compared with the hydrostatic pressure in veins, the total hydrostatic pressure is significantly greater in arterioles, venules, and capillaries (sources of blood obtained by finger or heel prick). When the capillary vessels of the finger or heel are cut with a lancet such as the Tenderlett[tm] device, a central and more rapid stream of concentrated particles, including the red blood cells, is likely to arise. Hence, a relatively red blood cell–enriched sample would be collected, causing hemoglobin concentration to be slightly elevated when compared with the hemoglobin concentration in the venous blood sample.

Most researchers agree that the difference in hemoglobin levels between capillary blood and venous blood is modest and has no practical importance—merely scientific importance. However, in situations requiring a comparison between the results of two surveys in which different sampling techniques were employed, a certain adjustment for either capillary sampling or venous sampling results is necessary. Uniformity of sampling techniques is especially important for anemia surveillance.

5.3 Determining Iron Status

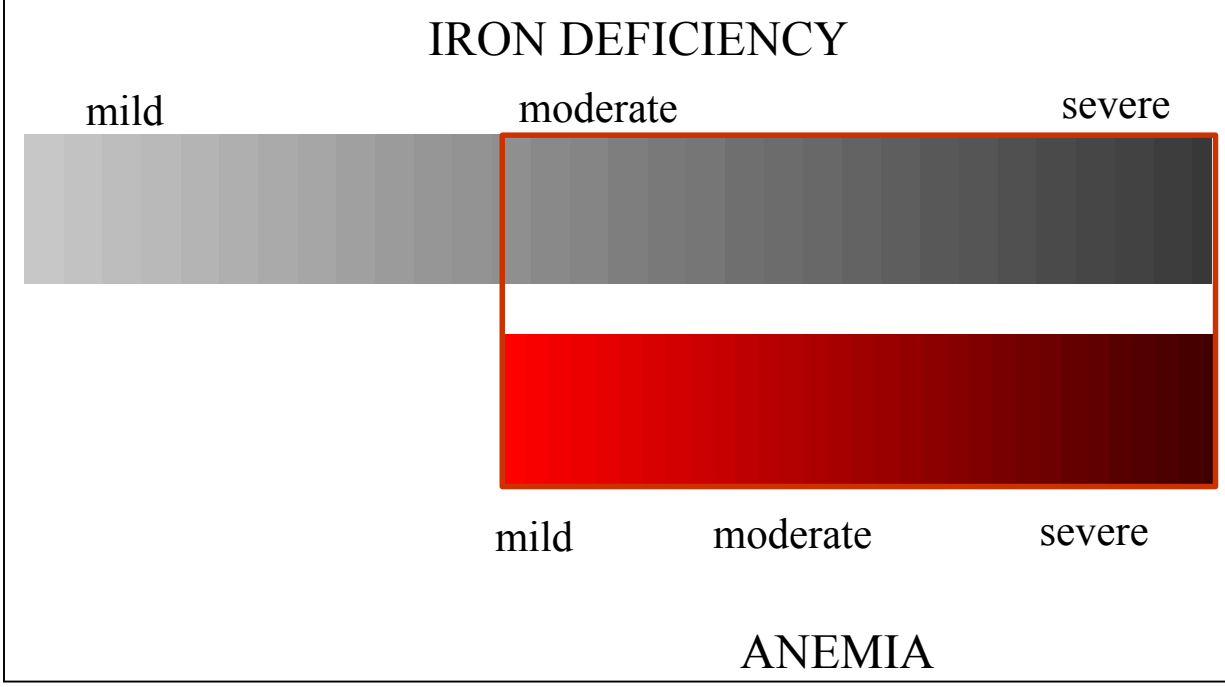
Until about a decade ago, iron deficiency was considered virtually synonymous with anemia. Technical advances in hematology, biochemistry, and molecular biology have demonstrated that iron de-

ficiency cannot be limited simply to anemia, and that the real magnitude of iron deficiency in anemic individuals is usually much greater. New methods have been developed to actually estimate iron status. As discussed in chapter 2, in many countries, especially in Africa and Southeast Asia, significant proportions of anemia are caused by parasite infestations, hemoglobinopathies, or factors other than iron deficiency. However, an important source of error is the assumption that all cases of anemia detected by hemoglobin measurement are caused by iron deficiency. Hemoglobin testing has to be envisioned as a proxy for measuring iron status in individuals.

It has been recognized that overt anemia detected by a low hemoglobin level usually represents advanced iron deficiency. Only 30 to 50 percent of biochemical iron deficiency will be detected when anemia is used as the measure (figure 5.1). Thus, in populations where most anemia is caused by iron deficiency and where a low hemoglobin level is determined in at least 40 percent of the population, virtually the entire population is iron deficient and universal iron supplementation is recommended (Yip, 1994).

For this reason, at least in a clinical setting, individual screening for anemia should also include further examination of iron status or a search for other potential causes of anemia. In population-based studies, determining the magnitude of iron deficiency is also important from the public health point of view, because it provides background information necessary to establish an appropriate protocol for anemia prevention and control.

Figure 5.1 Spectrum of iron deficiency and anemia



Clinical Approach

In clinical diagnosis, the principal measures of iron supply are serum iron, transferrin iron-binding capacity, and serum ferritin level. Other useful methods are the study of red blood cell protoporphyrin and serum transferrin receptor levels.

Serum iron and transferrin iron-binding capacity. Serum iron is a measure of the amount of iron bound to transferrin. Transferrin iron-binding capacity is a measure of the total binding capacity of transferrin. The normal level of serum iron is between 9 and 27 μmol/l (50 and 150 μg/dl); the normal level of transferrin iron-binding capacity is between 54 and 64 μmol/l (300 and 360 μg/dl). It is also routine to calculate the percent saturation of transferrin: serum iron ÷ transferrin iron-binding capacity = percent saturation. A normal individual has a percent saturation of between 30 and 50 percent. Iron deficiency states are associated with saturation levels below 20 percent, while clinically dangerous iron overload occurs if the saturation exceeds 50 to 60 percent (Hillman, 2000).

Serum ferritin level. In humans, iron is stored as a part of proteins called ferritin and hemosiderin. Iron in ferritin can be extracted for the synthesis of heme, whereas iron in hemosiderin is less reliably available for metabolic needs. Ferritin found in the circulation is in equilibrium with tissue ferritins; in other words, serum measurement correlates with the level of total-body iron stores. The normal value for a serum ferritin level varies according to the age and sex of the individual. The adult male has a serum ferritin level of 50 to 150 μg/l, while most adult women have levels of 15 and 50 μg/l. Once iron stores are depleted, the serum ferritin level declines to levels below 15 μg/l.

Detection of serum ferritin has been shown to be valid for evaluation of iron status, especially in pregnant women (Van den Broek et al., 1998). However, its validity can be limited in the presence of infection, which causes an increase in serum ferritin levels (Kuizon et al., 1996).

Red cell protoporphyrin level. Protoporphyrin is the molecule to which iron is added to form heme. The level of red cell protoporphyrin is another sensitive indicator of iron deficiency. Higher-than-normal levels indicate an inadequate iron supply to support the formation of heme. The assay can be performed on very small amounts of blood. Normal values fall below 0.53 μmol/l (30 μg/dl) of red blood cells, while iron-deficient individuals have values in excess of 1.77 μmol/l (100 μg/dl). Protoporphyrin levels also rise in children who have been exposed to lead. This increased level reflects the inhibition of heme synthetase, the enzyme required for the formation of heme (Hillman, 2000).

Serum transferrin receptor. Serum levels of transferrin receptors correlate with the level of maturation of the red blood cells and the adequacy of iron supply to the bone marrow, where red blood cells are produced. Serum levels increase progressively in response to functional iron deficiency. There are several commercial kits available on the market to test for serum transferrin receptors (Åkesson et al., 1999). Normal levels are 4 to 9 μg/l by immunoassay, and they increase rapidly in patients with iron deficiency anemia (Hillman, 2000).

The high diagnostic value of testing for transferrin receptors is related to the following factors. Unlike testing for ferritin and other conventional laboratory tests of iron status, serum transferrin receptors are unaffected by underlying acute or chronic infection. In addition, this test is not confounded by gestational effects, and therefore has high value in diagnosing iron deficiency in pregnant women (Kuizon et al., 1996; Ahluwalia, 1998). For these reasons, testing for serum transferrin receptors is important in situations when iron deficiency is simultaneously present with overt or subclinical infection, which is quite a common condition in people living in developing countries.

Population-Based Approach

Most tests for iron status assessment involve taking venous blood and using relatively sophisticated immunologic tests, such as an enzyme-linked immunosorbent assay (ELISA). Detailed morphologic analysis, genetic screening, and clinical assessment are necessary to diagnose hemoglobinopathies, such as thalassemia. Performing these tests can be quite cumbersome and often requires special technical skills. In addition, the facilities to perform such tasks are usually not available in the field, and therefore various options should be considered for population-based surveys.

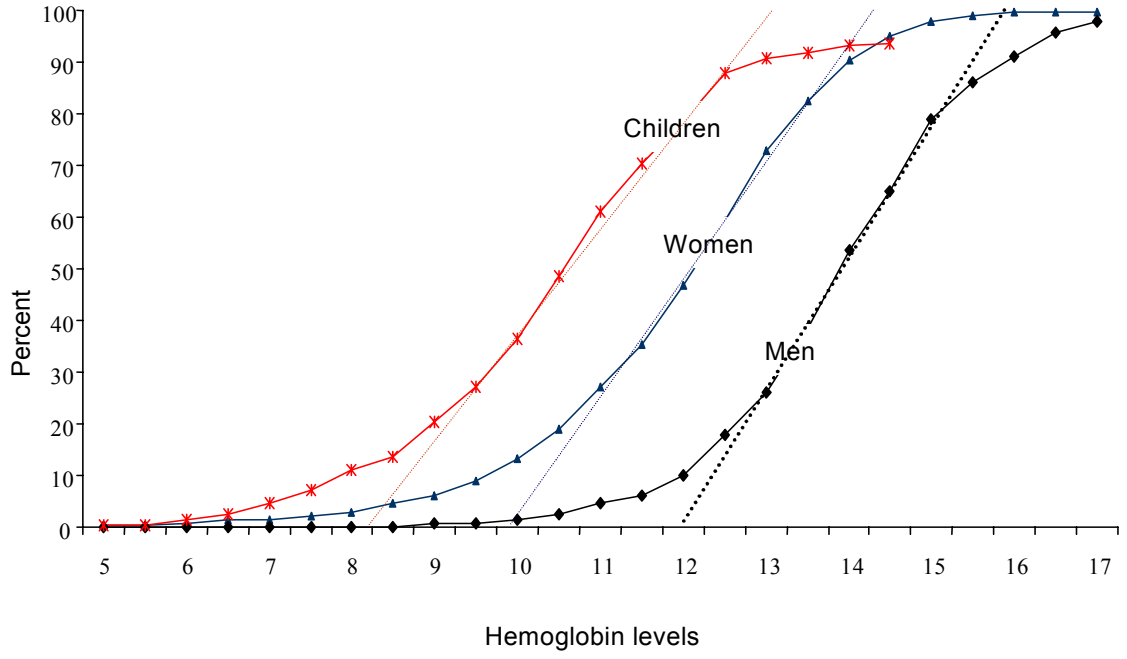
One option would be to conduct a small study to determine the iron status of a limited number of people in a sample population by testing venous blood for ferritin. Such testing was done in conjunction with the 1995 Kazakhstan Demographic and Health Survey (Sharmanov, 1998). Testing for ferritin helped to confirm the importance of iron deficiency causes of anemia in Kazakhstan.

Another approach in determining the causes of anemia among populations is to examine hemoglobin distribution. This approach helps to determine whether there are any disproportionately affected population groups, such as women and young children (Yip, 1994). In the case of a disproportionate shift in the hemoglobin distribution curve, iron deficiency may be assumed as a main etiologic factor of anemia. Where there is no disproportionate shift of the hemoglobin distribution curve, all population groups, including the adult male population, will be equally affected. In that case, parasite infestation or other factors may be considered as major contributing factors of anemia.

To test these assumptions, it is useful to select a relatively small sample of the adult male population and perform hemoglobin testing on them. The sample size should be large enough to construct a hemoglobin distribution curve comparable to those for women and children. Usually a subsample of approximately 200 adult males is sufficient to construct such a distribution curve. It is believed that this approach is feasible and cost-effective. This approach is also informative enough to assume whether or not iron deficiency is a leading cause of anemia among a certain population.

Comparative analysis of hemoglobin distribution curves was implemented during the 1999 Kazakhstan Demographic and Health Survey. In addition to women and children, the survey collected the anemia data for a subsample of men. As seen in figure 5.2, the hemoglobin distribution curves for women and children are shifted downward compared to those for men. As mentioned above, this pattern is characteristic of populations where iron deficiency is the main cause of anemia.

Figure 5.2 Probability plots of cumulative percent distributions for hemoglobin concentrations in children, nonpregnant/nonlactating women, and men, 1999 Kazakhstan



ANEMIA TRAINING GUIDELINES 6

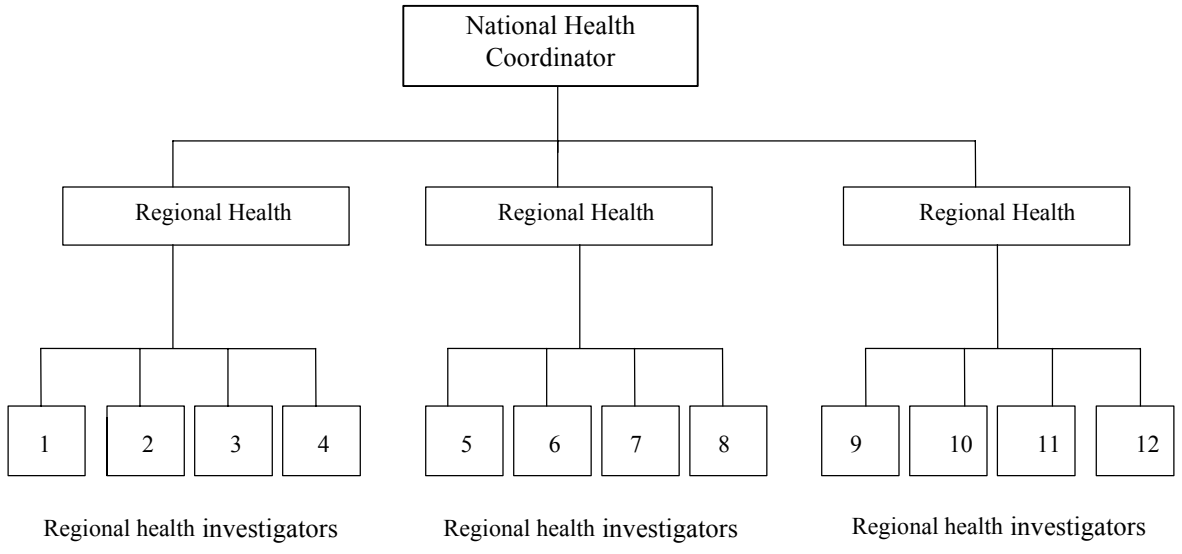
6.1 PERSONNEL REQUIREMENTS

Generally, personnel who are recruited as health investigators should have some medical background (nurses, laboratory technicians, physicians). The overall training is coordinated by an expert in the area of anemia testing. The trainer could be a person providing technical assistance for the survey or a local expert with extensive experience in HemoCue hemoglobin testing, universal blood precautions, and biohazardous-waste disposal.

During the training, health investigators (trainees) will receive formal instructions from, and be closely supervised by, a health coordinator. Health coordinators usually have a medical background and laboratory or clinical experience. One health coordinator can supervise four to five trainees during formal training and fieldwork (see chapter 7 for guidelines on field supervision), although it may be feasible for a health coordinator to train and supervise a larger number of trainees. In certain situations, survey team supervisors who have a medical background may supervise the work of health investigators. In such cases, survey team supervisors should undergo a short formal training program to ensure that they are capable of performing the tasks of health coordinators.

The maximum recommended number of trainees for each training session is 20. If more people need to be trained, organize additional training sessions. Split trainees into several groups with four or five trainees in each group. Each group must be supervised by a health coordinator (see figure 6.1 for personnel structure).

Figure 6.1 Recommended personnel structure for anemia training and field data collection



One set of hemoglobin-testing equipment and supplies is provided to each group. For example, if there are a total of 12 trainees, the class may be divided into three groups with four trainees in each group. Provide each class with at least three HemoCue photometers, as well as with enough supplies (microcuvettes, lancets, sterile gauze, alcohol preps, bandages, gloves) to perform approximately 600 hemoglobin tests. Under these guidelines, each trainee will be able to perform at least 50 tests during the training session.

6.2 TRAINING SCHEDULE

Four days of training are recommended for building the skills necessary for field anemia testing. If there is a gap of more than two weeks between training and the start of fieldwork, a refresher course may be added.

The anemia testing training should include the following:

- classroom presentations on anemia testing methods and disposal of biological wastes
- instructions for completing the informed consent form, the questionnaire, and the results form containing test results to be given to the respondent
- instructions for referring persons with severe anemia for medical treatment
- field practice in blood sampling and hemoglobin assessment

Day One

The first day should be devoted to classroom training. Classroom instructions are to include formal presentations (video and overhead) on the meaning and importance of anemia, and demonstrations of procedures for blood sampling, blood handling, HemoCue measurements, and biological-waste disposal. The importance of high-quality measurements, standardization of procedures, and appropriate data recording should be emphasized.

The trainees should also practice handling the HemoCue photometer and accessories (microcuvettes, lancets, gloves) to become familiar and comfortable with the use of the instrument and with universal precaution procedures. Under the supervision and guidance of the health coordinator, the trainees should practice blood sampling and hemoglobin measurement. Each trainee should determine the hemoglobin value of the next two trainees in the group by testing the third and fourth blood drops from each finger stick. With this procedure, each trainee will undergo two finger sticks.

Day Two

The second day of training will include visits to high schools located near the training site. Anemia testing will be performed on students of these schools under the close supervision and guidance of health coordinators. During the school visits, each trainee should be able to perform at least 20 hemoglobin measurements. At least 10 measurements should be done in duplicates: trainees perform two separate finger sticks on the same person and measure hemoglobin levels in two separate blood drops (the third and fourth from each finger stick). This procedure, referred to as testing of stick-to-stick precision, helps demonstrate how improper blood-sampling techniques can affect the results of hemoglobin measurement.

At the end of the second day of training, trainees will perform the procedure for biological-waste disposal, and will then return to the main training site for a classroom discussion of problems encountered during the school visits. During the discussion, special attention should be paid to 1) describing the potential causes of poor blood collection by means of capillary sampling and 2) following universal precautions for safe finger-stick blood collection (very important).

Day Three

The third day of training will include visits to a primary health center responsible for providing maternal and child health care services. In these centers, anemia testing is performed on women and their children under the age of five under the close supervision and guidance of the health coordinators. After training in the primary health center, trainees will visit households where they will perform anemia testing on women and their children. The health coordinators accompany the trainees to the homes to observe all aspects of measurements and to provide an immediate feedback. During the visits to the primary health center and households, each trainee should be able to perform at least 10 hemoglobin measurements on adult women and at least 10 measurements on their children, including the heel-puncture procedure. Special attention must be paid to the appropriateness of reading and obtaining consent. The heel-puncture procedure must also be closely supervised.

At the end of the third day of training, trainees will return to the main training site for a classroom discussion of problems encountered during the primary health center and household visits.

Day Four

The fourth day of training will include a performance-evaluation procedure using a quality assurance protocol. This procedure and protocol are discussed below in section 6.3. The health coordinator conducts a performance evaluation of each trainee based on the results recorded in the quality assurance form and on their personal observations during the previous days of training. On the basis of this evaluation, the health coordinator can identify trainees who need additional training and practice.

At least four days of training are recommended for building the skills necessary for field anemia testing. As mentioned above, if there is a gap of more than two weeks between training and the start of fieldwork, a refresher course may be added.

6.3 Quality Assurance

As mentioned above in section 5.3, there is significant within-subject variability in capillary blood sampling. One of the main purposes of the training is to reduce such variability by performing as many sampling procedures as possible under the close supervision of a skilled phlebotomist, i.e. a person with clinical experience in blood sampling techniques. The skilled phlebotomist in this case is usually the health coordinator who is in charge of certain survey teams during the training and fieldwork.

The quality assurance protocol is implemented based on a comparison exercise of multiple hemoglobin tests on a single subject. This could be done either by practicing on volunteers or by practicing on each other. In the latter case the trainees should pair up, with one acting as measurer and the other as a person to be tested. Each trainee then performs hemoglobin testing on the same person two or more times and records the results in the Quality Assurance form (appendix D). Afterwards, the trainees compute the values of precision (consistency among the three measurements) and discuss the results.

It is recommended that the health investigators should practice until they have collected duplicate blood samples that are within 0.5 g/dl of each other from at least two consecutive volunteers or trainees.

It is beneficial if the trainer/skilled phlebotomist participates in the quality assurance procedure. The trainee first performs the duplicate blood sampling (from two different fingers); then the skilled phlebotomist takes blood from a third finger. The results of the duplicate blood sampling done by the trainee and the blood sampling done by the skilled phlebotomist can then be compared for accuracy.

Health coordinators should encourage health investigators who need additional practice prior to fieldwork to pursue further training. The overall recommendation after the formal training is that health investigators should continue to practice anemia testing by performing it approximately 40 to 50 times prior to starting fieldwork. We recommend that the total number of anemia testing procedures performed by each health investigator during the formal training and post-training period prior to fieldwork be approximately 90 to 100.

PROCEDURES FOR IMPLEMENTING AN ANEMIA SURVEY 7

7.1 PLANNING A POPULATION-BASED ANEMIA SURVEY

During the planning stage of an anemia survey, the country's health authorities should play an important role in designing and implementing the study in close collaboration with institutions and international donor agencies providing technical assistance.

The leadership should not be limited just to providing training sites, selecting field teams, and designing the survey regions. The organization providing technical assistance, together with the country's implementing organization, needs to ensure the protection of the human subjects according to local and international laws and agreements by obtaining informed consent from study participants. The implementing organization must also make decisions on how to better organize and implement the referral system and whether to provide iron supplements to those who tested as anemic during the study. In some areas it may be necessary to obtain written consent from parents to allow their children to participate in the survey. The confidentiality of test results should also be considered.[2]

Such responsibilities will require a thorough review of the anemia testing procedure and may require formal government approval for the study. There may be a committee that must review the survey procedures and provide approval before the survey can be initiated. Institutional review board (IRB) approval is also necessary if foreign technical assistance is involved.[3] All these requirements and conditions need to be considered before the study to avoid misunderstandings or legal problems during its implementation.

An expert in the field of nutrition is usually appointed to coordinate all phases of the anemia study. This coordinator works closely with survey implementation organizations and with international organizations that provide technical assistance. The coordinator's responsibilities include ensuring that all necessary government approvals are obtained and properly accommodating requirements such as providing iron supplements for anemic respondents.

Another important task is informing national and regional government officials and communities about upcoming studies. This may be done by making announcements in the mass media and by distributing pamphlets explaining the objectives and benefits of the study. An example of such a pamphlet is given in appendix E. If they are well informed, respondents and community leaders will be better prepared for the study, thus ensuring good cooperation.

In general, national health coordinators should work closely with the implementing organization and the organization providing technical assistance to properly coordinate all phases of the anemia survey, including sample design and selection, training of health investigators, and field supervision.

[2] Standard DHS protocol requires that informed consent be obtained from participants in anemia testing and that confidentiality be ensured.

[3] All anemia studies conducted in conjunction with MEASURE *DHS*+ surveys have been approved by the Institutional Review Board of ORC Macro.

7.2 SAMPLING ISSUES

In order to generalize the results of anemia testing to the entire population, it is important to select a representative sample of target groups; for example, a sample of women of reproductive age and their children under five years of age. Many of the reported studies on anemia use samples heavily weighted with representatives of deprived socioeconomic groups. This results in a bias that increases the reported prevalence of anemia. Proper representativeness is also important because it allows for comparison with representative surveys performed in other countries, as well as in the same country over time.

Ideally, a population-based anemia study is done using a stratified cluster sample, which requires sequential selection of communities, eligible households, and eligible respondents within the households. Sample design and selection should be done by a person with professional training in sampling techniques.

However, in many situations, anemia testing is done in conjunction with a population-based survey, such as the Demographic and Health Survey, and the issue of sampling is limited to making decisions on whether to cover an entire sample or to select a subsample of population. The decision on selection of sample size is based on a number of factors. First, it depends on the country's needs for subnational estimates of anemia prevalence. Second, incorporating anemia testing in population-based surveys has implications for cost, logistics, and sample size. For this reason, the availability of resources necessary to hire technical personnel and to purchase equipment and supplies sufficient to cover the sample have to be well estimated.

Surveys that include more than 4,000 households provide enough blood samples to yield an accurate estimate of the national prevalence of anemia and some sub-national estimates. It is important to establish a minimum sample size that would provide accurate estimates of anemia prevalence. Selection of the minimum sample size required for anemia surveys is discussed in appendix F.

7.3 FIELD SUPERVISION

Ensuring proper supervision of anemia data collection throughout the survey is important. Health investigators often fail to pay enough attention to the problems that can occur in handling the HemoCue photometer or microcuvettes (see chapter 6, Common Problems of Field Hemoglobin Testing, in the *Anemia Testing Manual for Population-Based Surveys* [Sharmanov, 2000]). Such problems can cause significant variability in the results of anemia testing and may lead to underestimation or overestimation of the anemia rate. Health investigators also may not follow the requirements for universal blood precautions. For this reason, closely supervising health investigators is very important throughout the fieldwork.

If possible, all teams should start collecting data in a small area to allow for maximum supervision during the first few days of fieldwork. In some countries, the fieldwork may start simultaneously by all survey teams in a capital city, or in another place where the training site is located. In such situations, the initial phase of the fieldwork may be envisioned as an opportunity to identify those health investigators who still perform poorly and, if necessary, to schedule additional field training or even replace them. If arranging such close supervision of the fieldwork is not feasible, then emphasize close supervision by means of site visitation.

As mentioned in the previous section, during the training and throughout the fieldwork, one health coordinator is responsible for the performance of four to five health investigators and coordinates anemia testing in various survey regions. The coordinator can accomplish this by visiting the teams during the fieldwork and by observing how health investigators perform their tasks. Periodically evaluating test performance helps to ensure consistency and accuracy of hemoglobin testing procedures, compliance with universal blood precautions, and procedures for biohazardous-waste disposal.

Throughout the fieldwork, health coordinators should arrange to visit each field team at least once per month. Health coordinators and other senior survey technical staff need to develop a detailed travel plan to efficiently cover all necessary survey regions. The travel plan should include all information on team location and needed equipment and supplies for additional anemia testing. The health coordinators should make appropriate arrangements to take necessary equipment and supplies to the field.

During the team visit, the health coordinator first meets with the team supervisor to discuss potential problems with anemia data collection. Special attention should be paid to motivation and morale among the team members and health investigators in particular, and to such issues as lack of supplies, problems with response rates, or difficulties in assigning work to health investigators. If such problems occur, the health coordinator needs to work with the team supervisor in identifying possible origins of and solutions to the problems.

After contacting the team supervisor, the health coordinator should meet with the health investigator to discuss difficulties and problems with the field anemia data collection. The health coordinator should guide the health investigator on how to overcome these difficulties and solve the problems. The health coordinator should then go to the field to observe how the health investigator is collecting the anemia data. The purpose of the observation is to evaluate and improve the health investigator's performance and to look for mistakes and problems of which the health investigator may not be aware. During the observation, the health coordinator should sit close enough to see how the anemia testing is proceeding. That way, he or she can see whether the testing is done correctly and whether the health investigator is following universal blood precautions. The health coordinator should not intervene during the anemia testing if he observes minor mistakes. The coordinator should make note of problem areas and discuss them later with the health investigator.

Field Checklist

During the field observation, the health coordinator should pay special attention to the following components of anemia testing:

- Check the performance of the HemoCue photometer by means of the control microcuvette.

- Handling the photometer: Check to make sure the machine and holder for the microcuvette are clean and the batteries are functioning properly.

- Handling the HemoCue microcuvettes: Check that the date the container was opened is recorded and that the expiration date has not passed; observe how the health investigator takes microcuvettes from the container and how tightly he/she closes it.

- Wearing gloves: Check to ensure that one pair of gloves is used per person and that gloves are not reused for another person, even if that person is the child of the previous person.

- Availability of all necessary supplies for individual anemia testing: Check to make sure alcohol preps, sterile gauzes, bandages, and appropriate lancets are available and are used during the testing, but are not reused for another test.

- Appropriateness of microsampling technique: Check whether skin-puncture sites are selected correctly, whether the skin is treated with alcohol prep and dried with sterile gauze, and whether the lancet placed on the puncture site is triggered properly.

- Ensuring free flow of blood: Check whether a sufficient amount of capillary blood is available without "milking" the site or using other inappropriate procedures; make sure that the third or fourth drop of blood is used for the anemia testing.

- Filling in the microcuvette: Check to make sure the entire portion of the microcuvette covered by the reagent (including both circle and the tip) is filled in with the capillary blood and that excess blood on the outside of the microcuvette is wiped off properly.

- Measuring the hemoglobin level: Check whether the filled-in microcuvette is placed properly into the microcuvette holder and the HemoCue photometer displays the hemoglobin data.

- Check the proper placement of a sterile bandage on site of puncture.

- Recording the data in the questionnaire: Check whether the data are recorded in the appropriate boxes on the questionnaire.

Special attention should be given to disposal of used lancets, microcuvettes, gloves, gauze, and alcohol preps. During the anemia testing, all of them should be collected in a container for biohazardous-waste disposal. After the testing is done, the container must be securely stored and safely disposed of later. The health coordinator must ensure that the container is properly transported and destroyed (see chapter 7, Disposal of Biohazardous Wastes, *Anemia Testing Manual for Population-Based Surveys* [Sharmanov, 2000]).

The health coordinator should observe the anemia testing at least 10 times during each field team visit. This observation will require that the health coordinator stay at least one full day to observe the anemia testing. Additional time may be required for discussing problems and providing refresher training. If serious problems are evident, it may be necessary to replace or retrain the health investigator. Refresher training at regular intervals during the fieldwork can be valuable for maintaining the skills of health investigators.

At the end of the visit, the health investigator should meet the team supervisor again to brief him or her about the observations and conclusions. At survey headquarters, the health coordinator reports the results of the visit to senior technical staff, so they can take appropriate action in case problems occur.

7.4 Referral System

When an anemia survey identifies women and children with severe anemia, they should be referred to a local health care facility for follow-up treatment. As noted above in section 4.2, severe anemia is diagnosed when hemoglobin levels in the blood fall below 7g/dl. It is clinically defined as a condition leading to cardiac decompensation, i.e., a situation in which the heart cannot maintain adequate blood circulation. Individuals with severe anemia commonly complain of breathlessness at rest (Stoltzfus and Dreyfuss, 1998).

Cases of severe anemia usually comprise only a small proportion of overall cases (approximately 1 to 5 percent among women and children in developing countries). However, severe anemia can account for a large proportion of anemia-related morbidity and mortality. It can become a dangerous condition for women, causing severe complications during pregnancy and delivery, and can be dangerous for children when infectious diseases are present. For this reason it is important to notify a health facility of cases of severe anemia identified during the survey. This can be done by implementing a referral system.

Discussed below are the major elements of the referral system and issues that should be taken into account in setting up the system. A prototype of this system was established during the 2000 Egypt

Demographic and Health Survey. In MEASURE *DHS+* surveys, if a woman or a child is diagnosed as having severe anemia—with Hb levels less than 7 g/dl—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[4] about her condition (see appendix G for an example of text for the form requesting referral).

Designating Health Facilities to Serve as Referral Sites

The country's Ministry of Health should provide a list of health facilities to which severely anemic individuals can be referred. The facilities should be notified in advance by the Ministry of Health that there may be referrals during the survey, and they should be prepared to assist individuals referred by the survey field teams. A list of the nearest referral facilities organized according to cluster should be prepared for each survey field team.

In designing a referral system, the following questions need to be addressed:

1. How many facilities will be designated for referrals? There should be a sufficient number of referral sites so that individuals who are found to be severely anemic will not have to travel great distances to the facilities.

2. Who at the facility will be responsible for interaction with the survey staff? It would be helpful if a particular physician or other facility staff person were identified in advance as the contact for the survey staff at each facility.

3. What kind of follow-up is possible? If the survey staff provides a health facility with a list of the households in which individuals were identified as having a very low hemoglobin level, will the facility take responsibility for making contact with the household? Or will it be the household's responsibility to contact the facility for assistance?

4. What kind of anemia treatment protocols are implemented, and are there enough drug supplies available to implement such protocols? For oral iron preparations and iron supplementation options, refer to chapter 3 (Anemia Control and Prevention).

Establishing Procedures for Field Staff to Follow in Making Referrals for Individuals with Very Low Hemoglobin Levels

Procedures should be established to facilitate referrals, and all survey field staff (not only the physicians and health technicians) should be familiar with the procedures. The following represent suggested components of the referral procedures for individuals with very low hemoglobin levels:

1. *Inform woman or caretaker of low hemoglobin level and provide referral.* Whenever a woman or child is found to be severely anemic, the physician or health technician responsible for the testing should advise the individual or, in the case of a child or adolescent, the mother or other designated caretaker, of the need to consult a health facility. In each case of severe anemia, the facility referral form should be completed and given to the woman or caretaker. The facility referral form should include the following information (see appendix H, form A):

    - name and address of the person being referred
    - hemoglobin level at the time of the survey
    - name and location of the health facility to which the referral is being made

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

2. *Advise survey headquarters of the referral*. In clusters in which severely anemic individuals are identified, the physician or health technician should prepare and give to the survey team supervisor a list of all of the individuals who were referred in the cluster and the health facility to which the referral was made (see appendix H, form B). The team supervisor should send this list by fax or other means to the survey headquarters at the completion of the fieldwork in each cluster.

3. *Maintain a master list of all referrals.* The team supervisor should maintain a master list of all of the referrals of severely anemic individuals that were made by the team during the fieldwork (See appendix H, form C).

In establishing such procedures the following questions need to be addressed:

1. Should the survey field staff provide any additional assistance to individuals who are referred (e.g., arrange for transport to the facility)?

2. Who should have the responsibility for providing information to the health facility about individuals who were referred to the facility: the survey field staff or the survey headquarters? If a contact person is not designated by the Ministry of Health in advance, who should be informed at the facility? What information should be provided to the health facility about the individuals who are being referred?

3. Should there be any follow-up effort to determine whether the referred individuals received treatment? Can such checking be incorporated into the reinterviews for the survey?

Section 3.6 of this report describes the recommended management and treatment protocols for patients with severe anemia referred to a health facility. These protocols are applicable for most of the health care settings where an anemia survey and referral system are implemented. However, decisions on all issues of the management, treatment, and system of referral have to be made by the country's authorities based on the proximity of health facilities and professional staff near the survey clusters, as well as depending on the availability of appropriate drugs and supplies to treat severe anemia cases.

All these issues need to be discussed during the planning process for the survey. All procedures have to be approved by the Ministry of Health or the government agency responsible for providing health care. If iron supplements are provided by UNICEF or some other donor organization, it is important to ensure proper coordination between the parties involved, so that the procedures for procuring and distributing supplies are well established.

7.5 Analysis of the Results of the Anemia Survey and Report Writing

Data Entry and Analysis

The results of the anemia testing are entered into the survey questionnaire (see appendix H for the section of the MEASURE *DHS*+ questionnaire with the anemia data entry form). The anemia data and other data entered into the questionnaires have to be edited and corrected in the field before being sent to the survey headquarters for computer data entry and analysis.

There are several software packages that could be used for data entry, editing, tabulation, and analysis. For example, popular statistical packages such as SPSS, SAS, or STATA could be successfully used at the analysis stage. In the MEASURE *DHS*+ project, a program called ISSA (Integrated System for Survey Analysis) is used for data entry, editing, primary tabulations, and some statistical analyses. The process of data editing using the ISSA package usually includes the checking of ranges, structure,

and internal consistency. All errors detected during machine editing should be corrected before running tables.

A standard anemia tabulation plan used in the MEASURE *DHS*+ project is shown in appendix I. The tables usually include information on the prevalence of anemia among women and children by background characteristics. Additional tables can be developed to present results on country-specific issues of concern.

Report Writing

It is very important to write a report on the anemia survey and make it available to interested parties. In general, reports have the following format: introduction, methods, results, discussion, and, if desired, program implications.

The introduction usually has information on the detrimental effects of anemia on maternal and child health. Chapters 1 through 3 of this report may be useful in writing the introductory section. Information about previous surveys in the study area or nearby areas should be included.

The methods section should cover issues of sampling design and also present information on the methods used to diagnose anemia (usually the HemoCue system) or to assess iron status (for example, ferritin assessment). If a subsample of the population was used for the anemia survey, the total number of persons (women and children) sampled and tested should be included in the report. It is also important to present refusal rates and to state whether men, adolescents, or other population groups were tested.

The results section presents the results of the analyses. Appendix I shows how the anemia data collected during the survey can be tabulated and presented.

In the discussion section, the results of the survey should be discussed in the context of the results of previous surveys and any anemia control and prevention programs existing in the country. Potential policy implications of the anemia data collected during the survey should be discussed. For example, if the survey identifies areas with particularly high anemia prevalence levels, these areas could be considered for enhanced intervention programs.

In the discussion section of the report, any weaknesses or problems encountered during the survey planning or implementation should be identified, and recommendations for future activities should be provided.

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CLASSIFICATION OF ANEMIA

APPENDIX A

Table A1. Etiologic classification of macrocytic anemias (Lee, 1999)

Vitamin B12 deficiency
A. Dietary deficiency (rare)
B. Lack of Castle intrinsic factor
   1. Pernicious anemia
      a. Congenital form
      b. Adult form
   2. Gastric surgery
      a. Total gastrectomy
      b. Partial gastrectomy
      c. Gastric bypass
   3. Ingestion of caustic materials
C. Functionally abnormal intrinsic factor
D. Biologic competition
   1. Small-bowel bacterial overgrowth
      a. Small-bowel diverticulosis
      b. Anastomoses and fistulae
      c. Blind loops and pouches
      d. Strictures
      e. Scleroderma
      f. Achlorhydria
   2. Fish tapeworm disease
E. Familial selective vitamin B12 malabsorption (Imerslund syndrome)
F. Drug-induced vitamin B12 malabsorption
G. Chronic disease of the pancreas
H. Zollinger-Ellison syndrome
I. Hemodyalysis
J. Diseases especially affecting the ileum
   1. Ileum resection and bypass
   2. Regional enteritis

Folic acid deficiency
A. Dietary deficiency
B. Increased requirements
   1. Alcoholism requirements
   2. Pregnancy
   3. Infancy
   4. Diseases associated with rapid cellular proliferation
C. Congenital folate malabsorption
D. Drug-induced folate deficiency
E. Extensive intestinal resection, jejunal resection

Combined folate and vitamin B12 deficiency
A. Tropical sprue
B. Gluten-sensitive enteropathy

Inherited disorders of DNA synthesis
A. Orotic aciduria
B. Lesch-Nyhan syndrome
C. Thiamine responsive megaloblastic anemia
D. Deficiency of enzymes required for folate metabolism
   1. Methyl-tetrahydrofolate transferase
   2. Formiminotransferase
   3. Dihydrofolate reductase
E. Transcoloalamin II deficiency
F. Abnormal transcobalamin II
G. Homocystinuria and methylmalonic aciduria

Drug- and toxin-induced disorders of DNA synthesis
A. Folate antagonists (such as methotrexate)
B. Purine antagonists (such as 6-mercaptopurine)
C. Pyrimidine antagonists (such as cytosine arabinoside)
D. Alcylating agents
E. Zidovudine (AZT, Retrovir)
F. Trimetoprim
G. Oral contraceptives
H. Anticonvulsants (such as Dilantin)
I. Nitrous oxide
J. Arsenic
K. Chlordane

Erythroleukemia

Nonmegaloblastic macrocytic anemias
A. Disorders associated with accelerated erythropoiesis
   1. Hemolytic anemia
   2. Posthemorrhagic anemia
B. Disorders associated with increased membrane surface area (thin mabrocytosis)
   1. Hepatic disease
   2. Obstructive jaundice
   3. Postsplenectomy
C. Refractory anemias
   1. Myelodysplastic anemias
   2. Myelophthisic anemias
   3. Aplastic anemia
   4. Acquired sideroblastic anemia
   5. Hereditary dyserythropoietic anemia, type 1
   6. Idiopathic macrocytosis in the elderly
D. Alcoholism
E. Hypothyroidism
F. Chronic obstructive pulmonary disease
G. Benign familial macrocytosis

Table A2. Etiologic classification of microcytic and hypochromic anemias (Lee, 1999)

- Disorders of iron metabolism
  - Iron-deficiency anemia
  - Anemia of chronic disorders
  - Atransferrinernia
  - Shahidi-Nathan-Diamond syndrome
  - Familial microcytic anemia with impaired absorption and metabolism of iron
    - Antibodies to the transferrin receptor
    - Gallium administration
    - Aluminum intoxication
    - Experimentally induced copper deficiency in swine
- Disorders of globin synthesis
  - The thalassemias
  - Hemoglobin E trait and hemoglobin E disease
  - Hemoglobin C disease
  - Unstable hemoglobin diseases
- Disorders of porphyrin and heme synthesis
- Sideroblastic anemias
  - Defective aminolevulinic acid (ALA) synthesis
    - Vitamin B6 deficiency
    - Defective vitamin B6 metabolism induced by drugs or toxins
    - Defective ALA synthetase activity
  - Deficiency of coporphyrinogen oxiclase
  - Deficiency of heme synthetase (ferrochelatase)
  - Lead intoxication
  - Unknown cause

Table A3. Classification of normocytic, normochromic anemias (Lee, 1999)

- Anemia associated with appropriately increased erythrocyte production
  - Posthemorrhagic anemia
  - Hemolytic anemia
- Anemia with impaired marrow response
  - Intrinsic bone marrow disease
    - Hypoplasia
    - Hypoplastic or aplastic anemia (pancytopenia)
    - Erythroblastic hypoplasia
  - Disorders characterized by infiltration of the bone marrow
  - Leukemia
  - Myeloma
  - Other myelophthisic anemias
    - Dyserythropoietic anemias
    - Myelodysplastic anemias
    - Masked megaloblastic anemia
- Decreased erythropoietin secretion
  - Impaired source
    - Renal: Anemia of renal insufficiency
    - Hepatic: Anemia of liver disease
  - Reduced stimulus (decreased tissue oxygen need): Anemia of endocrine deficiency
  - Protein-calorie malnutrition
  - Anemia of chronic disorders
- Deficiency or unavailability of iron (early: normocytic, normochromic; later: hypochromic, microcytic)
  - Iron deficiency
  - Anemia of chronic disorders
- Anemia of space flight

HEMOCUE SYSTEM: TECHNICAL REQUIREMENTS AND COST

APPENDIX B

TECHNICAL REQUIREMENTS

Although the HemoCue system has proven to be quite durable and reliable in field conditions, there are some technical limitations in using the machine and cuvettes. In addition to having a good understanding of correct blood sampling techniques, it is important to know the requirements for handling and storing the HemoCue photometer and microcuvettes.

The HemoCue photometer can be safely operated between 15 and 40 degrees centigrade (59 to 104 degrees F). The storage temperature is 0 to 50 degrees centigrade (32 to 122 degrees F). It is important to allow the instrument to come to the ambient temperature before use and to protect it from direct sunlight.

The photometer is calibrated at the factory against the cyanomethemoglobin (HiCN) method, which is the international reference method for determining hemoglobin levels in the blood. In addition to the factory calibration, however, day-to-day calibration using a control cuvette is also required. The control cuvette is an optical interference filter, which is used to verify that the calibration is stable. During the measurement of the cuvette, the received value should not deviate from the assigned value by more than ±0.3 g/dl. The photometer comes with technical documentation on how to measure the control cuvette. It also has a troubleshooting guide and contact numbers for technical assistance.

The HemoCue microcuvettes should be stored at room temperature (15 to 30 degrees centigrade, or 59 to 86 degrees F). In unopened containers the microcuvettes are stable for two years from the date of manufacture. The expiration date is recorded on each container. Once the container is opened, it is important to keep the microcuvettes away from humidity. Generally, if the cuvette container is kept closed between uses, the cuvettes are stable for approximately two months.

COST OF ANEMIA TESTING EQUIPMENT AND SUPPLIES

The total cost of equipment and supplies depends on how many people will be tested. If a minimum sample size of approximately 2,000 women is selected (see chapter 6), the total cost of equipment and supplies will be based on testing approximately 4,800 subjects. This number includes testing 2,000 women and 2,000 children under the age of five, 600 tests during training, and 200 waste. Table B1 provides a breakdown of approximate costs for individual equipment and supplies from selected manufacturers.

The cost of the anemia testing equipment and supplies may vary depending on the manufacturer's and distributor's prices. The total cost of anemia testing should also include the cost of field implementation and technical assistance.

Table B1. Approximate cost of anemia testing equipment and supplies

Item	Manufacturer	Price/Unit (approximate)
Hemoglobin photometer	HemoCue AB Box 1204 S-262 23 Angelholm, Sweden Tel 46-431-458200 Fax 46-431-483035	$311.15 ea
Carrying case		$17.10 ea
Hemoglobin cleaning device	HemoCue AB, etc.	$6.10/5 pack
AA batteries for photometer	Corporate Express or other distributors	$14.48/12 pack
Microcuvettes	HemoCue AB, etc.	$62.25/box (200)
Safety lancets	HemoCue AB, etc.	$19.60/box (200)
Sterile sponge gauzes	Medical Connection 9689 Gerwig Lane, Columbia, MD 21046 Tel 301-621-3445	$3.05 box (100)
Adhesive bandage, sheer (small)	Medical Connection, etc.	$2.63/box (100)
Exam gloves, latex-free (medium)	Medical Connection, etc.	$11.11/box (100)
Alcohol preps	Medical Connection, etc.	$2.37/box (200)
Biohazard-waste bags (large)	Medical Connection, etc.	$48.85/case (500) 7-10 gallons

ADJUSTMENTS NECESSARY FOR POPULATION-BASED ANEMIA TESTING

APPENDIX C

There are several environmental factors, such as high altitude and smoking, which may affect the hemoglobin concentration in the blood. They need to be considered during the individual hemoglobin testing for the analysis of the population anemia studies.

ADJUSTMENT FOR HIGH ALTITUDE

Hemoglobin concentration in the blood is negatively regulated by the level of oxygen saturation of arterial blood. The oxygen partial pressure declines as altitude increases. It is accompanied by a decline in the oxygen-saturation level of arterial blood, a condition called hypoxia. One of the manifestations of hypoxia is the increased concentration of hemoglobin in the blood. Hypoxia also stimulates the release of a hormone from the kidney called erythropoietin, which increases the production of red blood cells and concentration of hemoglobin in the blood. Hypoxia also causes losses of sodium and water, which leads to decreased blood volume and further concentration of red blood cells.

For these reasons, high altitude is an important factor that can affect the level of hemoglobin in the blood and, therefore, should be considered in the calculation of anemia rates. Based on some of the relationships mentioned above, Hurtado et al. developed high-altitude hemoglobin-level adjustments, according to which the rates of anemia can be calculated using the following equations:

$$\textit{Adjusted level of altitude} = \textit{observed level} - \textit{adjustment coefficient}$$

Adjustment coefficient is calculated as follows:

$$\textit{Adjustment coefficient} = -0.032 \times (\textit{altitude}) + 0.022 \times (\textit{altitude}^2)$$

Altitude is measured as follows:

$$[\textit{altitude in meters}/1{,}000] \times 3.3$$

This approach has been used in the CDC Pediatric Nutrition Surveillance System and in some of the Demographic and Health Surveys (Kyrgyz Republic, Peru, and India). It is generally suggested that the cutoff values for different levels of anemia should be increased according to the values below (*Morbidity and Mortality Weekly Report*, vol. 47, no. RR-3, p. 13):

Altitude (feet)	**Hb concentration (<g/dl)**	**Hematocrit (%)**
Less than 3,000 feet	No adjustment	No Adjustment
3,000-3,999	+0.2	+0.5
4,000-4,999	+0.3	+1.0
5,000-5,999	+0.5	+1.5
6,000-6,999	+0.7	+2.0
7,000-7,999	+1.0	+3.0
8,000-8,999	+1.3	+4.0
9,000-9,999	+1.6	+5.0
10,000-11,000	+2.0	+6.0

The above adjustments are meant to increase the cutoff levels for each level of anemia. For example, for someone who lives at 3,500 feet, the cutoff level for severe anemia should be increased from 7.0g/dl to 7.2 g/dl.

ADJUSTMENT FOR SMOKING

As with long-term residency at high altitudes, cigarette smoking can cause a generalized upward shift in Hb concentration and hematocrit. An adjustment in the cutoff values for determining anemia levels allows the positive predictive value of anemia screening to be comparable between smokers and nonsmokers. The adjustment values are presented below.

Number of cigarette packs[1]	**Hb concentration (<g/dl)**	**Hematocrit (%)**
0.5 to less than 1 pack per day	+0.3	+1.0
1.0 to less than 2 packs per day	+0.5	+1.5
2 or more packs per day	+0.7	+2.0
All smokers	+0.3	+1.0

For example, if a person lives at an altitude of 3,500 feet and smokes 25 cigarettes per day, then the cutoff level for severe anemia would be raised to 7.7 g/dl for that person.

In a situation where someone smokes, but there is no information about how much that person smokes, it should be assumed that the person smokes from 0.5 packs per day to less than one pack per day.

[1] One pack = 20 cigarettes

QUALITY ASSURANCE FORM

APPENDIX D

QUALITY ASSURANCE FORM

Group #_____ Field Organization(s)_____

Health Coordinator_____ Resource Person_____

	CATEGORIES	NAME OF SUBJECT 1 _____		NAME OF SUBJECT 2 _____		NAME OF SUBJECT 3 _____		COMMENTS: NEEDS IMPROVEMENT
		Test 1	Test 2	Test 1	Test 2	Test 1	Test 2	
Name of Health Investigator #1 _____	Weight							
	Height							
	Hb							
Name of Health Investigator #2 _____	Weight							
	Height							
	Hb							
Name of Health Investigator #3 _____	Weight							
	Height							
	Hb							

Additional comments:

_____

_____

_____

ANEMIA PAMPHLET

APPENDIX E

Why is anemia DANGEROUS?

Anemia is dangerous because

- It reduces one's resistance to infections
- Severe anemia can lead to heart failure
- During childbirth, anemic women are more likely to die from excessive bleeding and have fewer live births
- Anemic children and young children have low birth weight, poor learning capacity, and less resistance to infections

How can anemia be PREVENTED?

- Pregnant mothers and infants should take oral iron supplements
- Eat a diet adequate in iron-rich foods such as dark green vegetables, liver, meat, fish, and fruits rich in vitamin C
- Avoid giving tea to infants and young children
- Avoid drinking tea with meals
- Prevent and treat worms

HEALTHY HOME PRACTICES

Improving mother & child nutrition

- Breastfeed infants exclusively for at least four, and if possible, up to six months.
- At about six months, add solid foods while continuing to breastfeed for up to two years or longer.
- Ensure that women and children receive enough vitamin A foods (carrots, green leafy vegetables, pumpkins, red or yellow squash, yams or red sweet potatoes), iron and other micronutrients through their foods or supplements.

Avoiding childhood illnesses

- Dispose of feces safely; always wash hands after defecation, before preparing meals, and before feeding children.
- Give children a full course of immunizations (BCG, DPT, OPV and measles) before their first birthday.

Treating illnesses

- When children are sick, continue to feed them, offering more breast milk and fluids.
- When children are sick treat them appropriately for infections.
- Seek medical care for sick children and women when needed.
- Follow the health worker's advice about treatment, follow-up and referral of sick children and women.

[ORGANIZATION]
[COUNTRY]

Name ..... Date.....

Dear Respondent,

[ORGANIZATION] [COUNTRY] is carrying out an anemia study. The study will help us to find out if there is a problem with anemia and other illnesses among mothers and young children in [COUNTRY].

We appreciate your allowing us to interview you and to test you and your child for anemia.

Thank you for your cooperation.

Please look inside for the results of the anemia testing

TEST RESULTS Date.....

Mother	*Youngest child*	*Next child*
Name.....	*Name.....*	*Name.....*
Hemoglobin level		
____ g/dl	____ g/dl	___ g/dl
Diagnosis (circle one)		
Normal (more than 11 g/dl)	Normal (more than 11 g/dl)	Normal (more than 11 g/dl)
Mild anemia (10-11 g/dl)	Mild anemia (10-11 g/dl)	Mild anemia (10-11 g/dl)
Moderate anemia (7-10 g/dl)	Moderate anemia (7-10 g/dl)	Moderate anemia (7-10 g/dl)
Severe anemia (less than 7g/dl)	Severe anemia (less than 7g/dl)	Severe anemia (less than 7g/dl)

What does the DIAGNOSIS mean?

Severe anemia: You have a seriously low level of hemoglobin in your blood. You need to visit your doctor immediately for treatment.

Moderate anemia: Your anemia needs treatment. You should visit your doctor as soon as possible.

Mild anemia: Take iron supplements daily. Treat malaria and worms. Eat more foods that enhance iron in the body, such as dark green vegetables, liver, meat, fish, and fruits rich in vitamin C (for example, oranges and lemons) which increase absorption of iron in the body.

Normal: Prevent anemia by eating foods that increase iron in the body, such as dark green vegetables, liver, meat, fish, and fruits rich in vitamin C (for example, oranges and lemons) which increase absorption of iron in the body.

WHAT IS anemia?

Anemia is a serious health condition in which there are insufficient red blood cells or hemoglobin in the blood.

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

What are the SYMPTOMS of anemia?

The following are some of the symptoms of anemia:

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

What CAUSES anemia?

Anemia is caused by:

- Loss of blood because of
  - parasites, especially hookworms
  - excessive menstrual losses
  - chronic diseases such as ulcers or tuberculosis
- Deficiency of iron in the diet
- Inadequate iron absorption from the gut

MINIMUM SAMPLE SIZE RECOMMENDED FOR POPULATION-BASED ANEMIA STUDIES

APPENDIX F

Sample sizes required for anemia testing can be determined based on 1) the prevalence level, 2) the desired level of precision, and 3) the design effect. The desired levels of precision and prevalence are used to compute the sample size needed, assuming simple sampling. The design effects of previously conducted surveys are used to adjust for the complexity of the actual sample design.

ESTIMATES OF ANEMIA PREVALENCE

In anemia studies conducted by the Demographic and Health Surveys (DHS) program, the rates of anemia among women (including mild, moderate, and severe forms) vary between 40 and 60 percent. According to the WHO, the average world estimate of anemia among adult women is 50 percent. Therefore, in determining the sample size of a study when no value of anemia prevalence is available, it is recommended to use the lower end of the range (40 percent).

DESIRED PRECISION

In a setting where most anemia is due to iron deficiency, and the prevalence of anemia reaches 40 percent, given the fact that only 30 to 50 percent of biochemical iron deficiency will be detected when anemia is used as the measure, virtually the entire population is iron deficient. In these populations, universal supplementation of iron would be the best choice for an anemia control program. Usually, for monitoring of health-intervention programs, such as iron fortification and supplementation, estimation of anemia prevalence requires 10 percent precision.

DESIGN EFFECT

Design effect (DEFT) is defined as the ratio between the standard error using a given sample design and the standard error that would result if a simple random sample had been used. Results from previous DHS surveys show that the average design effect is 1.408 for urban areas, 1.273 for rural areas, and 1.315 for the national level.

Sample sizes required for anemia testing can be determined in two steps:

1. Sample size assuming simple random sampling

Let p be the proportion of women with moderate anemia, and let c be the desired relative error. Under simple random sampling, the sample size is

$$n_{srs} = \frac{1 - p}{p \times c^2}$$

2. Actual sample size

Let *deft* be the design effect known from previous similar surveys. The actual sample size is estimated as follows:

$$n = n_{srs} \times deft$$

The minimum sample size required to estimate the prevalence level of anemia with a coefficient of variation not to exceed 10 percent ranges between 1,000 and 1,500 women for each domain of the study. For example, if an estimate is needed only at the national level, then the minimum size is 1,000. For estimates for urban, rural, and national levels, 2,000 women would be required: 1,000 in the urban areas and 1,000 in the rural areas. A larger sample is required for a precision of less than 10 percent.

SECTION OF MEASURE *DHS+* QUESTIONNAIRE WITH INFORMED CONSENT AND REFERRAL FORMS

APPENDIX G

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 1995 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	.		

* CONSENT STATEMENT

As part of this survey, we are studying anemia among women and children. Anemia is a serious health problem that 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 1995[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 2001, 2002 or 2003, the year should be 1996, 1997 or 1998, respectively.

50	CHECK 5, 47 AND 48: NUMBER OF USUAL RESIDENTS WITH HEMOGLOBIN LEVEL BELOW 7 G/DL 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	

FORMS USED IN ANEMIA REFERRAL SYSTEM APPENDIX H

Form A

Ministry of Health
Referral Form for Low Hemoglobin Level

Name of woman/child: _____

Hemoglobin level: _____ g/dl

The above individual was tested during the fieldwork for the [name of the survey] and found to have a low level of hemoglobin. A low level of hemoglobin is associated with severe anemia, a serious health condition. Therefore, it is very important that a medical evaluation be obtained for this individual as soon as possible.

The Ministry of Health has arranged for a physician at _____ [name/address of Ministry of Health facility] to be available to further evaluate this condition, or you may consult your own physician.

If there are further questions with regard to this referral, you may contact:

Address:
Telephone:

Name of Health Technician _____

Signature of Health Technician _____

Date of Referral _____

Form B

Cluster Report
Women/Children with Low Hemoglobin Level

Team Number: _____

Supervisor Number: _____

Health Technician: _____

Cluster Number: _____

Governorate: _____

Locality: _____

Ministry of Health facility where women/children were referred: _____

Household Number	Name of Household Head	Address for Household	Name of Woman/Child with Low Hemoglobin Level	Accepted Referral
				1 Yes 2 No
				1 Yes 2 No
				1 Yes 2 No
				1 Yes 2 No
				1 Yes 2 No
				1 Yes 2 No

Form C

Master List
Women/Children with Low Hemoglobin Level

Team Number:

Supervisor Number:

Cluster Number	Household Number	Name of Household Head	Address for Household	Woman/Child with Low Hemoglobin Level	Line Number	Accepted Referral
						1 Yes 2 No
						1 Yes 2 No
						1 Yes 2 No
						1 Yes 2 No
						1 Yes 2 No
						1 Yes 2 No
						1 Yes 2 No
						1 Yes 2 No
						1 Yes 2 No
						1 Yes 2 No
						1 Yes 2 No
						1 Yes 2 No
						1 Yes 2 No
						1 Yes 2 No
						1 Yes 2 No
						1 Yes 2 No
						1 Yes 2 No
						1 Yes 2 No

ANEMIA DATA TABULATION PLAN

APPENDIX I

This tabulation plan shows how the anemia data collected during population-based surveys can be presented by level of severity and background characteristics.

Table I.1 Percentage of women classified as having anemia, by background characteristics

Background characteristic	Percentage of women with:			Number of women measured	
	Severe anemia[a]	Moderate anemia[b]	Mild anemia[c]	Weighted	Unweighted
Age					
15-19					
20-24					
25-29					
30-34					
35-39					
40-44					
45-49					
Residence					
Urban					
Rural					
Region					
Region 1					
Region 2					
Region 3					
Education					
No education					
Primary					
Secondary					
Higher					
Ethnic group					
Group 1					
Group 2					
Pregnant/Breastfeeding					
Pregnant					
Breastfeeding					
Neither					
Total					

[a] Hemoglobin level less than 7.0 g/dl
[b] Hemoglobin level between 7.0 and 9.9 g/dl
[c] Hemoglobin level between 10.0 and 11.9 g/dl (10.0 and 10.9 g/dl for pregnant women)

Table I.2 Percentage of children age 6 to 59 months classified as having anemia, by background characteristics

Background characteristic	Percentage of children with:			Number of children measured	
	Severe anemia [a]	Moderate anemia [b]	Mild anemia [c]	Weighted	Un-weighted
Age					
6-23 months					
24+ months					
Sex					
Male					
Female					
Birth order					
1					
2-3					
4-5					
6+					
Birth interval					
Under 24 months					
24-35 months					
36-47 months					
48+ months					
Residence					
Urban					
Rural					
Region					
Region 1					
Region 2					
Region 3					
Education of mother					
No education					
Primary					
Secondary					
Higher					
Ethnic group					
Group 1					
Group 2					
Total					

[a] Hemoglobin level less than 7.0 g/dl
[b] Hemoglobin level (7.0 to 9.9 g/dl)
[c] Hemoglobin level (10.0 to 10.9 g/dl)

Table I.3 Percent distribution of children age 6 to 59 months by mother's anemia status at the time of the survey, according to child's anemia status

Mother's anemia status	Percentage of children with: Severe anemia [a]	Moderate anemia [b]	Mild anemia [c]	No anemia	Number of children measured
Severe anemia[a]					
Moderate anemia[b]					
Mild anemia[c]					
Not anemic					
Total	100.0	100.0	100.0	100.0	

[a] Hemoglobin level less than 7.0 g/dl
[b] Hemoglobin level between 7 and 9.9 g/dl
[c] Hemoglobin level between 10 and 11.9 g/dl (10.0 and 10.9 g/dl for pregnant women)

Nationally and regionally representative anemia levels among women and children, which are determined in conjunction with population-based surveys, could be tabulated to show the overall prevalence of anemia, as well as socioeconomic, residential, demographic, and ethnic differentials.

When presenting anemia data for women, it is important to take into consideration the role of pregnancy and breastfeeding status. A negative iron balance due to an imbalance of iron requirements versus iron intake often occurs during pregnancy and child growth. For this reason, when iron deficiency is highly prevalent in a population, pregnant women, who provide the fetus with a considerable amount of iron, are at greater risk of developing anemia than women who are not pregnant. In many regions, the prevalence of anemia among pregnant women is two to three times greater than among nonpregnant/nonlactating women. Our data have also shown that the percent distribution of nonpregnant and nonlactating women by the level of hemoglobin shifts toward the lower concentration of hemoglobin in the blood when compared to the corresponding reference sample population of healthy U.S. women (Sharmanov, 1998).

There are some demographic predisposing factors that increase the likelihood of anemia in children. These factors need to be reflected in the tables. They include the age group 6 to 23 months, high order births, and birth intervals of 24 to 47 months. Certain relationships are also observed between the prevalence of anemia among mothers and its prevalence in their children. A child with an anemic mother runs an increased risk of having moderate or severe anemia.