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**ADDIS ABABA UNIVERSITY  
SCHOOL OF GRADUATE STUDIES  
COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCES  
CENTER FOR FOOD SCIENCE AND NUTRITION**

**Status of Anemia, Zinc and Copper among Adolescent Girls in Lode-Hitosa  
wereda, Oromiya Region, Ethiopia**

**By  
Tigist Tullu**

**Supervisor: Kaleab Baye (PhD)**

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## DECLARATION

I, the undersigned, declare that this thesis is my original work and that all sources of materials used for the thesis have been acknowledged.

Signature: \_\_\_\_\_ Date: \_\_\_\_\_

The thesis has been approved for submission by:

| Name of supervisor         | signature | Date  |
|----------------------------|-----------|-------|
| KaleabBaye (PhD) (Advisor) | _____     | _____ |

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## **Abbreviations**

|        |                                                 |
|--------|-------------------------------------------------|
| AAS    | Atomic Absorption Spectroscopy                  |
| AGP    | Alphas-1-Acid Glycoprotein                      |
| ASF    | Animal source foods                             |
| BMI    | Body mass index                                 |
| CRP    | C - reactive protein                            |
| Cu     | Copper                                          |
| DDS    | Dietary Diversity Score                         |
| FAO    | Food and Agriculture Organization               |
| FNB    | Food and Nutrition Board                        |
| Hb     | Hemoglobin                                      |
| ID     | Iron Deficiency                                 |
| IDA    | Iodine Deficiency Anemia                        |
| IZiNCG | International Zinc Nutrition consultative Group |
| MD     | Micronutrient deficiency                        |
| MDD    | Minimum Dietary Diversity                       |
| NFCS   | National Food Consumption Survey                |
| NNP    | National Nutrition Programme                    |
| RDA    | Recommended Dietary Allowances                  |
| SPSS   | Statistical package for Social Science          |
| SF     | Serum Ferritin                                  |
| TE     | Trace elements                                  |
| VA     | Vitamin A                                       |
| WHO    | World Health Organization                       |
| WDDS   | Women's Dietary Diversity Score                 |
| Zn     | Zinc                                            |

## Abstract

**Background:** Adolescent growth and development is closely linked to the diet they receive during childhood. The physical and physiological changes that occur in adolescents place a great demand on their nutritional requirements and make them more vulnerable to micronutrient deficiency. Copper and zinc are essential trace elements and nutrients, which have important roles in physiology and disease and as cofactors in many metabolic pathways. Anemia is a widespread condition of known public importance.

**Objectives:** The aim of this study is to investigate the status of zinc, copper and anemia among adolescent girls aged 15-20 years old living in Arsi zone, Lode-Hitosa wereda, Ethiopia.

**Methodology:** A cross sectional study involving 257 participants was carried out in Lode-Hitosa wereda (Huruta), Oromiya region. An interviewer-administered questionnaire was used to gather socio-demographic characteristics and dietary pattern. Epiinfo was used to enter data from questionnaires. A total of (4ml) blood samples were collected from each study participants. Blood samples of participants were analyzed for hemoglobin (Hb), (ferritin, C-reactive Protein (CRP), and Acid Glycoprotein (AGP)); and serum zinc and copper. Serum Zinc and Copper status were assessed using atomic absorption spectrophotometric method. Data were analyzed using SPSS Version 20 software for Windows.

**Result:** The total prevalence of anemia among the adolescent girls was 8.7% (milder form of anemia accounts for nearly all the cases). Iron status was also determined using serum ferritin (SF) values after adjusting for the presence of infection/inflammation based on CRP and AGP values. The prevalence of clinical iron deficiency after adjusting was very low (8.7 %, SF < 12 µg/L). However, a significant proportion had a marginal iron store (41 %, SF < 50 µg/L). The prevalence of infection in the area was about 17 %, of which 0.8%, 1.5 % and 14.8 % were in incubation, early and late convalescence phase, respectively. The prevalence of low serum zinc (<65µg/dl) was 49.2% and lower serum copper (<90µg/dl) was about 21%. An important proportion of adolescent girls (35 %) were underweight Body Mass Index (BMI) < 18.5. The mean number of food groups consumed was about 3. The consumption of nuts and seeds, vitamin A rich fruits and vegetables, dark green leafy vegetables, flesh foods, and dairy products were very low.

**Conclusion:** The prevalence of low serum zinc concentration was high; Deficiency of Cu has been considered a rarity. Low zinc content of the diet and consumption of dietary and other substances that lower zinc bioavailability are important factors in the pathogenesis of primary zinc deficiency. However, in the context of Huruta, inclusion of foods rich in bioavailable zinc will be difficult to achieve since zinc rich foods are usually animal origin and high cost. In this study area, the consumption pattern are based on cereals and plants which have high content of phytate, so, processing techniques such as soaking, germination or fermentation at household based should be encouraged. Knowledge and practice on importance of dietary diversity is poor and need to be improved by educational classes.

**Keywords:** Anemia, Adolescent girls, Zinc, Copper, Ferritin, Ethiopia

# 1. INTRODUCTION

## 1.1. Background

Adolescence has been defined by WHO as the period of life spanning the age's between 10-19 (WHO, 2014). It is the foundational period next to infancy, where rapid physical growth, psychological and behavioral changes take place, which in turn creates an increased demand to various nutrients. During this particular life-cycle, unmet nutrients requirement predispose to a number of problems.

Limited resources in developing countries superimposed by adverse cultural treatments result in unfair intra-household distribution that, partly manifested by the provision of sub-optimal care to the adolescent girls. The added burden of menstrual blood loss (normal/abnormal) precipitates the crisis too often (Chaudhary *et.al*, 2008). Inappropriate care and dietary constraint during adolescence can substantially delay sexual maturation and growth; likewise, the excessive consumption of some foods can increase the risk for chronic diseases in adolescence. Zinc, copper and iron are essential trace elements involved in adolescent growth (Urbano *et al.*, 2002; Perrone *et al.*, 1998).

There is now increasing evidence that adequate trace element (TE) intake may be important in promoting optimum health for growth (Fraga, 2005) including regulation of gene expression, growth, immunity; endocrine function, cell protection and brain development. For instance, zinc is involved in several steps of the immune response, in the RNA and DNA synthesis (Stefanidou *et.al.*, 2006). Many physiological functions for Cu depend on its action in a number of metallo-enzymes containing this element (Choi *et al.*, 2005; Perrone *et al.*, 1998).

The human body has an elaborate system for managing and regulating the amount of key trace metals circulating in blood and stored in cells. Nutrient metals from our diet are integrated into blood if blood levels are depleted, transported into cells if cellular levels are inadequate, or excreted if blood and cell levels are sufficient or overloaded. When this system fails to function properly, abnormal levels and ratios

of trace metals can be observed. The ratio of these micronutrients is clinically more important than the concentration of either of these trace metals (Cerovic<sup>1</sup> *et al.*, 2008; Osredkar *et al.*, 2011).

Adolescent growth and development is closely linked to the diet they receive during childhood and adolescence. Adequate nutrition of any individual is determined by two factors (Caroline *et al.*, 2014). The first is the adequate availability of food in terms of quantity as well as quality, which depends on socioeconomic status, food practices, cultural traditions, and allocation of the food. The second factor is the ability to digest, absorb, and utilize the food. This ability can be hindered by infection and by metabolic disorders.

Micronutrients of known public health importance include the following: zinc, iodine, iron, selenium, copper, vitamins A, E, C, D, B2, B6, B12 and folate (Herrador *et al.*, 2014). More than 2 billion people in the world today are estimated to be deficient in key vitamins and minerals. Particularly in Africa, MD affects millions of people, especially the most vulnerable groups, which are children and adolescents. Despite the implementation of many micronutrients supplementation programs, only a few countries have undertaken comprehensive surveys on various MD in the continent.

In Ethiopia, deficiencies in key vitamins and minerals are placed among the major public health problems. In September 2008, a National Nutrition Strategy, and a National Nutrition Programme (NNP) were launched in the country. This NNP set the need for tackling MD while strengthening nutrition information systems and monitoring and evaluating mechanisms (Herrador *et al.*, 2014).

Although there are some recent studies that have discussed on insufficiencies of some micronutrients in Ethiopian adolescents, to the best of the researcher's knowledge there is no published studies to capture comprehensively the status of serum multiple-micronutrients (iron, zinc and copper), anemia, food groups intake, as well as health status among adolescent girls in Lode-Hitosa wereda, Arsi zone, Oromiya regional state. Therefore, the present study is aimed at describing the status of selected micronutrients and

the presence of anemia among adolescent girls living in this study area and their relationship with diet and health factors.

## **1.2. Statement of the problem**

Adolescents are the future generation of any country and their nutritional needs are critical for the well-being of society. In most developing countries, nutrition initiatives have been focusing on children and women of child bearing age, thus neglecting adolescents. Addressing the nutrition needs of adolescents could be an important step towards breaking the vicious cycle of intergenerational malnutrition, chronic diseases and poverty. Malnutrition remains a common health problem among adolescence worldwide. During the past decades, deficiencies of three micronutrients have received the attention globally, namely iodine, vitamin A and iron (FAO/WHO, 2002). Zinc deficiency is wide spread in low income countries. Copper is necessary in human nutrition for normal iron metabolism and the formation of red blood cells (Khambalia, 2009; Hegazy *et al.*, 2010).

The life-course provides an important perspective for public health action. Events in one phase of life both affect and are affected by events in other phases of life. Thus, what happens during the early years of life affects adolescents' health and development, and health and development during adolescence in turn affect health during the adult years and, ultimately, the health and development of the next generation. Therefore it is crucial to assess the status of these multiple micronutrients among adolescent girls and to correlate their status with socio-demographic characteristics and dietary factors.

### **1.3. Significance of the study**

Assessing the mineral status of a population especially, adolescent girls are critical in developing nutrition intervention programs that enhance human health and well-being. The results of serum zinc and copper assessment efforts are necessary both to determine the presence and magnitude of deficiency problems and when indicated by the results, to elicit public interest and collect information for appropriate action. Assessment data are used to determine the level of risk of deficiency and indicate the risk factors which contribute to low zinc and copper status in the selected population. Assessments can also be used to monitor changes in zinc and copper status of subjects after intervention, thereby permitting decisions on the effectiveness of intervention programs and need for their continuation.

Institution based data relating to prevalence of zinc, copper deficiency and factors influencing zinc and copper deficiency among adolescent girls in Ethiopia are scarce. Determining the magnitude of deficiencies and its association with risk factors among adolescent girls in Ethiopia can contribute to planning strategies to alleviate zinc and copper deficiency and the related adverse health consequences. Therefore, the study will provide valuable information to the Lode-Hitosa wereda and policy makers regarding the magnitude of deficiency of zinc and copper among adolescent girls of Huruta.

### **1.4. Research question**

- What proportions of adolescent girls are anemic?
- What are the concentrations of serum zinc and copper in adolescent girls?
- What would be the mean dietary diversity scores of adolescent girls?

## **1.5. Objective of the study**

### **1.5.1. General objective**

- To investigate the status of zinc& copper deficiencies, dietary diversity and anemia among adolescents aged 15-20 years old living in Arsi zone, Lode-Hitosa wereda, Ethiopia.

### **1.5.2. Specific objectives**

- ❖ To determine the prevalence of anemia
- ❖ To determine serum zinc and copper status of adolescent girls
- ❖ To determine the dietary diversity scores and food nutrient contents of adolescent girls

## 2. LITRATURE REVIEW

### 2.1. Micronutrients and Adolescents

Micronutrients or minerals and vitamins are needed by the body in small concentration. Minerals are generally categorized as major or trace. Major minerals which are relatively needed by the body in large amount include calcium, phosphorous, potassium, sulfur, sodium, chloride, and magnesium; while, iron, copper, zinc, manganese, iodine, and selenium are regarded as trace minerals.

Trace minerals comprise metals in biological fluids at concentrations  $<1\mu\text{g/g}$  of wet weight. Among the trace elements, iron, copper, and zinc are involved in the function of several enzymes and are essential for maintaining health throughout life; lead and cadmium are non-essential toxic metals. Trace minerals can cause diseases through deficiency, imbalance, or toxicity (Goldhaber 2003; Underwood *et.al*, 2012.). Trace mineral deficiencies usually occur when dietary intake is inadequate or result from metabolic imbalances produced by antagonistic or synergistic interactions among metals. For instance, zinc absorption in small intestine is decreased by calcium, phosphate, and copper. On the other hand, excess zinc ingestion is a cause of copper deficiency (Choi *et al*, 2005).

Early adolescence is a period of rapid growth in females (Dahl *et.al*, 2004). Hence the recommended Dietary Allowances for Mature females. However, the dietary habits of teenagers are often poor (Greger *et al*, 1978). The process of sexual maturation and higher growth rate in adolescence usually start and finish in the second decade of life. Developmental age, as assessed by the stage of sexual maturation and/or bone age, is more accurate for the study of adolescents than chronological age. Given the normal variability of sexual maturation during puberty, same-age adolescents can be impubescent, pubescent, or present adult phenotypes (Urbano *et al*, 2002).

The maximum rate of muscle growth occurs during the peak growth spurt in boys and after the growth spurt, along with menarche, in girls. Maturation and growth stages are involved in the nutritional

diagnosis and prognosis and determine the nutritional requirements for each phase of adolescence. Therefore, appropriate nutrition is a basic health requirement for adolescents to properly express their genetic potential in terms of growth and development (Urbano *et al*, 2002).

## **2.2. Micronutrients and Dietary Diversity**

Nutrition is a main component of health and development. Healthy eating is related to the infant evolution, maternal and child health, healthier pregnancy and delivery, lower risk of chronic diseases and better academic achievement (WHO, 2011). Dietary diversity (DD) and the amount of animal source foods that an individual consume are two commonly used measures for dietary quality. Healthy growth and development essentially need a balanced diet of nutrients and vitamins which includes a variety of foods from different food groups (vegetables, fruits, grains, and animal source foods) (Belachew *et al.*, 2004). Dietary diversity has been shown to be strongly and positively correlated with nutrient adequacy (Arimond *et al.*, 2010) in low-income countries. Nutrient adequacy is one important element of diet quality.

Dietary diversity can be measured at the household or individual level through use of a questionnaire. Most often it is measured by counting the number of food groups rather than the food items consumed. At the household level, dietary diversity is usually considered as a measure of access to food (e.g., of households' capacity to access costly food groups); while at the individual level it reflects dietary quality, mainly the micronutrient adequacy of the diet. Although the reference period can vary, it is most often the previous day or week (FAO, 2011).

The lack of dietary diversity is a severe problem among poor people in the developing countries suggests that they feed mostly on starchy staples without or with minimal use of animal products, fresh fruits and vegetables (Popkin, 1994). The FAO's third version of the guidelines for measuring household and individual dietary diversity which was revealed in August 2007 is introduced as an expedient instrument for DDS measures (FAO, 2007).

Recently the association between dietary diversity and micronutrient adequacy of diets of women in reproductive age was assessed in five countries. Dietary diversity was significantly associated with micronutrient adequacy in all sites (Arimond *et al.*, 2010).

### **2.3. Iron**

Iron is the most abundant element on earth, yet only trace elements are present in living cells. Iron is involved in many enzymatic reactions of a cell; hence it is believed that the presence of iron was obligatory for the evolution of aerobic life on earth. Furthermore, the propensity of iron to catalyze the oxygen radicals in aerobic and facultative anaerobic species indicates that the intracellular concentration and chemical form of the element must be kept under tight control (Sarika *et al.*, 2012).

Iron is essential for the expansion of blood volume and muscle mass. It has enzyme or metabolic functions, in addition to storing iron in the form of ferritin and hemosiderin in order to maintain homeostasis. When dietary iron is inadequate, iron stores are mobilized to maintain the production of hemoglobin and other iron-containing components. During puberty, serum ferritin levels increase in boys, while they stay stable in girls, both in adolescence and adult life, and will only reach the same levels presented by boys after menopause. Attention deficits, poor performance in intelligence tests, behavioral and mood changes, tiredness, and below-average school performance are associated with iron deficiency in adolescents. Young individuals, just like infants, contain much more copper per body weight than adults (Urbano *et al.*, 2002).

Iron is an essential element in the body but its effect in the body is like a two-edged sword. At one end it is essential for maintaining most of the body functions and at the other end it becomes potentially toxic if in excess. Thus, elaborate physiological mechanisms have evolved for regulation of uptake and disposition of iron. The earlier concept of regulation of iron levels by absorption could not explain several clinical conditions like hemochromatosis and severe anemias associated with chronic diseases and malignancies. However, a newer insight into the understanding of iron metabolism has been provided in

the past few years, mainly as a result of the discovery of hepcidin, a key regulator of whole-body iron homeostasis (Sarika *et al.*, 2012; Vyorol *et.al*, 2005; Collins *et.al*, 2008; Vokurka *et.al*, 2006).

### **2.3.1. Iron requirement and food source**

Daily requirements for iron vary depending on the person's age, sex and physiological status. Although iron is not excreted in the conventional sense, about 1 mg is lost daily through the normal shedding of skin epithelial cells and cells that line the gastrointestinal and urinary tracts (Wick, 2003). Small numbers of erythrocytes are lost in urine and feces as well. Humans and other vertebrates strictly conserve iron by recycling it from senescent erythrocytes and from other sources. Such conservation of iron is essential because many human diets contain just enough iron to replace the small losses. However, the blood lost in each menstrual cycle drains 20 to 40 mg of iron (McCance, 1937), so women in their reproductive years need to absorb approximately 2 mg of iron per day (Gautam, *et al.*, 2008). However, when dietary iron is more abundant, absorption is appropriately attenuate (Sarika *et al.*, 2012).

### **2.3.2. Iron deficiency**

Iron is an essential element for the growth and well-being of almost all-living organisms. Human iron dietary intake is carefully controlled such that 1–2 mg of iron is absorbed from our diet and 1–2 mg is excreted per day (Freitas *et al.*, 2001). Slight alterations in both of process can elicit depletion of iron (anemia), while excessive intake may cause iron overload.

An extensive literature has focused on the study of iron deficiency and iron overload related diseases. We are still far from understanding the complexity that iron homeostasis represents for health in humans. Small imbalance in iron metabolism can originate harmful conditions. An interesting report has shown the effect of iron (14 mg/day) supplementation in healthy volunteers. In one group of volunteers, with higher mean initial levels of plasma Vitamin C, there was a significant rise in several oxidative base damage products and in total oxidative DNA damage after 6 weeks of supplementation. Given the suggestion that

oxidative DNA damage is a risk for the development of cancer, these results call for caution in regard to the use of dietary supplements containing both iron salts and ascorbates (Freitas *et al.*, 2001).

## **2.4. Zinc**

Zinc is the most abundant intracellular trace element required for a number of cellular processes, including cell proliferation, reproduction, immune function and defense against free radicals. It is a constituent of more than 300 metalloenzymes (Valle and Falchuk 1993). Insufficient dietary intake of zinc causes the appearance of pathogenic features including growth retardation, dermal lesions, immunodeficiency, male and female infertility and neurosensory disorders (Cerovic *et al.*, 2008). As a result, supplements containing zinc are used worldwide (Cerovic *et al.*, 2008).

### **2.4.1. Path and biological role of zinc**

Zinc is a component of over 200 enzymes and is essential for normal immune function, DNA and RNA synthesis, and gene transcription (Frassinetti *et.al*, 2006; Singh 2004). Zinc is absorbed along the length of the small intestine and is transported to the liver in the portal circulation. It is excreted into the gut (endogenous fecal zinc excretion), and pancreatic and biliary secretions contain large amounts of zinc, most of which is subsequently reabsorbed. Zinc homeostasis is maintained by changes in fractional zinc absorption and in endogenous fecal zinc excretion. Endogenous fecal zinc excretion increases during periods of high zinc intake and decreases during periods of zinc restriction (Griffin *et al.*, 2004).

Zinc is involved in numerous aspects of cellular metabolism. It was estimated that about 10% of human proteins potentially bind zinc, in addition to hundreds which transport and traffic zinc. It is required for the catalytic activity of more than 200 enzymes and it plays a role in immune function, wound healing, protein synthesis, DNA synthesis and cell division. Zinc is required for proper sense of taste and smell and supports normal growth and development during pregnancy, childhood, and adolescence (Bhowmik *et.al*, 2010; Deshpande *et al.*, 2013). It is believed to possess antioxidant properties, which may protect

against accelerated aging and helps speed up the healing process after an injury; however, studies differ as to its effectiveness.

Zinc ions are effective antimicrobial agents even at low concentrations. Gastroenteritis is strongly attenuated by ingestion of zinc and this effect could be due to direct antimicrobial action of the zinc ions in the gastrointestinal tract, or to the absorption of the zinc and re-release from immune cells [all granulocytes secrete zinc], or both. Cells in the salivary gland, prostate, immune system and intestine use Zn signaling as one way to communicate with other cells. In the brain, zinc is stored in specific synaptic vesicles by glutamatergic neurons and can modulate brain excitability. It plays a key role in synaptic plasticity and so in learning. It also can be a neurotoxin, suggesting zinc homeostasis plays a critical role in normal functioning of the brain and central nervous system (A.Cerovic<sup>1</sup> *et al.*, 2008; Osredkar *et al.*, 2011).

There are 2-4 grams of Zn distributed throughout the human body. Most zinc is in the brain, muscle, bones, kidney and liver, with the highest concentrations in the prostate and parts of the eye. It is the second most abundant transition metal in organisms after iron and it is the only metal which appears in all enzyme classes (Osredkar *et al.*, 2011).

Since the early reports of human zinc deficiency, and particularly during the past 10 to 15 years, a considerable number of well-designed clinical trials have been completed to examine the relationships between zinc supplementation and human health. Zinc is especially important during periods of rapid growth, both pre and postnatally, and for tissues with rapid cellular differentiation and turnover, such as the immune system and the gastrointestinal tract. Critical functions that are affected by zinc nutriture include pregnancy outcome, physical growth, susceptibility to infection, and neurobehavioral development, among others (Brown *et al.*, 2001).

Zinc supplemented diet increases the intestinal synthesis of cellular proteins, called metallothioneins (Mocchegiani *et.al*, 2013). They bind metals and do not allow their absorption by intestinal cells.

Metallothioneins have a stronger affinity for copper than for zinc, so high levels of metallothioneins due to increased zinc can cause reduced absorption of copper. High additional intake of zinc - 50 mg/d or more for an extended period of time can also lead to copper deficiency (Monte *et.al*, 2005).

#### 2.4.2. Food source and recommended intake

A wide variety of foods contain zinc. Oysters contain more zinc per serving than any other food, but red meat, especially beef, lamb and liver have some of the highest concentrations of zinc in food (Scherz *et.al*, 2006; Deshpande *et.al*, 2013). Other good food sources include beans, nuts, other types of seafood (such as crab and lobster), whole grains, cereals, almonds, pumpkin seeds, sunflower seeds. Supplements contain several forms of zinc, including zinc gluconate, zinc sulfate, and zinc acetate. The percentage of elemental zinc varies by form of supplement. Scientists have not determined whether differences exist among forms of zinc in absorption, bioavailability, or tolerability (Osredkar *et al.*, 2011).

Intake recommendations for Zn are provided in the Dietary Reference Intakes developed by the Food and Nutrition Board (FNB) at the Institute of Medicine of the National Academies. The current Recommended Dietary Allowances [RDA] for zinc is listed in Table below.

**Table 1:** Recommended Dietary Allowances [RDAs] of Zn per day.

| Age         | Male (mg) | Female (mg) | Pregnancy (mg) | Lactation (mg) |
|-------------|-----------|-------------|----------------|----------------|
| 0-6 months  | 2         | 2           | —              | —              |
| 7-12 months | 3         | 3           | —              | —              |
| 1-3 years   | 3         | 3           | —              | —              |
| 4-8 years   | 5         | 5           | —              | —              |
| 9-13 years  | 8         | 8           | —              | —              |
| 14-18 years | 11        | 9           | 12             | 13             |
| >19 years   | 11        | 8           | 11             | 12             |

### 2.4.3. Suggested cutoffs for assessing serum zinc

Currently, the serum or plasma zinc concentration is the most widely used biochemical indicator of zinc status and is the only biochemical indicator of zinc status for which adequate reference data are available. The second National Health and Nutrition Examination Survey (NHANES II) of the United States (1976–1980) included serum zinc concentration in its biochemical assessments. The analysis of these data led to the derivation of cutoffs for low serum zinc concentrations, which have been used since to assess zinc status. Separate cutoffs were derived on the basis of the fasting state and the time of day of sample collection. It is apparent, however, that the serum zinc concentration also varies with age and sex and health status, so the use of a single cutoff for all age and sex groups may not be appropriate (Christine *et.al*, 2003).

**Table 2:** Suggested lower cutoffs (2.5<sup>th</sup> percentile) for the assessment of serum zinc concentration in population studies (1)

| Lower cutoffs of serum zinc concentration |                         |                       |                     |                     |
|-------------------------------------------|-------------------------|-----------------------|---------------------|---------------------|
|                                           | Children aged<br>< 10 y | Females aged<br>≥10 y | Males aged<br>≥10 y | Pilch and Senti (3) |
| Time/fasting<br>status(2)                 | –                       | μg/dl                 |                     | μg/dl               |
| Morning                                   | –                       | 70                    | 74                  | 70                  |
| fasting(4)                                |                         |                       |                     |                     |
| Morning non<br>fasting                    | 65                      | 66                    | 70                  | 65                  |
| Afternoon                                 | 57                      | 59                    | 61                  | 60                  |

(1)Data derived from the second National Health and Nutrition Examination Survey (NHANES II).

(2)Time of day of blood sample collection and fasting status during blood sample collection

(3)From a previous analysis of NHANES II serum zinc data for all age and sex groups combined.

(4) Based on data from subjects aged ≥20 y only.

#### **2.4.4. Zinc deficiency**

Zinc deficiency is among the most important causes of morbidity in developing-country settings. There is an urgent need to begin to identify countries and regions that are at increased risk for zinc deficiency and to quantify the number of individuals at risk on a global scale. To date, there are very limited data available at the country level on the risk of zinc deficiency. Nearly two billion people in the developing world are deficient in zinc (Prasad, 2003).

Zinc deficiency is characterized by growth retardation, loss of appetite, and impaired immune function. In more severe cases, zinc deficiency causes hair loss, diarrhea, delayed sexual maturation, impotence, hypogonadism in males, and eye and skin lesions. Weight loss and impaired appetite, delayed healing of wounds, taste abnormalities, and altered cognition can also occur (Deshpande *et.al*, 2013). When zinc deficiency does occur, it is usually due to inadequate zinc intake or absorption, increased losses of zinc from the body, or increased requirements for zinc (Osredkar *et al.*, 2011). It can be associated with malabsorption, acrodermatitis enteropathica, chronic liver disease, chronic renal disease, sickle cell disease, diabetes, malignancy, and other chronic illnesses (Osredkar *et al.*, 2011). The WHO advocates zinc supplementation for severe malnutrition and diarrhea.

Severe Zn deficiency depresses immune function, and even mild to moderate degrees of zinc deficiency can impair macrophage and neutrophil functions, natural killer cell activity, and complement activity (Shankar *et.al.*, 1998; Prasad 2008). The body requires zinc to develop and activate T-lymphocytes (Deshpande *et.al.*, 2013). Individuals with low zinc levels have shown reduced lymphocyte proliferation response to mitogens and other adverse alterations in immunity that can be corrected by zinc supplementation (Osredkar *et al.*, 2011). These alterations in immune function might explain why low zinc status has been associated with increased susceptibility to pneumonia and other infections in children in developing countries. However, zinc supplements should not be administered alone, since many in the developing world have several deficiencies, and zinc also interacts with other micronutrients.

## **2.5. Copper**

Copper is necessary for growth, is an important factor for several enzyme systems, and is involved in the synthesis of hemoglobin, oxidizing ferrous iron into ferric iron, and in the formation of transferrin by means of ceruloplasmin. Zinc, which is an essential cofactor for nearly 200 enzymes, participates in cellular growth as a cofactor for enzymes necessary for the synthesis of ribonucleic acid (RNA) and deoxyribonucleic acid (DNA), and controls, among other functions, gonadal growth and development. Adolescents need more zinc/kg than adults, due to the role of this metal in sexual maturation and growth. According to what has been mentioned, it is important to assess the levels of iron, copper and zinc in adolescents during the pubertal spurt (Urbano *et al.*, 2002).

### **2.5.1. Biological role of Cu**

Copper plays an important role in our metabolism, largely because it allows many critical enzymes to function properly. Copper is essential for maintaining the strength of the skin, blood vessels, epithelial and connective tissue throughout the body. Cu plays a role in the production of hemoglobin, myelin, melanin and it also keeps thyroid gland functioning normally. Copper can act as both an antioxidant and a pro-oxidant. Free radicals occur naturally in the body and can damage cell walls, interact with genetic material, and contribute to the development of a number of health problems and diseases. As an antioxidant, Cu scavenges or neutralize free radicals and may reduce or help prevent some of the damage they cause. When copper acts as a pro-oxidant at times, it promotes free radical damage and may contribute to the development of Alzheimer's disease. Maintaining the proper dietary balance of Cu, along with other minerals such as zinc and manganese, is important (Osredkar *et al.*, 2011).

### 2.5.2. Food sources

Rich sources of copper include oysters and other sea food, beef and organ meats (especially liver), dark green leafy vegetables, enriched cereals, nuts and sunflower seeds, green olives, avocados, dried legumes, chocolate, cocoa, and black pepper (Osredkar *et al.*, 2011; Gharibzahedi *et.al.*, 2017).

### 2.5.3. Recommended intakes for Cu

The 10th edition of Recommended Dietary Allowances (RDA) did not include an RDA for copper; rather a safe and adequate daily intake was suggested. Some nutritionists think, committees that establish RDAs should return to the traditions of the first nine editions and make recommendations that meet functional needs, because lack of a recommended dietary allowance for copper may be hazardous to health. The following Table 2 provides the recommended daily dietary intake (RDI) of copper for children and adults and Tolerable upper intake levels for copper (Osredkar *et al.*, 2011).

**Table 3:** Recommended daily intakes (RDIs) Tolerable intake levels for CU

| Age         | Male (mcg) | Female (mcg) | Tolerable upper intake level (mcg)                                                 |
|-------------|------------|--------------|------------------------------------------------------------------------------------|
| 0-6 months  | 200        | 200          | Not possible to establish a TUL, sources of CU must be from food and formula only. |
| 7-12 months | 220        | 220          |                                                                                    |
| 1-3 years   | 340        | 340          | 1000                                                                               |
| 4-8 years   | 440        | 440          | 1000                                                                               |
| 8-13 years  | 700        | 700          | 5000                                                                               |
| 14-18 years | 890        | 890          | 8000                                                                               |
| 19+ years   | 900        | 900          | 10000                                                                              |
| Pregnancy   |            | 1000         | 8000 [14-18 years], 10000 [19+ years]                                              |
| lactation   |            | 1300         |                                                                                    |

#### **2.5.4. Copper Deficiency**

Because copper is involved in many functions of the body, copper deficiency can produce an extensive range of symptoms. Deficiencies of copper can result in hernias, aneurysms, blood vessel breakage manifesting as bruising or nosebleeds, iron deficiency anemia, osteoporosis and joint problems, brain disturbances, abnormalities in glucose and cholesterol metabolism, increased susceptibility to infections due to poor immune function [neutropenia], loss of pigment, weakness, fatigue, skin sores, poor thyroid function, irregular heart beat and low body temperature (Osredkar *et al.*, 2011). If copper is important in cellular membrane structure, then a copper deficiency could seriously alter the movement of nutrients through cell walls.

### **3. MATERIAL AND METHODS**

#### **3.1. Study design**

A community based, cross sectional study was designed to investigate the status of Anemia, Zinc, Copper and others (socio-demographic characteristics and dietary factors) under the aid of health facility. The study population was drawn household randomly selected to be representative of Lode-Hitosa wereda. The study was carried out between October, 2016 and April, 2017.

#### **3.2. Study area**

This study was conducted in Lode-Hitosa wereda, Arsi zone, Oromiya region situated at 8°09' north and 39°21' east with an elevation of 1978 meters. It has a total population of 15,298 of whom 7,463 were males and 7,835 were females (CSA, 2007). Considering a rate of 2.6% population growth annually currently total populations of 19,275 are living in Huruta. Lode-Hitosa wereda is rural area and major production site for wheat and barley. Huruta is the main town of Lode-Hitosa wereda. Huruta is 136 km far from Addis Ababa.

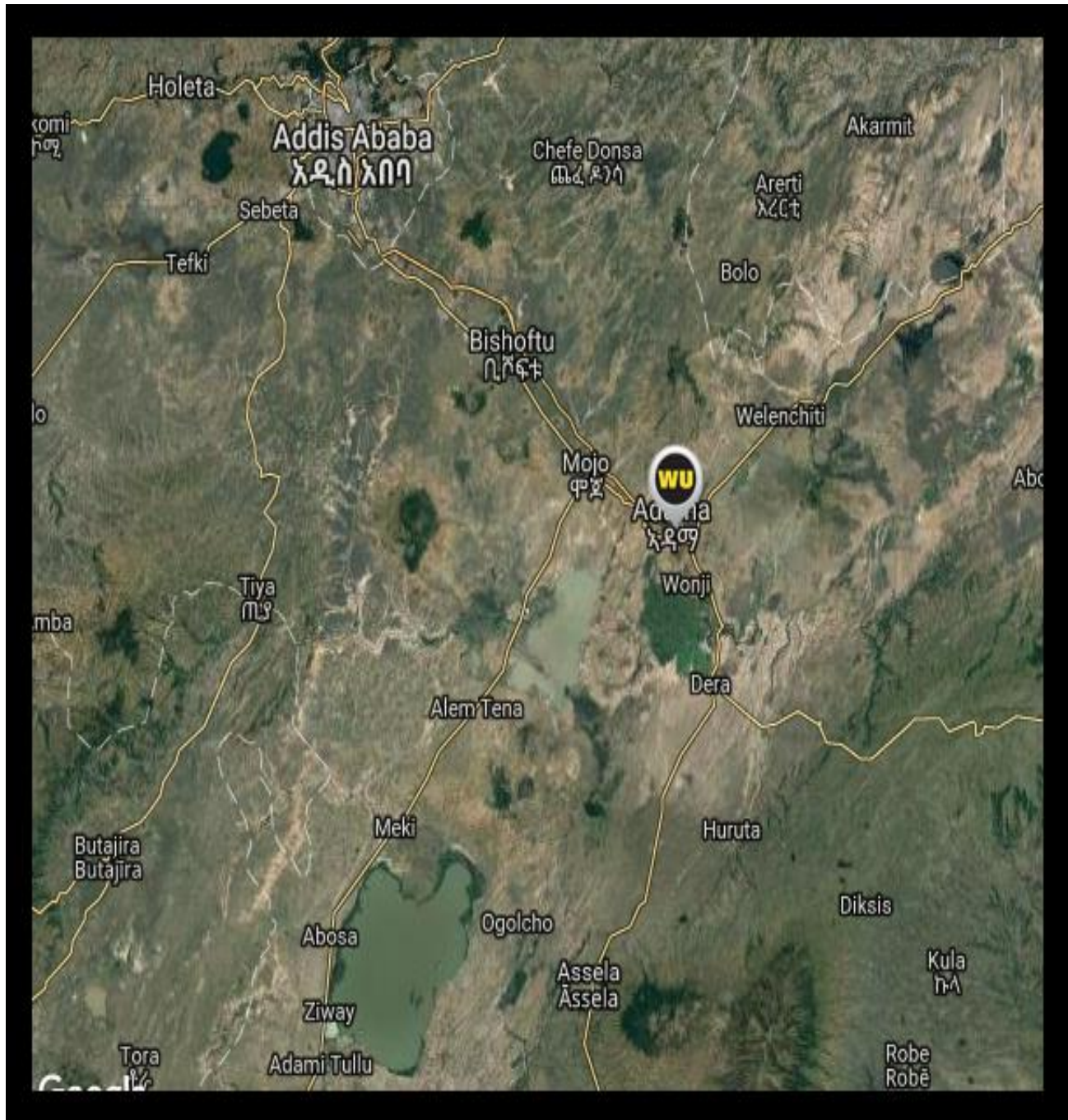


Figure 1: Map of the location Huruta, Lode-Hitosawereda (source, <http://www.ikimap.com/map/administrative-area-hitosa-ethiopia>)

### **3.3. Data Source and Study population**

All adolescent girls aged 15-20 years in the eleven clusters (Kebeles) were considered as the study population. All the subjects were selected randomly from resident of Huruta area of Lode-Hitosa wereda. Adolescent girls those resided in the study area, and willing to give blood were included. The exclusion criteria was adolescent girls who are seriously ill, pregnant (probed by asking the last menstrual period), married, had children and absent during the data collection period and Adolescent girls who were pregnant before or had abortion.

### **3.4. Sample size and sampling techniques**

Convenience sampling was used to select a total of 257 adolescent girls from 11 different kebeles using random technique. The study participants were selected randomly. Considering prevalence of anemia in oromiya region was 27% (DHS, 2016).

### **3.5. Study variables**

- Anemia and inflammation (CRP, AGP) status
- Serum zinc status
- Serum copper status
- Dietary diversity score
- Socioeconomic factors (educational level including family, family composition, household income source, and facilities available in the household, and access to healthcare)
- Anthropometry (weight, height, BMI)

### **3.6. Ethical considerations**

The study protocol was approved by the Human Ethics Committee of the University. Verbally Informed consent was obtained from parents/guardians (under 18 years old) and participants (above 18 years old) after fully explaining the nature, benefits and side effects of the study

### **3.7. Data collection**

#### **3.7.1. Socio-demographic and anthropometric measurements**

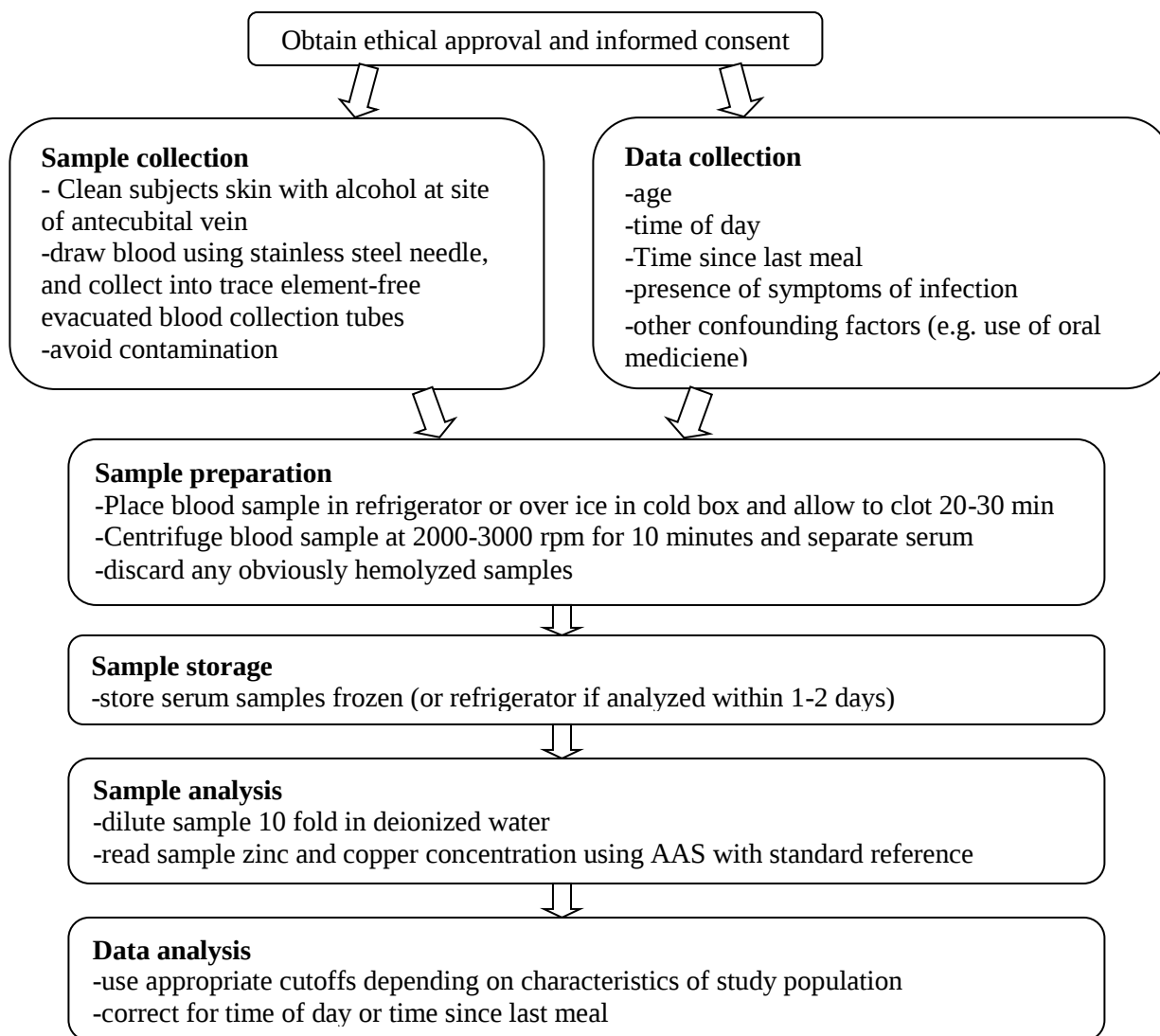
A data on socio-demographic characteristics was collected through personal interview using pre-designed, pretested questionnaires which includes educational level (including family), family composition, and facilities available in the household, and access to healthcare. Weight and height of each respondent was measured at the beginning of the survey using standardized techniques and calibrated equipment, with the subjects wearing light clothing and no shoes. The body mass index (BMI) was calculated as the weight in kilograms divided by the square of the height in meters ( $\text{kg/m}^2$ ). Accordingly, BMI values were categorized as underweight ( $< 18.5$ ), normal ( $18.5 - 24.9$ ) and overweight/obese ( $> 25$ ) (WHO, 2007; Mercedes *et al*, 2007).

#### **3.7.2. Dietary diversity score**

Individual dietary diversity score was estimated using 24-hour recall. Respondents were asked to recall the foods they had consumed in the previous 24h, first spontaneously followed by probes to ascertain that no meal or snack was left out. All food eaten, inside and/or outside the house, was probed and grouped into ten major food groups. A detailed list of all ingredients of the dishes, snacks, or other foods consumed was generated to enable better classification of mixed dishes. Then, the foods were categorized into ten (10) groups as 1) Grains, white roots and tubers, and plantains 2) Pulses (beans, peas and lentils) 3) Nuts and seeds 4) Dairy 5) Meat, poultry and fish 6) Eggs 7) Dark green leafy vegetables 8) Other vitamin A-rich fruits and vegetables 9) Other vegetables 10) Other fruits (FAO, 2016). Finally, the dietary diversity score (DDS) (0-10) was calculated by summing the number of food groups.

### 3.8. Blood sample collection and analysis

Non-fasting venous blood sample (~ 4 ml) was collected in serum separate tube (SST) to determine anemia and the biochemical indices of zinc and copper deficiencies. A few drops of blood were taken immediately and used for hemoglobin (Hb) analyses using Hemocue 201 (Hb 201, Angelholm, Sweden). The remaining samples were centrifuged in the field to separate the serum and transferred into 2 ml Eppendorf tubes. The serum then transported to Center for Food Science and Nutrition laboratory and stored at -20<sup>0</sup>c until further analysis.



**Figure 2:** Schematic presentation of steps of preparation and measurement of serum zinc and copper concentration

### 3.8.1 Hemoglobin test

Hemoglobin test was performed from whole blood using portable hemoglobinometer (Hemocue 201) device and the reading was adjusted for altitude according to the WHO recommended Hb adjustment formula described below.

$$\text{Hb adjustment} = -0.032 * (\text{Altitude} * 0.0033) + 0.022 * (\text{Altitude} * 0.0033)^2$$

$$\text{Hb adjustment} = -0.032 * (1976 * 0.0033) + 0.022 * (1976 * 0.0033)^2$$

$$= 0.8$$

The adjustment value (0.8) was subtracted from each Hb value. WHO recommended Hb concentration cut-offs value for adolescent girls was used, where anemia was defined as Hb value <12g/dl non anemic.

### 3.8.2. Biochemical assessment

Zinc and copper determinations were carried out using (Atomic Absorption Spectroscopy) AAS in Food science and Nutrition laboratory. Iron and iron biomarkers (ferritin, AGP, and CRP) were analyzed first in EPHI. Zinc and copper were determined by AAS. The serum and standards was diluted with de-ionized water at the ratio of 1:10, and the concentrations of copper and zinc were determined as follows: wavelength of 324.8nm (Cu) and 213.9nm (Zn) respectively; duplicate reading.

#### 3.8.2.1. Blood sample and standard preparation

The reagents used for the preparation of standard solutions were ultra-pure grade. The water used for standard solution and dilution of serum was doubly distilled and de-ionized. Standard solutions were prepared in glass bottles, previously washed with diluted nitric acid (10%) and rinsed with de-ionized water. Pipettes and other materials used for reagent preparation and sample dilution had been previously acid soaked overnight, then rinsed with de-ionized water and dried in an oven.

A stock standard solution was prepared with concentrations of 5µg/ml for each Zn and Cu and then a series of calibration standard were prepared by diluting the stock standard with de-ionized water. The volumes of stock standard and resultant concentration in each calibration standard were as follows:-

Table 4. Volume of standard stock solutions and resultant concentrations used for calibration

|                                  | 0  | 1    | 2    | 3    | 4    | 5    |
|----------------------------------|----|------|------|------|------|------|
| Concentration of element (µg/ml) |    |      |      |      |      |      |
| Cu                               | 0  | 0.10 | 0.20 | 0.30 | 0.40 | 0.50 |
| Zn                               | 0  | 0.10 | 0.20 | 0.30 | 0.40 | 0.50 |
| Total volume (ml)                |    |      |      |      |      |      |
|                                  | 50 | 50   | 50   | 50   | 50   | 50   |

### 3.8.2.2. Determination of serum zinc and Copper concentration

The serum samples were stored at -20<sup>0</sup>c until the analysis. So prior to analysis the samples get ambient temperature and ready for analysis. Once the calibration is done the measurement is automatically carried out according to the standard calibration method. Zinc and Copper were determined in the same diluted solution. 1 ml of serum placed in a test tube was diluted to 10ml with de-ionized water. The test tube was placed in the auto sampler after mild shaking by using vortex. The measurement is automatically carried out according to the standard calibration method and the results are average of two replicates.

We calibrated the instrument with the blank and a standard. After the initial calibration, separately for Zinc and Copper samples were measured consequently and the values reported by the system were the concentration so the final calculation was only for dilution factor.

### 3.9. Food samples analysis

Freeze-dried samples (approximately 5 g) of homogenized diet were accurately weighed into silica crucibles, dry ashed at 480<sup>0</sup>c for 48 h. The ash was dissolved by 5ml of 6M HCL at low temperature on hot plate for about 2hrs. Then 7ml of 3M HCL was added and heated on hot plate until the solution boils. The digest was cooled and filtered through a filter paper (42mm, whataman) into a 50ml volumetric flask. Then 5ml of 3M HCL was added to the dish and heated to dissolve the residue in the dishes and then transferred to the volumetric flask. Then the filter paper was washed thoroughly and the washing collected in the flask made to the mark.

Afterwards the mineral concentration (for Fe, Zn and Cu analyses) was determined by atomic absorption spectrometry (AAU). Analyses were performed in duplicate on 5 kind of food samples, Injera (just baked injera), Shiro stew (mainly prepared with chekpea and beans), kik stew (mainly of split pea), and Injera dibilik (Injera which is baked of teff, wheat, barely) and meat stew (made of some piece of meat and pepper that is all purpose spice).

The mineral contents of each sample were calculated using the following formula:-

$$\text{Metal content (mg/100gm)} = \frac{(a-b)*V}{10*W}$$

Where: W = Weight in gm of the sample

a = concentration in ppm of the sample solution

V = Volume in ml

b = concentration in ppm of blank solution

### **3.10. Statistical analysis**

Statistical analysis were carried out using SPSS version 20 (IBM SPSS), was used to enter and analyze data. Frequencies, means and standard deviations (SD) were used to summarize the data. Frequencies were used to summarize categorical data Mean and standard deviation were used for non-categorical/discrete data.

The data collected through 24hr diet recall were computed into 10 food groups; cereals; roots and tubers; vitamin A-rich fruits and vegetables; other fruit; other vegetables; legumes and nuts; meat, poultry and fish; fats and oils; dairy; and eggs. The Dietary Diversity Score (DDS) was calculated by summing the number of unique food groups. This index is based on a simple count of food groups consumed in the previous day to the survey. Accordingly, the level of diet diversity was computed out of the score of 10.

## **4. RESULTS**

### **4.1. Household and respondent's characteristics**

A total of 255 adolescent girls aged (15-20 years) enrolled in this study (Table 5). The family size of the study participants ranged from 1 to 15, with an average of 6 persons per household, and most of the study participants had a household size of 5 and only 1 household has a size of 15 persons. The majority of the participant's household were male headed (75.9%), of which 23% of household head had no formal education.

Majority of the households own agricultural land (83 %) and livestock (72 %). Accordingly, agricultural production (74 %) was the main income source for the households. Furthermore, about 50 % of the households cultivate fruits and vegetables, of which 80 % is used for home consumption. Nearly all households had access to public tap water and had a toilet facility (pit latrine with slab) in their premises. 50% of the household have access of electricity. However, wood and charcoal (75 %) are the ones mostly used for cooking fuel. The majority of adolescent girls who participated in this study were primary school students with no history of smoking and drug addiction

**Table 5:** Household and respondent's characteristics

|                                                        | N   | Proportion (%) | Mean (min-max) |
|--------------------------------------------------------|-----|----------------|----------------|
| Age of participants (in years)                         | 248 |                | 16 (15-20)     |
| House hold size                                        | 255 |                | 6 (2-15)       |
| Household headship                                     | 249 |                |                |
| Male                                                   |     | 75.9           |                |
| female                                                 |     | 24.1           |                |
| Household head education                               | 248 |                |                |
| No formal education                                    |     | 23.4           |                |
| Primary                                                |     | 48             |                |
| Secondary                                              |     | 22.2           |                |
| University or college diploma and degree               |     | 6.4            |                |
| Household income source                                | 255 |                |                |
| Wage income                                            |     | 5.9            |                |
| Pension                                                |     | 1.2            |                |
| Salary                                                 |     | 10.2           |                |
| Business                                               |     | 9              |                |
| Agricultural production                                |     | 73.7           |                |
| Source of drinking water                               | 237 |                |                |
| Improved source                                        |     | 95.4           |                |
| Non-improved source                                    |     | 4.6            |                |
| Type of toilet facility                                | 238 |                |                |
| Improved facility (pit latrine with slab)              |     | 88.7           |                |
| Non-improved facility                                  |     | 11.3           |                |
| Electricity                                            | 238 | 50             |                |
| Source of cooking fuel                                 | 255 |                |                |
| Electricity                                            |     | 17.6           |                |
| Charcoal                                               |     | 78             |                |
| Wood                                                   |     | 84.7           |                |
| Straw/Shrubs/Grass                                     |     | 3.5            |                |
| Animal dung                                            |     | 3.9            |                |
| Fruits and vegetables cultivation                      | 237 | 49.8           |                |
| Use of cultivated fruits and vegetables (out of 49.8%) | 123 |                |                |
| For sale                                               |     | 20.3           |                |
| For home use                                           |     | 79.1           |                |
| Ownership of agricultural land                         | 237 | 66.1           |                |
| Ownership of livestock                                 | 228 | 54.7           |                |
| Respondents education                                  | 237 |                |                |
| No formal education                                    | -   |                |                |
| Primary                                                |     | 77.6           |                |
| secondary                                              |     | 22.4           |                |

## 4.2 Anthropometry and dietary diversity score of adolescent girls

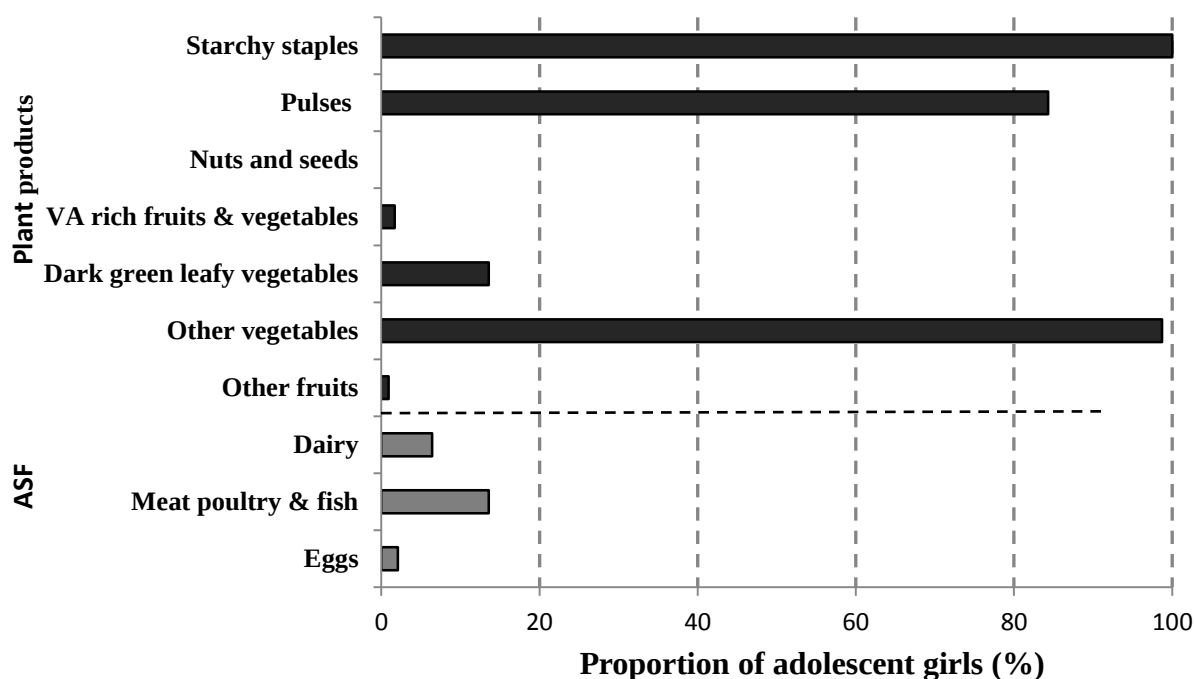
An important proportion of adolescent girls (35 %) were underweight (BMI < 18.5) and about 3 % were overweight. The mean height and weight of the participants were 155 cm and 47 Kg, respectively (**Table 6**).

**Table 6.** Anthropometry and women's dietary diversity score of adolescent girls

| Variables                              | N   | Mean±SD    | Proportion (%) |
|----------------------------------------|-----|------------|----------------|
| Height (cm)                            | 228 | 155.2±6    |                |
| Weight(kg)                             | 253 | 47.5 ± 6.9 |                |
| Body Mass Index(kg/cm <sup>2</sup> )   | 228 | 19.9 ± 2.8 |                |
| Underweight (< 18.5)                   |     |            | 35.1           |
| Normal (18.5-25)                       |     |            | 61.8           |
| Over weight (>25)                      |     |            | 3.1            |
| MUAC(cm)                               | 248 | 22.4±3.9   |                |
| < 21                                   |     |            | 11             |
| > 21                                   |     |            | 86.3           |
| Women's dietary diversity score (WDDS) | 235 | 3.2 ± 0.6  |                |
| WDDS category                          |     |            |                |
| Adequate (≥ 5)                         |     |            | 4.3            |
| Inadequate (< 5)                       |     |            | 95.7           |

Furthermore, the dietary pattern of the participants was assessed using women's dietary diversity score (WDDS) by asking foods consumed for the past 24 hrs. WDDS revealed that nearly all of adolescent girls consumed fewer than five food groups (WDDS < 5) out of the ten food groups included in the questionnaire. Further insight into the food groups revealed that, the diet was mainly composed of grains and tubers (100 %), other vegetables (mainly onion, 99 %), and pulses (84 %) (**Figure 3**).

The mean number of food groups consumed was about 3. The consumption of nuts and seeds, vitamin A rich fruits and vegetables, dark green leafy vegetables, flesh foods, and dairy products were very low. The consumption of organ meats, eggs, fish and vitamin A rich fruits and vegetables is almost zero. This limited variation in food groups among the target groups may represent their cultural eating habits and also low availability of different foods during the survey was conducted.



**Figure 3.** Food groups consumed according to Women's dietary diversity score (WDDS) classification (n=235). VA, vitamin A; ASF, animal source foods

#### 4.4 Anemia, zinc and copper status of adolescent girls

Hemoglobin level was measured to determine anemia status; the mean value was 14.2 g/L. Despite a significant, proportion of the study population being underweight, and the low consumption of animal source foods (ASF), the prevalence of anemia (8.7 %) was very low with 0.8 % moderate and 7.9 % mild anemia. Iron status was also determined using serum ferritin (SF) values after adjusting for the presence of infection/inflammation based on CRP and AGP values. The prevalence of clinical iron deficiency after adjusting was very low (8.7 %, SF < 12 µg/L). However, a significant proportion had a marginal iron store (41 %, SF < 50 µg/L). The prevalence of infection in the area was about 17 %, of which 0.8%, 14.8 % and 1.5 % were in incubation, early and late convalescence phase, respectively. Furthermore, the presence of infection did not affect the prevalence of ID.

Furthermore, serum zinc and copper level was determined, the mean values are 72 mg/dL and 155.8 µg/dL. The latter value had been found to be highly variable. Accordingly, half of the population was zinc deficient and about 20 % were copper deficient.

Serum Zinc level in our population was 72±26.55 mg/dL. But, the deficiency rate was high (49.2%). This study found that younger children presented a significantly higher prevalence of zinc deficiency (ZD) in this study area (Lode-hitosa wereda).

Similarly, for the mean serum level of copper in this study population was 155.8±105 mg/dL. The standard deviation for this mean value was quite large despite that no outlier value was identified. It is common to find this kind of wide ranges for this parameter in the literature. The prevalence of copper was 20.9%.

**Table 7:** Hb, anemia classification, zinc and copper deficiency, and infection in adolescent girls

| Parameters                | N   | Mean ± SD | Proportion (%) |
|---------------------------|-----|-----------|----------------|
| Hemoglobin (g/L)          | 252 | 14.2 ± 1  |                |
| Anemia classification     |     |           |                |
| Mild (100 - 119 g/L)      |     |           | 7.9            |
| Moderate (70 - 99 g/L)    |     |           | 0.8            |
| Severe (< 70 g/L)         |     |           |                |
| Zinc deficiency (mg/dL)   | 248 | 72±26.55  | 49.2           |
| Copper deficiency (mg/dL) | 248 | 155.8±105 | 20.97          |
| Infection groups          | 248 |           |                |
| Late convalescence        |     |           | 14.8           |
| Early convalescence       |     |           | 1.5            |
| Incubation                |     |           | 0.8            |

**SD, standard deviation, zinc deficiency, serum zinc < 65 µg/dL, copper deficiency, serum copper < 90 µg/dL, Clinical iron deficiency (ID), serum ferritin < 15 mg/L, marginal iron store, SF < 50 mg/L.**

#### 4.5. Nutrient content in food sample

The amount of micronutrients (iron, zinc, and copper) per 100grams of different food samples is shown in table below. The result is the average of triplicate measurement. So the meal pattern in lode-Hitosa wereda consists mainly of this components and the recommended dietary allowance of Iron, Zinc and Copper are 15, 12 and 1.5 mg respectively (ref ....). The amount of each food groups are calculated through amount of nutrients found in each component to get daily nutrient consumption to meet Estimated Dietary Allowance daily.

**Table 8:** analyzed and calculated nutrient values from food samples

| Minerals         | Injera | Shiro stew | Kik stew | Meat stew | Injera diblik |
|------------------|--------|------------|----------|-----------|---------------|
| Iron (mg/100g)   | 12.76  | 9.4        | 17.5     | 3.7       | 20.7          |
| Zinc (mg/100g)   | 2.6    | 1.5        | 2.5      | 2.2       | 2.05          |
| Copper (mg/100g) | 0.5    | 0.3        | 0.5      | 0.3       | 0.35          |

## 5. Discussion

Copper and zinc are essential metals for biochemical processes in the body. Assessment of copper (Milne, 1998; Harvey *et.al.*, 2009) and zinc (Hess *et.al.*, 2007) status of individuals is dependent on quantification of total copper and zinc concentrations in serum. These are the most widely used laboratory tests for diagnosis of severe deficiency and to monitor adequacy of provision.

This study investigated the prevalence of dietary pattern, anemia, micronutrient deficiency (zinc and copper), and infection in adolescent girls. The dietary pattern had shown over-reliance on plant-based diets and low animal source food consumption. Surprisingly, the prevalence of anemia, and copper and iron deficiency was very low. No case of ID and severe anemia were reported. However, the prevalence of zinc deficiency was high.

The observed dietary pattern in this study is in agreement with reports that showed reliance of adolescent girls, especially in developing countries, on plant-based diets with a low consumption of animal source food (Aurino, 2017). Studies in Ethiopia, both regional and national, food consumption surveys reported similar findings (DHS, 2011, Belachew *et al.*, 2013). It is well known that plant-based diets contain poorly bioavailable form of iron (non-heme) and absorption inhibitors such as phytates and tannins. The latter significantly reduces absorption by forming insoluble complexes with non-heme iron. These factors will likely results in iron deficiency.

Surprisingly, the prevalence of anemia and iron deficiency in this study was found to be low (8 %), which is indicative for mild public health problem as per the WHO (2008). This value is even lower than national prevalence of anemia in adolescent girls (14.5 – 19.9 %) (2011 EDHS). Furthermore, low prevalence of iron deficiency (10.5 %) is also reported in adolescent and non-pregnant women of reproductive age groups in Ethiopia (NMNS, 2011). Other studies also reported low prevalence of iron deficiency in population consuming mainly plant based diets (Gashu *et.al.*, 2016; Gebreegziabher *et.al.*, 2017). This might be due to the contribution of contaminant iron (extrinsic iron) introduced to the food

during traditional processing steps to the iron status. For instance, in Ethiopia, it is reported that about 63 % of women of reproductive age is consuming iron in excess of their daily requirement. Furthermore, animal study has shown the potential of contaminant iron joining common non-heme iron pool and thus available for absorption (Habtamu and Kaleab, 2018).

Even if, no clinical iron deficiency was observed, about half of the adolescent girls' had a marginal iron store. Since, adolescent girls are future mothers and about 21 million adolescent girls in developing countries become pregnant (WHO, 2018), appropriate intervention strategies to restore the iron status are needed. Furthermore, this study reported significantly higher prevalence of zinc deficiency (49 %). Results reported by the IZiNCG, stating that 21.1%, using stunting prevalence (> 20 %) as a proxy, of the Ethiopian population was at risk of inadequate dietary zinc intake.

Zinc deficiency results from an insufficient intake of zinc, which is primarily found in animal protein as well as in whole-grain cereals, pulses, and legumes, but, plant sources are considered to be less bioavailable because of the presence of phytic acid that binds to zinc-forming insoluble complexes, which thus inhibits zinc's absorption. Zinc deficiency can also be caused by poor absorption or excessive loss of the mineral. Because the human body cannot store zinc, a consistent supply of the mineral in the diet is necessary to fulfill zinc requirements (Lowe, Fekete, and Decsi 2009). Generally, the main reason is in most of rural areas of Ethiopia consumption of animal source food is mostly limited to occasional public holidays, which indicates minimal intake.

Low zinc content of the diet and consumption of dietary and other substances that lower zinc bioavailability are important factors in the pathogenesis of primary zinc deficiency. Inhibitors of zinc absorption include phytate, dietary fiber, oxalate, and products of Millard browning, casein, iron, calcium, and cadmium (Sandstead, 1991; Sandstead, et.al, 1972; Pellett, et.al., 1999). Phytate, which is present in whole-grain cereals and legumes, is probably the most important dietary inhibitor of zinc absorption. Ferguson *et al.* (1993) confirmed that a high intake of phytic acid relative to zinc was a risk factor for

inadequate zinc nutrition and stunted growth in Malavian children. Zinc supplementation studies in stunted children from non-industrialized countries with or without biochemical signs of zinc deficiency or low intake of highly bioavailable zinc, yield conflicting results. Zinc supplementation produced highly significant, positive responses in height and weight increments.

About 20 % of the population was also copper deficient. The mean serum level of copper in this study population was  $155.8 \pm 105$  mg/dL. In a previous research carried in Gondar town, in Amhara Regional State, Ethiopia, Amare *et al*, (2012) found a mean value of  $191.30 \pm 50.17$  mg/dl in 100 urban school-aged children, and in other study carried out in LiboKemkem and Fogera Districts, Amhara Regional State was  $135.60 \pm 28.20$  mg/dl; figures which are higher than this finding (Herrador *et.al*, 2014).. This difference may be partially accounted for by the lack of rural communities in their sample, taking into account that rural-urban disparities for this micronutrient have been previously reported in the literature.

Common dietary sources of copper include animal liver, seafood (oysters, lobster), sesame seeds, cocoa products, and nuts. Deficiency of Cu has been considered a rarity. However, reports of increased Cu requirements in association with fructose consumption, and observations from the United States of America that the intake of Cu is often below the recommended intake (Laitinen *et al*. 1989), suggest that subclinical Cu deficiency may be rather common.

## 6. Conclusion and recommendation

Results from our study demonstrate that the prevalence of low serum zinc level ( $<65\mu\text{g/dl}$ / $<10.7\text{ }\mu\text{mol/l}$ ) was high and the prevalence of low serum copper level ( $<90\mu\text{g/dl}$  / $<14.2\text{ }\mu\text{mol/L}$ ) was low in a sample of adolescent girls. The magnitude of anemia determined in this study (8.7%) is considered as a moderate public health problem according to WHO standards.

Zinc deficiency is prevalent in the study area due to low intake of zinc rich food. Direct approaches to increase the zinc status of adolescent girls should aim at increasing the amount of zinc intake either through dietary diversification, or reducing intake of inhibitors of zinc absorption through different processing techniques. Dietary diversification to include more micronutrient rich foods in diet is generally considered to be a sustainable approach in addressing most micronutrient deficiencies. However, in the context of Huruta, inclusion of foods rich in bioavailable zinc will be difficult to achieve since zinc rich foods are usually animal origin and high cost. In this study area, the consumption pattern are based on cereals and plant based which have high content of phytate, so, processing techniques such as soaking, germination or fermentation at household based should be encouraged.

Copper deficiency states are relatively rare in human but have been associated with premature infants, patients on total parental nutrition and severely malnourished children.

Up to date, only few countries have conducted surveys on micronutrients status at the national or sub national level, and most of them are focused on limited number of micronutrients. Given the paucity of previous data, our results represent a starting point for future research. Zinc deficiency is a commonly overlooked yet widespread public health problem with nearly half of the world's population at risk of insufficient intake. Diet diversification strategies are far less common, despite of dietary diversity being universally recognized as a key component of nutrition programs.

A daily intake of Cu and Zn is required to maintain a steady state because the body has no specialized storage system of these two elements. Therefore, major efforts are needed to close critical knowledge gaps, to test and scale-up improved and affordable interventions, and to disseminate evidence to decision makers and stakeholders to fully respond to this key public health problem. This will help to rapidly and sustainably improve the diet diversity and the nutritional status in the study setting, which would have a positive impact on economic growth and development.

## 7. References

- Amare, B., Moges, B., Moges, F., Fantahun, B., Admassu, M., Mulu, A., & Kassu, A. (2012). Nutritional status and dietary intake of urban residents in Gondar, Northwest Ethiopia. *BMC public health*, 12(1), 752.
- Arora, S., & Kapoor, R. K. (2012); Iron Metabolism in Humans: An Overview in Iron Metabolism. InTech.
- Arimond, M., Wiesmann, D., Becquey, E., Carriquiry, A, Daniels, C. M., Deitchler, M., ... Torheim, L. E. (2010) Simple food group diversity indicators predict micronutrient adequacy of women's diets in 5 diverse, resource-poor settings. *Journal of Nutrition*, 140(11), 2059S-2069S. <http://dx.doi.org/10.3945/jn.110.123414>
- Aurino, E., Fernandes, M., & Penny, M. E. (2017). The nutrition transition and adolescents' diets in low- and middle-income countries: a cross-cohort comparison. *Public health nutrition*, 20(1), 72-81.
- Balcı, Y. I., Karabulut, A., Gürses, D., & Çövüt, İ. E. (2012). Prevalence and risk factors of anemia among adolescents in Denizli, Turkey. *Iranian journal of pediatrics*, 22(1), 77.
- Belachew, T., Haley, C., & Lindstorm, D. (2008). Differentials in measures of dietary quality among adolescents in Jimma zone, Southwest Ethiopia. *Ethiop Med J*, 46(2), 133-42.
- Bhowmik, D., Chiranjib, K.P., and Kumar, S. (2010). A potential medicinal importance of zinc in human health and chronic. *Int J Pharm Biomed Sci* 1 (1), 05-11.
- Brown, K. H., Wuehler, S. E., & Peerson, J. M. (2001). The importance of zinc in human nutrition and estimation of the global prevalence of zinc deficiency. *Food and Nutrition Bulletin*, 22(2), 113-125.
- Central Statistical Agency (CSA) [Ethiopia] and ICF. 2016. Ethiopia Demographic and Health Survey 2016. Addis Ababa, Ethiopia, and Rockville, Maryland, USA: CSA and ICF.

- Cerovic, A., Miletic, I., Blagojevic, D., Sobajic, S., Vasiljevska, M., Poznanic, M., & Radusinovic, M. (2008). Effects of Low, Adequate and High Dietary Zinc Intake on Metabolic Interactions between Zinc, Copper and Iron in Different Mongolian Gerbil Tissues. *Acta Veterinaria Brno*, 77(1), 17-23.
- Chaudhary, S. M., & Dhage, V. R. (2008). A study of anemia among adolescent females in the urban area of Nagpur. *Indian journal of community medicine: official publication of Indian Association of Preventive & Social Medicine*, 33(4), 243.
- Choi, J. W., & Kim, S. K. (2005). Relationships of lead, copper, zinc, and cadmium levels versus hematopoiesis and iron parameters in healthy adolescents. *Annals of Clinical & Laboratory Science*, 35(4), 428-434.
- Collins, J. F., Wessling-Resnick, M., & Knutson, M. D. (2008). Hepcidin regulation of iron transport. *The Journal of nutrition*, 138(11), 2284-2288.
- Dahl, R. E. (2004). Adolescent brain development: a period of vulnerabilities and opportunities. Keynote address. *Annals of the New York Academy of Sciences*, 1021(1), 1-22.
- De Freitas, J. M., & Meneghini, R. (2001). Iron and its sensitive balance in the cell. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, 475(1), 153-159.
- Deshpande, J. D., Joshi, M. M., & Giri, P. A. (2013). Zinc: The trace element of major importance in human nutrition and health.
- FAO, F. (2016). Minimum dietary diversity for women: a guide for measurement. Rome: FAO.
- FHI, F. (2016). 360. Minimum dietary diversity for women: a guide for measurement. Rome (Italy): FAO.
- Ferguson, E. L., Gibson, R. S., Opare-Obisaw, C., Ounpuu, S., Thompson, L. U., & Lehrfeld, J. (1993). The zinc nutriture of preschool children living in two African countries. *The Journal of nutrition*, 123(9), 1487-1496.

- Fraga, C. G. (2005). Relevance, essentiality and toxicity of trace elements in human health. *Molecular aspects of medicine*, 26(4-5), 235-244.
- Frassinetti, S., Bronzetti, G. L., Caltavuturo, L., Cini, M., & Della Croce, C. (2006). The role of zinc in life: a review. *Journal of environmental pathology, toxicology and oncology*, 25(3).
- Gashu, D., Stoecker, B. J., Adish, A., Haki, G. D., Bougma, K., & Marquis, G. S. (2016). Ethiopian pre-school children consuming a predominantly unrefined plant-based diet have low prevalence of iron-deficiency anaemia. *Public health nutrition*, 19(10), 1834-1841.
- Gallagher, C. M., Black, L. J., & Oddy, W. H. (2014). Micronutrient intakes from food and supplements in Australian adolescents. *Nutrients*, 6(1), 342-354.
- Gautam, C. S., Saha, L., Sekhri, K., & Saha, P. K. (2008). Iron deficiency in pregnancy and the rationality of iron supplements prescribed during pregnancy. *The Medscape Journal of Medicine*, 10(12), 283.
- Gebreegziabher, T., & Stoecker, B. J. (2017). Iron deficiency was not the major cause of anemia in rural women of reproductive age in Sidama zone, southern Ethiopia: A cross-sectional study. *PloS one*, 12(9), e0184742.
- Gharibzahedi, S. M. T., & Jafari, S. M. (2017). The importance of minerals in human nutrition: Bioavailability, food fortification, processing effects and nanoencapsulation. *Trends in Food Science & Technology*, 62, 119-132.
- Goldhaber, S. B. (2003). Trace element risk assessment: essentiality vs. toxicity. *Regulatory toxicology and pharmacology*, 38(2), 232-242.
- Greger, J. L., Higgins, M. M., Abernathy, R. P., Kirksey, A., DeCorso, M. B., & Baligar, P. (1978). Nutritional status of adolescent girls in regard to zinc, copper, and iron. *The American journal of clinical nutrition*, 31(2), 269-275.

- Griffin, I. J., Kim, S. C., Hicks, P. D., Liang, L. K., & Abrams, S. A. (2004). Zinc metabolism in adolescents with Crohn's disease. *Pediatric research*, 56(2), 235.
- Habtamu Guja, Kaleab Baye. (2018). extrinsic iron from soil contributes to the hemoglobin regeneration of anemic rats: implication for foods contaminated with soil iron. *British Journal of Nutrition*, 119, 880-886.
- Harvey, L. J., Ashton, K., Hooper, L., Casgrain, A., & Fairweather-Tait, S. J. (2009). Methods of assessment of copper status in humans: a systematic review—. *The American journal of clinical nutrition*, 89(6), 2009S-2024S.
- Hegazy, A. A., Zaher, M. M., Abd el-hafez, M. A., Morsy, A. A., & Saleh, R. A. (2010). Relation between anemia and blood levels of lead, copper, zinc and iron among children. *BMC research notes*, 3(1), 133.
- Herrador, Z., Sordo, L., Gadisa, E., Buño, A., Gómez-Rioja, R., Iturzaeta, J. M., & Cañavate, C. (2014). Micronutrient deficiencies and related factors in school-aged children in Ethiopia: a cross-sectional study in LiboKemkem and Fogera districts, Amhara Regional State. *PLoS One*, 9(12), e112858.
- Hess, S. Y., Pearson, J. M., King, J. C., & Brown, K. H. (2007). Use of serum zinc concentration as an indicator of population zinc status. *Food and nutrition bulletin*, 28(3\_suppl3), S403-S429.
- Hotz, C., Pearson, J. M., & Brown, K. H. (2003). Suggested lower cutoffs of serum zinc concentrations for assessing zinc status: reanalysis of the second National Health and Nutrition Examination Survey data (1976–1980). *The American journal of clinical nutrition*, 78(4), 756-764.
- Jian-Xin, Q. (1990). Determination of Cu, Zn, Fe, Ca, Mg, Na and K in serum flame by atomic absorption spectroscopy. *Varian Instruments at Work's, Palo Alto*, 93, 1-2.
- Khambalia, A. (2009). Periconceptional Iron Supplementation and Iron and Folate Status among Pregnant and Non-pregnant Women in Rural Bangladesh (Doctoral dissertation).

- Laitinen, R., Vuori, E., Dahlström, S., & Åkerblom, H. K. (1989). Zinc, copper, and growth status in children and adolescents. *Pediatric research*, 25(4), 323.
- Lowe, N. M., Fekete, K., & Decsi, T. (2009). Methods of assessment of zinc status in humans: a systematic review-. *The American journal of clinical nutrition*, 89(6), 2040S-2051S.
- Macro, I., & Measure, D. H. S. (2011). Ethiopia demographic and health survey 2011: preliminary report. Central Statistical Agency and ICF Macro: Addis Ababa, Ethiopia and Calverton, Maryland, USA.
- Milne, D. B. (1998). Copper intake and assessment of copper status. *The American journal of clinical nutrition*, 67(5), 1041S-1045S.
- Mocchegiani, E., Romeo, J., Malavolta, M., Costarelli, L., Giacconi, R., Diaz, L. E., & Marcos, A. (2013). Zinc: dietary intake and impact of supplementation on immune function in elderly. *Age*, 35(3), 839-860.
- Namaste, S. M., Rohner, F., Huang, J., Bhushan, N. L., Flores-Ayala, R., Kupka, R. ... & Northrop-Clewes, C. A. (2017). Adjusting ferritin concentrations for inflammation: Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) project. *The American journal of clinical nutrition*, 106(suppl\_1), 359S-371S.
- Onis, M. D., Onyango, A. W., Borghi, E., Siyam, A., Nishida, C., & Siekmann, J. (2007). Development of a WHO growth reference for school-aged children and adolescents. *Bulletin of the World health Organization*, 85, 660-667.
- Osredkar, J., & Sustar, N. (2011). Copper and zinc, biological role and significance of copper/zinc imbalance. *J Clin Toxicol S*, 3(001), 2161-0495.
- Pellett, P., & Ghosh, S. (1999). Global nutritional problems with special attention to the role of animal foods in human nutrition. *Global Agenda for*, 25.

- Perrone, L., Gialanella, G., Moro, R., Feng, S.L., Boccia, E., Palombo, G., Carbone, M.T. and Di Toro, R., (1998). Zinc, copper, and iron in obese children and adolescents. *Nutrition research*, 18(2), pp.183-189.
- Plich, S. M., & Senti, F. R. (1984). Assessment of the iron nutritional status of the US population based on data collected in the second National Health and Nutrition Examination Survey. 1976-1980. Rockville MD: Life Science Research Office, Federation of American Societies for Experimental Biology.
- Popkin, B. M. (1994). The nutrition transition in low-income countries: an emerging crisis. *Nutr Rev*, 52, 285-298. <http://dx.doi.org/10.1111/j.1753-4887.1994.tb01460>.
- Prasad, A. S. (2003). Zinc deficiency: has been known of for 40 years but ignored by global health organisations.
- Prasad, A. S. (2008). Zinc in human health: effect of zinc on immune cells. *Molecular medicine*, 14(5-6), 353.
- Sandstead, H. H., Gillespie, D. D., & Brady, R. N. (1972). Zinc Deficiency. *Pediatric Research*, 6(2), 119-125.
- Sandstead, H. H. (1991). Zinc deficiency: a public health problem?. *American Journal of Diseases of Children*, 145(8), 853-859.
- Siddharam, S. M., Venketesh, G. M., & Thejeshwari, H. L. (2011). A study of anaemia among adolescent girls in rural area of Hassan district, Karnataka, South India. *Int J Biol Med Res*, 2(4), 922-4.
- Singh, M. (2004). Role of micronutrients for physical growth and mental development. *The Indian journal of pediatrics*, 71(1), 59-62.

- Scherz, H., & Kirchhoff, E. (2006). Trace elements in foods: zinc contents of raw foods—a comparison of data originating from different geographical regions of the world. *Journal of Food Composition and Analysis*, 19(5), 420-433.
- Stefanidou, M., Maravelias, C., Dona, A., & Spiliopoulou, C. (2006). Zinc: a multipurpose trace element. *Archives of toxicology*, 80(1), 1.
- Underwood, E. (2012). *Trace elements in human and animal nutrition*. Elsevier.
- Urbano, M. R., Vitalle, M. S., Juliano, Y., & Amancio, O. (2002). Iron, copper and zinc in adolescents during pubertal growth spurt. *Jornal de pediatria*, 78(4), 327-334.
- Vallee, B. L., & Falchuk, K. H. (1993). The biochemical basis of zinc physiology. *Physiological reviews*, 73(1), 79-118.
- Vokurka, M., Krijt, J., Sulc, K., & Necas, E. (2006). Hepcidin mRNA levels in mouse liver respond to inhibition of erythropoiesis. *Physiological Research*, 55(6), 667.
- Vyoral, D., & Petrák, J. (2005). Hepcidin: a direct link between iron metabolism and immunity. *The international journal of biochemistry & cell biology*, 37(9), 1768-1773.
- Wick, M., Pinggera, W., Pinggera, W., & Lehmann, P. (2003). *Clinical Aspects and Laboratory. Iron Metabolism, Anemias: Iron Metabolism, Anemias: Novel Concepts in the Anemias of Malignancies and Renal and Rheumatoid Diseases*. Springer Science & Business Media.
- Widdowson, E. M., & McCance, R. A. (1937). The absorption and excretion of iron before, during and after a period of very high intake. *Biochemical Journal*, 31(11), 2029.
- Willis, M. S., Monaghan, S. A., Miller, M. L., McKenna, R. W., Perkins, W. D., Levinson, B. S. ... & Kroft, S. H. (2005). Zinc-induced copper deficiency: a report of three cases initially recognized on bone marrow examination. *American journal of clinical pathology*, 123(1), 125-131.

World Health Organization. (2014). Health for the world's adolescents: a second chance in the second decade: summary.

## **Annex 1: Consent form (Afan oromo)**

**Addis Ababa University (Yuunivarsiitii Addis Ababaa)**

**College of Natural and Computational Sciences (Kolleejjii saayinsii uumamaafi madaallii)**

**Center for Food Science and Nutrition (Goddu gala saabinsii nyaataafi sirna nyaataa)**

### **Questionnaire for the study on “Investigation of the etiology of anemia among adolescent girls (15 - 20 years of age) in Ethiopia” (Gaaffiiwwan qu’annoo Qorannoo ka’uumsa dhukkuba hir’ina dhiigaa Sharreewwan ga’eevyii “umurii 15-20” gidduu jiruu” kan Ethiophiyaa keessa jiranii)**

Nagaa jirtuu, maqaankoo walii galtee jedhama. Yuunivarsiitii Addis Ababaatti giddugaa saainsii nyaataafi sirna nyaataan hojjadha. Qorannoo ka’umsad hukuba hindhina dhiigaa shamarree itaphiyaa keessa jiraatan kan ummuriin isaanii 15-20 ta’aniifi dahanii kan hin beekne akkasumas ulfan kan hintaane gidduutti kan mudatu gaggessaa jirra.

Hirmaannaa isin gootaniif baayyee isin galateeffanna. Hirmaannaan keessan saba dhukkuba hirdhina dhiigaa akka hubannuuf hedduu nu gargaara. Warra dhimmi ilaallatuf karoora agarsiisuunis biyyi keenyaafi addunyaa irratti sababa dhukkuba kanaan kan rakkatan gargaaruuf hedduu gargaara.

Qorannoo kana keessatti hirmaachuuf fedhii yoo qabaattan, dhiiga mililifra 6 (faldhaana sukkaasa tokko kan ta’u) akka kennitaniifi qorannoo hirdhina dhiigaa akka gootan gaafanna. Bobbaa keessan fudhannees raammolee garaa keessa hammaayiranii fi ilbiisota biroo qu’achuu barbaanna. Ulfaatina, dheerinaafi furdina hirree keessaniis safaruu malla. Dhumarratti waliigala qabeenya, nyaataafi sirna nyaataa keessan isin gaafanna.

Hirmaannaan isin gootan fedhiidhaani. Gaaffii isin hin barbaanne yoo isin gaafanne nutty himaa irra ceena gara itti aanutti. Ykn ammo gaaffiif deebii yeroo barbaaddan dhaabuu dandeessu.

Hirmaannaan keessan baayyee waan barbaachisuuf akka hirmaattan abdii qabna. Yeroo dhiiga fudhannu dhukkubbiin xiqqoon isinitti dhagahama. Bakki isaas xiqqoo diimachuu fi dhiiga maddee dhiiguu, dhiitawuu ni danda’a. namni tokko tokko xiqqoo mataan naannawuu fi wallaaluus ni danda’a. haa ta’u malee namni baay’een akkas hin ta’u.

Qapxiin dhiiga keessani fi ragaan isin kennitan icitiin qabama, nama kamiifuu dabarsinee hin himnu warren qorannoo gaggeessan qofatu arga. Hirmaachuu keessaniin dura gaaffii qabdan yoo jiraate gaafadhaa. Qoratamaa osoo jirtaniis gaafachuu dandeessu.

Maqaa hirmaataa \_\_\_\_\_

Mallattoo hirmaataa (waggaa 18%) \_\_\_\_\_

Maqaa warraa (guddiftuu)\_\_\_\_\_

Maqaa warraa (guddiftuu) \_\_\_\_\_

## Annex 2: Consent form (Amharic)

aë^S ab² y,,n@vRs^t  
ytfEÄ a³ xMp#Wt†}³L ±YNS x1\_J  
yMGB ±YNS A³ n@WTP}N iOkL

bw½T s Ä{ (15 - 20 ODì... KLL WSE @lf) @Y lan...i@¶ yi@¶-Le& MKN¶Ä{N liwQ ytz-j meYQ

### SMMnT

An... \_\_\_\_\_ A²@lu'f kaë^S ab² y,,n@vRs^t# yMGB ±YNS³ n@WTP}N iOkL  
-R bw½T s Ä{ (ODì...¶[W k 15 - 20 yòñ&mkz^U bÜT LJ ¶Lwlë& A³ YU E³T bi@·¾...DbT wQT ARg&Z ¶Lòñ&)  
@Y lan...i@¶ (dM ìñS) yi@¶-Le& MKN¶Ä{N A³e³lN' yARS¿ t†TÄ lan...i@¶ a-@X yòñ& MKN¶Ä{N bELqT ANDNré  
¶Gz³L' kE³t>M yi@giWN We\_T ltl¶y,, y²Ldrá a·@T A³±WšlN' YUM an@i¶N bkFt¾ dré lmqnS BÉM liE''T  
bugR aqF dré Ayt·¾...d ¶lWN ErT ligZ YrëL' bz^UM MKN¶T yARS¿ bz^U E³T @Y m†tF kFt¾ eqi...ª alW'

bz^U E³T @Y lm†tF s^wSn& kKND¿ @Y 6 i@l^ (uflT yáy ìnk^¶) dM ANwSëln' YUM an...i@¶ ANëlb¿T A³  
ANDl\_lB¿T lir-gE ANë^ufM yaYrN A³ y<Yªi@Ñ{ meN ¶±Wq³L' kz^UM btxiþ Eg¾ yòñ& T@TÉ{ mÑR almnÑ™[WN  
lir-gE yaYrN meN ANë^ufM yrq#Q t\$S^¶N SBER liwQ sg™ ANwSëln' btxiþM KBdT¿TN q>mT¿TN A³ yKND¿N  
a-i} men z&þ¶ (mf\$K) ANl·lN' aNëND b\_ts²\$# A³ a^×Ñi¶\$#@ t×R yòñ& kMGB \$ST³ A³ kam-gB SBER -R  
yt¶¶z& E¶qt¿{N ANeYÖªlN'

bz^U E³T @Y yi@¶dRg&T t†TÄ bFšdilt @Y Bê ytmsrt nW' mmlS yiYfLg&T E¶qt† -em¿ An...N ¶±Wq>I A³  
wdi@qElW E¶qt† aL''luf' btxiþM biN¾WM g^z\_ E¶qt† A³ mLs&N iÖM Y{öl'f' òÑM GN yARS¿ t†TÄ bAJg& aSf@g^  
Slòñ lm†tF YSiillf BlN tS'' A³dR-lN'

dM bi@wsDbT g^z\_ mRf\_W yi@w-¿T a·²b^ men¾ UmMª mQ@Tª men¾ dM mFsSª ABeT A³ ysMbR aYnT MLKT  
l^¶±y,, Y{öl' btxiþM b¾M bEq#T s¿{ @Y ymFzZ wYM ym°L Si...T l^¶-Ei[W Y{öl' òÑM GN Bz& s¿{ MNM aYnT  
{GR a¶-Ei[WM' bE³t> @Y yi@¶qRb&T uflfM mré¿{ A³ ydM MRm™ We\_Ä{ bEBQ i@SERnT Y¶°lf'

lm†tF kmwsN¿ bÜT iN¾WM aYnT E¶qt† ·l¿T ¶±Wq>N' btxiþM bE³t> ¾@dT WSEM iN¾WNM aYnT E¶qt† meYQ  
Y{öl'f' kz^U bª{ SM¿N :fW kfrmf bz^U E³T WSE t†ªÜ lmòN tSiMt\$SL ilt nW'

yt†ªÜ SM \_\_\_\_\_

yt†ªÜ ÜRi (k 18 ODì... b@Y lòn&) \_\_\_\_\_

yw@J wYM a±ëg^ SM \_\_\_\_\_

yw@J wYM a±ëg ÜRi (k 18 ODì... bª{ lòn&) \_\_\_\_\_

## **Part I. General information (eQ®® mré)**

## Part II. Anthropometry, hookworm infection and hemoglobin measurements

**Please, if the participant's hemoglobin level is below 7.6 g/dl (severely anemic) refer to the nearest health facility** (ytl<sup>a</sup>Ü\$ y<sup>3</sup>...ÀGÉb<sup>N</sup> meN k 7 G/d\_s<sup>a</sup> l<sup>a</sup>TR b<sup>a</sup>{ kÒn baS[èY bQRB wdi@gI e\_<sup>3</sup> ½b<sup>a</sup>¶ ANë<sup>¾</sup>...ë& ¶DRq&)

➤  $yt^{\pm a} \ddot{U}_S \ y^{\frac{3}{4}} \dots \dot{A}GEb^{\wedge N} \ meN \ k \ 12.6 \ G/d\_s^{\wedge} \ 1^{\wedge TR} \ b^a \{ \ k\ddot{O}n \ ban \dots i@ \mathbb{I} \ wYM \ ydM \ i\grave{n}S \ \hbar\hbar\eta\tau\omega \cdot i\grave{l}T$   
 $n\dot{W}$

➤  $yt \pm^a \ddot{U}S \ y^{\mathfrak{A}} \dots \grave{A}G\acute{E}b^{\wedge}N \ meN \ k \ 12.6 \ G/d_s^{\wedge} \ 1^{\wedge}TR \ b^{\circledast}Y \ k\grave{O}n \ an \dots i@ \mathfrak{T} \ wYM \ ydM \grave{i}nS \ y^{1^2} [wM \grave{i}lT$   
 $n\vec{W}$

### Part III. Household characteristics (yb\_tsB ²UR¶T)

| No.<br>(ጥ.ቁ.) | Question (፫፻፲ታ)                                                                                                                                                                            | Response (ጠረፍ)                                                                                                                                                                                                                                                                                                                                                                   | Code<br>(×D) |
|---------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------|
| 1             | Household composition<br>(yb_tsB SBER)                                                                                                                                                     | <p>Number</p> <p>Total household size<br/>(eQ® yb_tsB B°T) _____</p> <p>Head of the household      Father (□□□) (01)<br/>(yb_tsb&amp; ,®Ü)      Mother (□□□) (02)</p> <p>Another male (□□□□ □□□□) (03)</p> <p>Another female (□□□□□□□□) (04)</p> <p>Relationship to head of the household (kb_tsb&amp; ቆ፻፻<br/>-R ¶l፻T GNi,,nT (yቢተሰቡ ቆ፻፻ ኣላባት ወይም ኣእናት ውጪ ከሆነ)</p> <p>_____</p> |              |
| 2             | What is the main occupation of the household head?<br>(yb_tsb& ,®Ü §³ S™[W/§ MNDN nW?                                                                                                      | <p>Wage income (S™ የሌለው) (01)</p> <p>Housewife (የቤት እመቤት) (02)</p> <p>Personal work/small business (ሃግል ስራ/አነስተኛ ንግድ) (03)</p> <p>Government employee (ሃገራዊ ስራ ተቀጣሪ) (04)</p> <p>Private organization employee (ሃግል ድርጅት ተቀጣሪ) (05)</p> <p>NGO (mNGSª§# ¶LÖn DRJT ተቋም) (06)</p> <p>Daily laborer (የቀን ስራተኛ) (07)</p> <p>Other (1_®) (08) _____</p>                               |              |
| 3             | Has (Name of the household head) ever attended school?<br>(yb_tsb& ,®Ü SM) TMURT b_T gBÄ tMÄaL?) If the answer is no, please go to question No. 5 (ጠረፍ aY ኣስከረ ለ²K፻ wd ፫፻፲ታ ቁ፻ER 5 ሃ፻፳፻፳፻) | <p>Yes (a፻) (01)</p> <p>No (aY) (02)</p>                                                                                                                                                                                                                                                                                                                                         |              |
| 4             | What is the highest level of school (Name of the household head) attended? (yb_tsb& ,®Ü SM) ytkªtlfT kFt፻ yTMURT dré MNDN nW?                                                              | <p>Primary (mjጠ፻፻ dré) (01)</p> <p>Secondary (ufl፻፻ dré) (02)</p> <p>Technical/Vocational (፻፻Kn®K³/፻፻፻) (03)</p> <p>University/College Diploma (□□□□□□/□□□□□□) (04)</p> <p>University/College Degree and above (□□□□□□/□□□□ □□□ □□□ □□□ □□□) (05)</p> <p>Other (1_®) (05) _____</p>                                                                                              |              |
| 5             | What is the primary source of income of the household?<br>(yb_tsb& §³ ygb^ MNX MNDN nW?                                                                                                    | <p>Wage income (yg&amp;LbT S™) (01)</p> <p>Pension (e&amp;rª) (02)</p> <p>Salary (dmwZ) (03)</p> <p>Business (NGD) (04)</p> <p>Agricultural production (yARá MRT) (05)</p> <p>Remittances (kWä yì®®K gNzB) (06)</p> <p>Other (1_®) (07) _____</p>                                                                                                                                |              |



|   |                                                                                                                                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |  |
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|   |                                                                                                                                  | Don't know (□□□□□) (02)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |  |
| 8 | <p>What kind of toilet facility do members of your household usually use?</p> <p>yARSḡ b_tsB yi@eqMbT y}NT b_T aYnT MNDN nW?</p> | <p>Flush or pour flush toilet (iFsá ḡlW }NT b_T)</p> <ul style="list-style-type: none"> <li>➤ Piped sewer system (bt&gt;İ F±} iSwgé) (01)</li> <li>➤ Septic tank (yF±} ie™qi@ḡ) (02)</li> <li>➤ Pit latrine (yg&amp;DæD m'ëé b_T) (03)</li> <li>➤ Elsewhere (l_® İª) (04)</li> <li>➤ Unknown place (ḡLªwq İª) (05)</li> </ul> <p>Pit latrine (g&amp;DæD m'ëé b_T)</p> <ul style="list-style-type: none"> <li>➤ Ventilated improved pit latrine (VIP) (ytál yN``S mW´ ḡlW) (06)</li> <li>➤ Pit latrine with slab (wlL ḡlW yg&amp;DæD m'ëé b_T) (07)</li> <li>➤ Pit latrine without slab/open pit (wlL yl_lW yg&amp;DæD m'ëé b_T) (08)</li> </ul> <p>Composting toilet (bሴራሚክ የተሰራ (ከፍ ያለ መቀመጫ ያለው})NT b_T) (09)</p> <p>Bucket toilet (bÆDæë Aš WSE) (10)</p> <p>Hanging toilet/Hanging latrine (ግድግዳ ላይ ytsq1 bሴራሚክ የተሰራ }NT b_T) (11)</p> <p>No facility/Bush/Field (}NT b_T y1M/bq&gt;EåÖ/bi...ë ©Y) (12)</p> <p>Other (l_®) (13) _____</p> |  |

| No.<br>(ጥ.ቀ>) | Question (Eḡqt)                                                                                         | Response (mLS)                           | Code<br>(×D) |
|---------------|---------------------------------------------------------------------------------------------------------|------------------------------------------|--------------|
| 9             | <p>Do you share this toilet facility with other households?</p> <p>□□□□ □□□□□ □□□□□ □□ □□□ □□ □□□□?</p> | <p>Yes (aḡ) (01)</p> <p>No (aY) (02)</p> |              |
| 10            | <p>Does anyone smoke inside your house often?</p> <p>bb_Tḡ WSE aB°¼WN g^z_ s^-™ yi@ḡxS</p>              | <p>Yes (aḡ) (01)</p> <p>No (aY) (02)</p> |              |

|    |                                                                            |                       |  |
|----|----------------------------------------------------------------------------|-----------------------|--|
|    | sW al?                                                                     |                       |  |
| 11 | Does your household have:<br>yARS¿ b_tsB alWN¥                             | Yes (a¿)      No (aY) |  |
|    | ➤ Electricity (a_l_KT¿K)?                                                  | 1      2              |  |
|    | ➤ A watch/clock (s¹T)?                                                     | 1      2              |  |
|    | ➤ A radio (™¿^ÇN)?                                                         | 1      2              |  |
|    | ➤ A television (t†l_v^ N)?                                                 | 1      2              |  |
|    | ➤ A mobile phone (yÀ²YL SLK)?                                              | 1      2              |  |
|    | ➤ A non-mobile phone (yb_T SLK)?                                           | 1      2              |  |
|    | ➤ A refrigerator (ìqZqì)?                                                  | 1      2              |  |
|    | ➤ A table (er/_°)?                                                         | 1      2              |  |
|    | ➤ A chair (wNbR)?                                                          | 1      2              |  |
|    | ➤ A bed with cotton/sponge/spring<br>mattress (aL¬ yEE/SÍNJ/SP¿NG<br>F™})? | 1      2              |  |
|    | ➤ An electric "mitad" (ya_l_KT¿K<br>M¿D)?                                  | 1      2              |  |
|    | ➤ A kerosene lamp/pressure lamp<br>(¨ÑS)?                                  | 1      2              |  |
| 12 | Does any member of this household<br>own:<br>yb_tsb& a²L yi@ktl†T alW?     | Yes (a¿)      No (aY) |  |
|    | ➤ A bicycle (BSKl_T)?                                                      | 1      2              |  |
|    | ➤ A motorcycle or motor scooter (yÀ†R<br>BSKl_T)?                          | 1      2              |  |
|    | ➤ An animal-drawn cart (bANs±                                              | 1      2              |  |

|  |                                                                 |   |   |
|--|-----------------------------------------------------------------|---|---|
|  | $y_i @ \pm B^{\wedge} \neg P) ?$                                |   |   |
|  | ➤ A car or truck ( $y_b\_T \ wYM \ yXnT$<br>$mk^{\wedge 3} ) ?$ | 1 | 2 |

| No.<br>(ተ.ቁ.) | Question (ጥያቄ)                                                                                                                                                                                                                         | Response (ጠረጴጤ)                                                                                                                                                                                                                                                                                                                                                                                                                      | Code<br>(×D) |
|---------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------|
| 13            | <p>What type of fuel does your household mainly use for cooking?</p> <p>This question might have more than one answer</p> <p>b_tsB፤ b፳³nT MGB līBsL yī@eqMbT īgī MN aYnT nW?</p> <p>ኦገገ፣ በላይ መልስ ሊኖር ይችላል</p>                          | <p>Electricity (a_l_KT፡K) (01)</p> <p>LPG (f±} nēJ) (02)</p> <p>Natural gas (y፲fEÅ ፋZ) (03)</p> <p>Biogas (²ÇፋZ) (04)</p> <p>Kerosene (ፋZ) (05)</p> <p>Charcoal (ksL) (06)</p> <p>Wood (ANxT) (07)</p> <p>Straw/Shrubs/Grass (g1²/q&gt;EåÖ፤{/±R) (08)</p> <p>Agricultural crop (yGBR³ sBL) (09)</p> <p>Animal Dung (yANs፡፡ AbT) (10)</p> <p>No food cooked in household (bb_T WSE MGB aYbSLM) (11)</p> <p>Other (l_®) (12) _____</p> |              |
| 14            | <p>Does your household have a home garden for cultivation of fruits and vegetables?</p> <p>አትክልትና ፍራፍሬ የሚያመርቱበት የ□□ □□□ □□□?</p>                                                                                                       | <p>Yes (a፤) (01)</p> <p>No (aY) (02)</p>                                                                                                                                                                                                                                                                                                                                                                                             |              |
| 15            | <p>If the answer is yes; how do you use the produced fruits and vegetables?</p> <p>□□□□ □□ □□ □□□□□ □□□□□ □□□□ □□□□ □□□□?</p>                                                                                                          | <p>All the product is for sale (□□□□ □□□□) (01)</p> <p>Part of the product is for sale (□□□□ □□□□) (02)</p> <p>All the product is for food use (□□□□ □□ □□□□□ □□□□□) (03)</p> <p>Others (please mention) (□□□□ □□ □□□)</p> <p>_____</p>                                                                                                                                                                                              |              |
| 16            | <p>Does any member of this household own any agricultural land? If the answer is No, skip the next question (no. 13) and go to question (no.14).</p> <p>yb_tsB፤ a²L yÒn GlSB yARá mr_T alW? mLS&amp; aY kÒn yī@qElWN E፲q፡፡ YZllfT.</p> | <p>Yes (a፤) (01)</p> <p>No (aY) (02)</p>                                                                                                                                                                                                                                                                                                                                                                                             |              |

|    |                                                                                                                                          |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |  |
|----|------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--|
| 17 | <p>How many (local units) of agricultural land do members of this household own?</p> <p>ymr_t&gt; meN (ba·²b^¶\$# ml·¶{ } MN ¶UL nW?</p> | _____                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |  |
| 18 | <p>Does this household own any livestock, herds, other farm animals, or poultry?</p> <p>b_tsb&amp; kBÄ{¤ yRb^ ANs±T wYM yİÄ²lb_T nW?</p> | <p>Yes (ač) (01)</p> <p>No (aY) (02)</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |  |
| 19 | <p>Main material of the floor</p> <p>የቤትዎ ወለል ከምን የተሰራ የተሰራ ነው?</p>                                                                      | <p>Natural floor (ytfEÄ wlL)</p> <ul style="list-style-type: none"> <li>➤ Earth/Sand (afR/a]§) (01)</li> <li>➤ Dung (AbT) (02)</li> </ul> <p>Rudimentary floor (ባህላዊ ወለል)</p> <ul style="list-style-type: none"> <li>➤ Wood planks (yANxT ½W®) (03)</li> <li>➤ Palm/Bamboo (yzN²²/yqR·¾) (04)</li> </ul> <p>Finished floor (yte³qq wlL)</p> <ul style="list-style-type: none"> <li>➤ Parquet or polished (pRk_ (ANxT ymsl bQR©QR: yterb) (05)</li> <li>➤ Wood (ANxT) (06)</li> <li>➤ Vinyl or asphalt strips (yaS¨LT q&gt;™È{ ) (07)</li> <li>➤ Ceramic tiles (s_™i@K) (08)</li> <li>➤ Cement (s^i@NÄ) (09)</li> <li>➤ Carpet (MN½F) (10)</li> </ul> <p>Other (l_®) (11) _____</p> |  |
|    | <p>Main material of the roof</p> <p>የቤትዎ ጣሪያ ከምን የተሰራ የተሰራ ነው?</p>                                                                       | <p>Natural roofing (ytfEÄ ½P¶)</p> <ul style="list-style-type: none"> <li>➤ No roof (½P¶ ylWM) (01)</li> <li>➤ Thatch/Leaf/Mud (y±R/QeL/Xš) (02)</li> </ul> <p>Rudimentary roofing (²U©\$# ½P¶)</p> <ul style="list-style-type: none"> <li>➤ Rustic mat/Plastic sheets (yugR b_T MN½F/P©St#K) (03)</li> <li>➤ Reed/Bamboo (qRk¾) (04)</li> </ul>                                                                                                                                                                                                                                                                                                                                   |  |

|  |                                                                                   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |  |
|--|-----------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--|
|  |                                                                                   | <ul style="list-style-type: none"> <li>➤ Wood planks (yANxT ፯W፬) (05)</li> <li>➤ Cardboard (·RÄN) (06)</li> </ul> <p>Finished roofing (yte³qq ፯፻፱)</p> <ul style="list-style-type: none"> <li>➤ Corrugated iron/metal (yBrT ÖRÖÄ) (07)</li> <li>➤ Wood (ANxT) (08)</li> <li>➤ Asbestos/Cement fiber (yaSb_sÄS/s^i@NÄ) (09)</li> <li>➤ Cement/Concrete (s^i@NÄ/×NK፻T) (10)</li> <li>➤ Roofing shingles (y] ^NGL ፯፻፱) (11)</li> </ul> <p>Other (l_®) (12) _____</p>                                                                                                                                                                                                                                                                                                                                        |  |
|  | <p>Main material of the exterior walls</p> <p>የቤትዎ የውጭ ግድግዳ ከምን የተሰራ የተሰራ ነው?</p> | <p>Natural walls (ytfeÄ GDGë)</p> <ul style="list-style-type: none"> <li>➤ No walls (GDGë y1WM) (01)</li> <li>➤ Cane/Trunks/Bamboo/Reed (agë/GNÎ{/yqR፯) (02)</li> <li>➤ Dirt (Öää) (03)</li> </ul> <p>Rudimentary walls (²U®\$# GDGë)</p> <ul style="list-style-type: none"> <li>➤ Bamboo/Wood with mud (qRK፯/ANxT bXš) (04)</li> <li>➤ Stone with mud (Xš A³ DN¬Y) (05)</li> <li>➤ Uncovered adobe (¶Lt]fn e&amp;B) (06)</li> <li>➤ Plywood (×MÍN±Ä) (07)</li> <li>➤ Cardboard (·RÄN) (08)</li> <li>➤ Reused wood (bD¬i@ EQM ®Y y\$1 ANxT) (09)</li> </ul> <p>Finished walls (yte³qq GDGë)</p> <ul style="list-style-type: none"> <li>➤ Cement (s^i@NÄ) (10)</li> <li>➤ Stone with lime/Cement (bs^i@NÄ A³ Ñ™ DN¬Y) (11)</li> <li>➤ Bricks (b]K®) (12)</li> <li>➤ Cement blocks (BÉk_T) (13)</li> </ul> |  |

|  |  |                                                                                                                                    |  |
|--|--|------------------------------------------------------------------------------------------------------------------------------------|--|
|  |  | <p>➤ Covered adobe (yt]fn e&amp;B) (14)</p> <p>➤ Wood planks/shingles (yANxT ½W®{ /] ^NGLS) (15)</p> <p>Other (1_®) (16) _____</p> |  |
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#### **Part IV. Individual questionnaire (yGlsB meYQ)**

| No. | Question (E¶qt)                                                                                                                                                                  | Response (mLS)                                                                                                                                                                                                                                                                   | Code (×D) |
|-----|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------|
| 1   | <p>Have you ever attended school?</p> <p>If the answer is No, go to Question No. 3</p> <p>TMURT b_T gBtW tMrW ¶Wšlf?</p> <p>mLs&amp; aY kÒn A²Kç wd E¶qt q&gt;ER 3 Y¾@ë&amp;</p> | <p>Yes (aç) (01)</p> <p>No (aY) (02)</p>                                                                                                                                                                                                                                         |           |
| 2   | <p>What is the highest level of school you attended?</p> <p>ydrs&amp;bT kFt¼ yTMURT dré MN ¶UL nW?</p>                                                                           | <p>Primary (mjmb¶ dré) (01)</p> <p>Secondary (uflt¼ dré) (02)</p> <p>Technical/Vocational (t†Kn@K³/mf¶) (03)</p> <p>University/College Diploma (□□□□□□/□□□□□□) (04)</p> <p>University/College Degree and above (□□□□□□/□□□□ □□□ □□□ □□□□) (05)</p> <p>Other (1_®) (06) _____</p> |           |
| 3   | <p>What is your occupation, that is, what kind of work do you mainly do?</p> <p>S™çT MNDN nW (baB°¼W g^z_ MNDN nW yi@sÝT)?</p>                                                   | <p>Wage income (S™ ¶ΛΛው) (01)</p> <p>Student (t¶¶) (02)</p> <p>Personal work/small business (Y¶Δ ñç-/አነስተኛ ንግድ) (03)</p> <p>Government employee (ymንግስት ñç- ተቀጣሪ) (04)</p> <p>Private organization employee (Y¶Δ ድርጅት ተቀጣሪ) (05)</p>                                             |           |

|   |                                                                                                                                                                                                                |                                                                                                                                                                                                     |  |
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|   |                                                                                                                                                                                                                | NGO (mNGS <sup>a</sup> \$# ¶LÒn DRJT tqḡḡḡ) (06)<br>Daily laborer (የቀን ሰራተኛ) (07)<br>Other (l_Ⓢ) (08) _____                                                                                         |  |
| 4 | What is your religion?<br>yARSḡ uYiÑT MNDN nW?                                                                                                                                                                 | Orthodox (ÁRÄÏKS) (01)<br>Catholic (·Äl^K) (02)<br>Protestant (PÄt+S^aNT) (03)<br>Muslim (mfSl^M) (04)<br>Traditional (^UⓈ\$#) (05)<br>Other (l_Ⓢ) (06) _____                                       |  |
| 5 | What is your ethnicity?<br>yARSḡ B³⁴...R MNDN nW?                                                                                                                                                              | Oromo (ÁÄÄ) (01)<br>Amhara (ai <sup>TM</sup> ) (02)<br>Guragie (g& <sup>TM</sup> g_) (03)<br>Sidamo (s^ëÄ) (04)<br>Tigray (TGr_) (05)<br>Welaita (w®Y <sup>a</sup> ) (06)<br>Other (l_Ⓢ) (07) _____ |  |
| 6 | ቀጥሎ ከተጠቀሱት ልጎች ውስጥ የትኛውን ልግ ይናገራሉ                                                                                                                                                                              | Oromiffa (ኦሮምኛ) (01)<br>Amharic (ኦማርኛ) (02)<br>Tigirigna (ትግርኛ) (03)<br>Guragigna (ጉራጊኛ) (04)<br>Sidamegna (ሲዳሞኛ) (05)<br>Other (l_Ⓢ) (06) _____                                                    |  |
| 7 | Can you indicate your mode of birth?<br>yARSḡN yaw@lD uf³t† ¶mLKt>?<br>በቤት ውስጥ ወይም በሆስፒታል እንደተወለዱ ያረጋግጡ<br>የተሳታፊዎ መልስ በቤት ውስጥ የሚል ከሆነ ቁጥር 2 (በቤት<br>ብልት) የሚለውን ይምረጡ። መልሱ በሆስፒታል ከሆነ የአወላጊድ<br>ሁኔታውን በድጋሚ ይጠይቁ። | Cesarean section (ሲዜዕ¶N s_K}N) (01)<br>Vaginal (bs_T BLT) (02)<br>Don't remember (aⓈS^WSM) (03)                                                                                                     |  |

| No. | Question (ፎቃተ)                                                                                                                                                                                                                                | Response (ጠረጴጤ)                                                                                                                                                                                          | Code (×D) |
|-----|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------|
| 8   | <p>Can you indicate if you were breastfed during your infancy?</p> <p>If the answer is yes, how long the breastfeeding has lasted?</p> <p>bU;NnTḡ wQT ሃA³T e&amp;T Yeb&amp; nbR?</p> <p>mLSḡ aḡ kòñ e&amp;T mE²t&gt; ASkm[† DrS yz1q nbR?</p> | <p>Yes (aḡ) (01)</p> <p>No (aY) (02)</p> <p>Don't know (a@SªWSM) (03)</p> <p>Duration (if breastfed) (ለምን ያህል ጊዜ የእናት ጡት ወተት ጠብተዋል?)_____</p>                                                            |           |
| 9   | <p>What is your marital status?</p> <p>y→BÊ ufn...ªḡ MNDN nW?</p>                                                                                                                                                                             | <p>Never married (ፕፀግ²/{) (01)</p> <p>Married (ፕግ²/{) (02)</p> <p>Living together (k→BÊ Wā aBĀ mÑR) (03)</p> <p>Divorced/Separated (bF[፡ ytlፕy,,) (04)</p> <p>Widowed (yTÈR a→™[WN bĀT ፕe&amp;) (05)</p> |           |
| 10  | <p>Have you ever given birth?</p> <p>kz^U bÜT aRGzW ፕWšlf?</p>                                                                                                                                                                                | <p>Yes (aḡ) (01)</p> <p>No (aY) (02)</p>                                                                                                                                                                 |           |
| 11  | <p>When was your last menstrual period?</p> <p>m[† nbR ymxráWN ywR ab² ፕy,,T?</p>                                                                                                                                                             | <p>Date (qN) _____</p>                                                                                                                                                                                   |           |
| 12  | <p>Did you have diarrhea in the last two weeks?</p> <p>²lÛT uflT ±MNªT WSE btQìE ªmW nbR?</p>                                                                                                                                                 | <p>Yes (aḡ) (01)</p> <p>No (aY) (02)</p>                                                                                                                                                                 |           |
| 13  | <p>Have you taken antibiotics in the last three months?</p> <p>²lÛT ÎsT w™T WSE aNt#²Çt#KS ('r-²Kt†ፀ mDun@T) wSdW ፕWšlf?</p>                                                                                                                  | <p>Yes (aḡ) (01)</p> <p>No (aY) (02)</p> <p>Don't remember (a@SªWSM) (03)</p>                                                                                                                            |           |
| 14  | <p>Have you taken any medicine other than antibiotics in the last three months</p> <p>ከባክቴሪያ መከላከያ ውጪ የሚወስዱት ሌላ መድሃኒት አለ</p> <p>መልሱ አዎ ከሆነ እባክዎ የመድሃኒቱን ስም ይጠይቅዎ</p>                                                                          | <p>Yes (aḡ) (01)</p> <p>No (aY) (02)</p> <p>Don't remember (a@SªWSM) (03)</p> <p>የመድሃኒቱ ስም kteqs _____</p>                                                                                               |           |
| 15  | <p>Have you been very sick (hospitalized or had surgery) for a long term (at least two</p>                                                                                                                                                    |                                                                                                                                                                                                          |           |



|    |                                                                                                                                                                                                                                                  |                                                                                                                                                                                                                                                                                                  |  |
|----|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--|
|    | ይቅማሉ?                                                                                                                                                                                                                                            |                                                                                                                                                                                                                                                                                                  |  |
| 22 | <p>Have you ever taken a drink that contains alcohol (such as Tella/Tegi/Areke/Beer/Wine, etc...)? if the answer is yes go to the next question.</p> <p>yaL×L meÖ{N (e@ eJ arq† wYN³ ym±slfTN) eጥተው ያውቃሉ?</p> <p>መልስዎ አዎ ከሆነ ወደሚቀጥለው ጥያቄ ይሂዱ</p> | <p>Yes (aḲ) (01)</p> <p>No (aY) (02)</p>                                                                                                                                                                                                                                                         |  |
| 23 | <p>How often do you drink alcoholic drinks in the last 30 days?</p> <p>ባለፉት 30 ቀናት WSE ምን ያህል ጊዜ የአልኮል መጠጥ ጠጥተዋል?</p>                                                                                                                            | <p>Every day (በየቀኑ) (01)</p> <p>5 – 6 times a week (5 - 6 ጊዜ በሳምንት) (02)</p> <p>3 – 4 times a week (3 - 4 ጊዜ በሳምንት) (03)</p> <p>twice a week (ሁለት ጊዜ በሳምንት) (04)</p> <p>once a week (አንድ ጊዜ በሳምንት) (05)</p> <p>2 – 3 times a month (2 - 3 ጊዜ በወር) (06)</p> <p>once a month (አንድ ጊዜ በወር) (07)</p> |  |

### Part V. Household Food Insecurity Questionnaires (yMGB \$ST³ Eፍገታ፡፤)

| No.<br>(ጥ.ቁ.) | Question<br>(Eፍገታ)                                                                                                                                            | Response options<br>(yM@} a፲፡፡{)                                                                                                                                                                                                                                                                                                                             | Code<br>(×D) |
|---------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------|
| 1             | <p>In the past four weeks, did you worry that your household would not have enough food?</p> <p>²lÛT a፡፡T ±MNªT WSE b_tsBḲ bq# MGB aፍገIM BlW txNqW ፍፂፂፂፂ?</p> | <p>0 - No (aY) (Skip to question 2) (wd Eፍገታ ቁ፡፡ER 2 Y³ፋ@፡፡)</p> <p>1 - Yes (aḲ) (Proceed to the next question) (wdi@qElW Eፍገታ Y³ፋ@፡፡)</p>                                                                                                                                                                                                                   |              |
|               | <p>How often did this happen?</p> <p>□□□□ □□□ □□□ □□□□□</p> <p>□□□□□ □□□□ □□□?</p>                                                                            | <p>1 - Rarely (once or twice in the past four weeks)</p> <p>aLÂ aLÂ (²lÛT a፡፡T ±MNªT WSE aND wYM uflT g^z_)</p> <p>2 - Sometimes (three to ten times in the past four weeks)</p> <p>aNëND g^z_ (²lÛT a፡፡T ±MNªT WSE k 3 Ask 10 g^z_)</p> <p>3 - Often (more than ten times in the past four weeks)</p> <p>aB°፡፡WN g^z_ (²lÛT a፡፡T ±MNªT WSE k 10 ጊዜ b@Y)</p> |              |

|   |                                                                                                                                                                                                                                                                                     |                                                                                                                                                                                                                                                                                                                                                            |  |
|---|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--|
| 2 | <p>In the past four weeks, were you or any household member not able to eat the kinds of foods you preferred because of a lack of resources?</p> <p>²¹ÛT a™T ±MNªT WSE ARS; wYM l_® yb_tsB; a²L ¶ìR;TN (□□□□□□) MGB baQM ìnS (ñ□□□ □□□□ □□□□ □□□□) MKN¶T mmgB ±Y{lf qRtW ¶Wšlf?</p> | <p>0 - No (aY) (Skip to question 3) (wd E¶q† q&gt;ER 3 Y³¼@ë&amp;)</p> <p>1 - Yes (a; ) (Proceed to the next question) (wdi@qElW E¶q† Y³¼@ë&amp;)</p>                                                                                                                                                                                                      |  |
|   | <p>How often did this happen?</p> <p>□□□□ □□□ □□□ □□□□□<br/>□□□□□ □□□□ □□□?</p>                                                                                                                                                                                                     | <p>1 - Rarely (once or twice in the past four weeks)</p> <p>aLÂ aLÂ (²¹ÛT a™T ±MNªT WSE aND wYM uflT g^z_)</p> <p>2 - Sometimes (three to ten times in the past four weeks)</p> <p>aNëND g^z_ (²¹ÛT a™T ±MNªT WSE kÎST Ask aSR g^z_)</p> <p>3 - Often (more than ten times in the past four weeks)</p> <p>aB°¼WN g^z_ (²¹ÛT a™T ±MNªT WSE kaSR ²½ b©Y)</p> |  |
| 3 | <p>In the past four weeks, did you or any household member have to eat a limited variety of foods due to a lack of resources?</p> <p>²¹ÛT a™T ±MNªT WSE ARS; wYM l_® yb_tsB; a²L □□□□ MKN¶T □□□□□ □□□□□ □□□□ □□□□ □□ □□□□□□ □□□ □□□□□□ □□□□?</p>                                    | <p>0 - No (aY) (Skip to question 4) (wd E¶q† q&gt;ER 4 Y³¼@ë&amp;)</p> <p>1 - Yes (a; ) (Proceed to the next question) (wdi@qElW E¶q† Y³¼@ë&amp;)</p>                                                                                                                                                                                                      |  |
|   | <p>How often did this happen?</p> <p>□□□□ □□□ □□□ □□□□□<br/>□□□□□ □□□□ □□□?</p>                                                                                                                                                                                                     | <p>1 - Rarely (once or twice in the past four weeks)</p> <p>aLÂ aLÂ (²¹ÛT a™T ±MNªT WSE 1 wYM 2 g^z_)</p> <p>2 - Sometimes (three to ten times in the past four weeks)</p> <p>aNëND g^z_ (²¹ÛT a™T ±MNªT WSE k 3 Ask 10 g^z_)</p> <p>3 - Often (more than ten times in the past four weeks)</p> <p>aB°¼WN g^z_ (²¹ÛT a™T ±MNªT WSE k 10 ²½ b©Y)</p>        |  |
| 4 | <p>In the past four weeks, did you or any household member have to eat some foods that you really did not want to eat because of a lack of</p>                                                                                                                                      | <p>0 - No (aY) (Skip to question 5) (wd E¶q† q&gt;ER 5 Y³¼@ë&amp;)</p> <p>1 - Yes (a; ) (Proceed to the next question) (wdi@qElW</p>                                                                                                                                                                                                                       |  |

|   |                                                                                                                                                                                                                                                                                              |                                                                                                                                                                                                                                                                                                                                                    |  |
|---|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--|
|   | <p>resources to obtain other types of foods?</p> <p>²¹ÛT a™T ±MNªT WSE ARS; wYM<br/>l_® yb_tsB; a²L □□□□ □□n□□<br/>□□□□□ □□□□□□□<br/>□□□□□□□□ □□□□ □□□□□<br/>□□□□?</p>                                                                                                                       | <p>E¶q† Y³@ë&amp;)</p>                                                                                                                                                                                                                                                                                                                             |  |
|   | <p>How often did this happen?</p> <p>□□□□ □□□ □□□ □□□□□<br/>□□□□□ □□□□ □□□?</p>                                                                                                                                                                                                              | <p>1 - Rarely (once or twice in the past four weeks)</p> <p>aLÂ aLÂ (²¹ÛT a™T ±MNªT WSE 1 wYM 2 g^z_)</p> <p>2 - Sometimes (three to ten times in the past four weeks)</p> <p>aNëND g^z_ (²¹ÛT a™T ±MNªT WSE k 3 Ask 10 g^z_)</p> <p>3 - Often (more than ten times in the past four weeks)</p> <p>aBº¼WN g^z_ (²¹ÛT a™T ±MNªT WSE k 10 ½ b@Y)</p> |  |
| 5 | <p>In the past four weeks, did you or any household member have to eat a smaller meal than you felt you needed because there was not enough food?</p> <p>²¹ÛT a™T ±MNªT WSE ARS; wYM<br/>l_® yb_tsB; a²L bq# MGB<br/>²LnbrbT ufn...ª ki@¶SfLG; meN<br/>bª{ (b¶ ½ΔU'½ ½º½·½) tmGbW ¶Wšlf?</p> | <p>0 - No (aY) (Skip to question 6) (wd E¶q† q&gt;ER 6 Y³@ë&amp;)</p> <p>1 - Yes (a; ) (Proceed to the next question) (wdi@qElW E¶q† Y³@ë&amp;)</p>                                                                                                                                                                                                |  |
|   | <p>How often did this happen?</p> <p>□□□□ □□□ □□□ □□□□□<br/>□□□□□ □□□□ □□□?</p>                                                                                                                                                                                                              | <p>1 - Rarely (once or twice in the past four weeks)</p> <p>aLÂ aLÂ (²¹ÛT a™T ±MNªT WSE 1 wYM 2 g^z_)</p> <p>2 - Sometimes (three to ten times in the past four weeks)</p> <p>aNëND g^z_ (²¹ÛT a™T ±MNªT WSE k 3 Ask 10 g^z_)</p> <p>3 - Often (more than ten times in the past four weeks)</p> <p>aBº¼WN g^z_ (²¹ÛT a™T ±MNªT WSE k 10 ½ b@Y)</p> |  |
| 6 | <p>In the past four weeks, did you or any other household member have to eat fewer meals in a day because there was not enough food?</p> <p>²¹ÛT a™T ±MNªT WSE ARS; wYM<br/>l_® yb_tsB; a²L bq# MGB<br/>²LnbrbT ufn...ª bqN □□□</p>                                                          | <p>0 - No (aY) (Skip to question 7) (wd E¶q† q&gt;ER 7 Y³@ë&amp;)</p> <p>1 - Yes (a; ) (Proceed to the next question) (wdi@qElW E¶q† Y³@ë&amp;)</p>                                                                                                                                                                                                |  |

|               |                                                                                                                                                                                                                              |                                                                                                                                                                                                                                                                                                                                                     |              |
|---------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------|
|               | <p>ki@ŦSfLG¿ yMGB meN bª{ tmGbW ŦWšlf?</p>                                                                                                                                                                                   |                                                                                                                                                                                                                                                                                                                                                     |              |
|               | <p>How often did this happen?</p> <p>□□□□ □□□ □□□ □□□□□</p> <p>□□□□□ □□□□ □□□?</p>                                                                                                                                           | <p>1 - Rarely (once or twice in the past four weeks)</p> <p>aLÂ aLÂ (²lÛT a™T ±MNªT WSE 1 wYM 2 g^z_)</p> <p>2 - Sometimes (three to ten times in the past four weeks)</p> <p>aNëND g^z_ (²lÛT a™T ±MNªT WSE k 3 Ask 10 g^z_)</p> <p>3 - Often (more than ten times in the past four weeks)</p> <p>aBº¼WN g^z_ (²lÛT a™T ±MNªT WSE k 10 2H b©Y)</p> |              |
| No.<br>(t.q>) | Question<br>(EŦqt)                                                                                                                                                                                                           | Response options<br>(yM©} aì™Ó{)                                                                                                                                                                                                                                                                                                                    | Code<br>(×D) |
| 7             | <p>In the past four weeks, was there ever no food to eat of any kind in your household because of lack of resources to get food?</p> <p>²lÛT a™T ±MNªT WSE □□□□</p> <p>□□□□□ □□□ □□□□ □□□</p> <p>□□□□□□□ □□□ □□□□?</p>       | <p>0 - No (aY) (Skip to question 8) (wd EŦqt q&gt;ER 8 Y¾@ë&amp;)</p> <p>1 - Yes (a¿) (Proceed to the next question) (wdi@qElW EŦqt Y¾@ë&amp;)</p>                                                                                                                                                                                                  |              |
|               | <p>How often did this happen?</p> <p>□□□□ □□□ □□□ □□□□□</p> <p>□□□□□ □□□□ □□□?</p>                                                                                                                                           | <p>1 - Rarely (once or twice in the past four weeks)</p> <p>aLÂ aLÂ (²lÛT a™T ±MNªT WSE 1 wYM 2 g^z_)</p> <p>2 - Sometimes (three to ten times in the past four weeks)</p> <p>aNëND g^z_ (²lÛT a™T ±MNªT WSE k 3 Ask 10 g^z_)</p> <p>3 - Often (more than ten times in the past four weeks)</p> <p>aBº¼WN g^z_ (²lÛT a™T ±MNªT WSE 10 2H b©Y)</p>   |              |
| 8             | <p>In the past four weeks, did you or any household member go to sleep at night hungry because there was not enough food?</p> <p>²lÛT a™T ±MNªT WSE ARS¿ wYM l_® yb_tsB¿ a²L bq# MGB ²LnbrbT □□□□□ □□□□□</p> <p>□□□□□□□?</p> | <p>0 - No (aY) (Skip to question 9) (wd EŦqt q&gt;ER 9 Y¾@ë&amp;)</p> <p>1 - Yes (a¿) (Proceed to the next question) (wdi@qElW EŦqt Y¾@ë&amp;)</p>                                                                                                                                                                                                  |              |
|               | How often did this happen?                                                                                                                                                                                                   | 1 - Rarely (once or twice in the past four weeks)                                                                                                                                                                                                                                                                                                   |              |

|   |                                                                                                                                                                                                                                                        |                                                                                                                                                                                                                                                                                                                                                                                  |  |
|---|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--|
|   | <p>□□□□ □□□ □□□ □□□□□</p> <p>□□□□□ □□□□ □□□?</p>                                                                                                                                                                                                       | <p>aLÂ aLÂ (²lÛT a™T ±MNªT WSE 1 wYM 2 g^z_)</p> <p><b>2 - Sometimes (three to ten times in the past four weeks)</b></p> <p>aNëND g^z_ (²lÛT a™T ±MNªT WSE k 3 Ask 10 g^z_)</p> <p><b>3 - Often (more than ten times in the past four weeks)</b></p> <p>aBº¼WN g^z_ (²lÛT a™T ±MNªT WSE k 10 ½b b©Y)</p>                                                                         |  |
| 9 | <p>In the past four weeks, did you or any household member go a whole day and night without eating anything because there was not enough food?</p> <p>²lÛT a™T ±MNªT WSE ARS; wYM l_® yb_tsB; a²L bq# MGB ²LnbrbT MKN¶T ተርባቹህ ወላቹህና አድራቹህ ታወቃላቹህን?</p> | <p><b>0 - No (aY) (questionnaire is finished) meYq&gt; aLåL</b></p> <p><b>1 - Yes (aç)</b></p>                                                                                                                                                                                                                                                                                   |  |
|   | <p>How often did this happen?</p> <p>□□□□ □□□ □□□ □□□□□</p> <p>□□□□□ □□□□ □□□?</p>                                                                                                                                                                     | <p><b>1 - Rarely (once or twice in the past four weeks)</b></p> <p>aLÂ aLÂ (²lÛT a™T ±MNªT WSE aND wYM uflT g^z_)</p> <p><b>2 - Sometimes (three to ten times in the past four weeks)</b></p> <p>aNëND g^z_ (²lÛT a™T ±MNªT WSE kîST Ask aSR g^z_)</p> <p><b>3 - Often (more than ten times in the past four weeks)</b></p> <p>aBº¼WN g^z_ (²lÛT a™T ±MNªT WSE kaSR g^z b©Y)</p> |  |

## Part VI. Dietary Diversity Score (ë¶Tþ ë¶vRs^t# SxR)

- ```

> Is yesterday a regular day or a special one (is there any holiday, fasting or social/family gathering)?
   □□□□□□ □□ □□□□ □□ □□□□ □□ (□□□□ □□□□ □□ □□□□ □□□□ □□□□) □□□?
       Fasting day (□□□ □□) (01)
       Holiday/social or family gathering (□□□□/□□□□□□ □□□□ □□) (02)
       Regular day (□□□□□ □□) (03)

```

Please describe the foods (meals and snacks) that you ate or drank yesterday during the day and night, whether at home or outside the home. Start with the first food or drink of the morning. Write down all foods and drinks mentioned.

A<sup>2</sup>K<sub>2</sub> bT®NT<sup>3</sup>W AlT ytmgb&TN MGB<sup>3</sup> meE (b\_T WSEM Òn kb\_T Wã) YNgÝN/ke\$T ktmgb&T MGB wYM meE jMÅ uflfNM YmZGñ

- When composite dishes are mentioned, ask for the list of ingredients.

ytd<sup>2</sup>lq> MGİ{ bteqs& g^z\_ yNEr ngÅ[>N ZRZR YeYq> kz^U btxiBM men&N bkhqQsf mlk^M\_{ (yǎe እና የኸርሳ ማንኪያ ወይም ሌላችን) YeYq>

- When the respondent has finished, probe for meals and snacks not mentioned.

M®} sXW s^xRs& ¶Lteqs& MGİ{ wYM mKsS ·lf dGmW ¶r¬Ge&

| Breakfast (q>RS) | Snack (mKsS) | Lunch (M±) | Snack (mKsS) | Dinner (A <sup>TM</sup> T) |
|------------------|--------------|------------|--------------|----------------------------|
|                  |              |            |              |                            |

- When the respondent recall is complete, **fill in the food groups (see the next page) based on the information recorded above.** For any food groups not mentioned, ask the respondent if a food item from this group was consumed. (bt±ªÜW yt;gl;&TN yMGB aYnÄ{ bi@qElW g: ktzrzÝT yMGB MDİ{ -R ¶°Më;[W yMGB MDb& WSE ¶Ltgl;& MGİ{ bi@¶-Emf g^z t±ªÜWN YeYq>)

እባክዎ የተጠቀሱትን የምግብ አይነቶች ከ ምግብ ምድቡ ጋር ሲያዛምዱ አንድ የሻይ ማንኪያ እና ከዛ ቢታች የተጨመሩ የምግብ አይነቶች መመዝገብ የለባቸውም፤ ለምሳሌ ማኪያዎ ላይ የሚጨመር ውተት ውተት እና የውተት ተዋዕጾች ውስጥ አይካተትም

## Major food groups

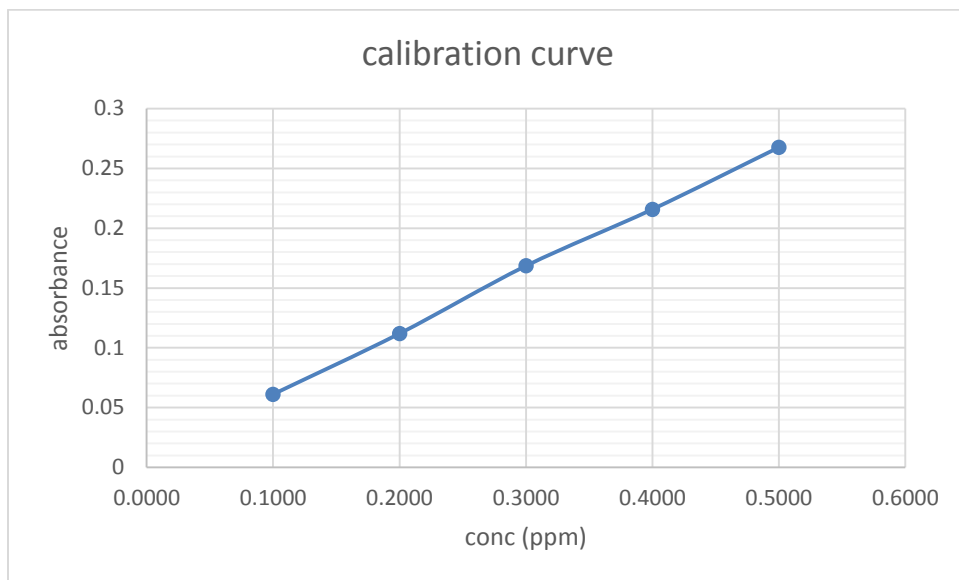
| Question number<br>(t.q>) | Food group<br>(yMGB b&DN)                                                  | Examples<br>(M±l_ç{)                                                                                                                                                                                                                                                                                                                                  | Yes (aç) – 1<br><br>No (aY) – 0 |
|---------------------------|----------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------|
| 1                         | Cereals<br>yE™Er_ sBÉ{                                                     | Corn/maize, rice, wheat, sorghum, millet or any other grains or foods made from these (e.g. bread, noodles, porridge or other grain products) + <i>insert local foods e.g. porridge or pastes or other locally available grains</i><br><br>bÖÉ¤ ÝZ¤ SNd_¤ ì}©¤ ëg&± (yëg&± gNÂ)¤ l_®<br>E™Er_ç{ wYM kAnz^U ytsÝ MGİ{ lM±l_ ëİ¤<br>°Sª¤ gNÂ A³ ym±slfT |                                 |
| 2                         | Vitamin A rich vegetables and tubers<br>b<Yªì@N a_ ybl;g&<br>aªKL T³ S™SÅ{ | Pumpkin, carrots, squash, or sweet potatoes that are orange inside + <i>other locally available vitamin A rich vegetables (e.g. red sweet pepper)</i><br><br>ë&²¤ ·ÅT¤ Sè}¤ SèR DN{ (Wse& BRt>·³ì yÒn)<br>btxiİM ba·²b^W yi@gi,, b<Yªì@N a_ ybl;g&<br>aªKLÄ{ (lM±l_ qY ½¨X bRbr_)                                                                     |                                 |
| 3                         | White roots and tubers<br>nX S™SÅ{³ GNİ{                                   | White potatoes, white yams, white cassava, or other foods made from roots<br><br>nX DN{¤ nX İY³¤ nX yfrNJ ANxT wYM kl_É{<br>S™SÅ{ ytz-é& MGİ{                                                                                                                                                                                                         |                                 |
| 4                         | Dark gree leafy vegetables<br>diQ arNæd_ Qe®ì<br>aTKLÄ{                    | Dark green/leafy vegetables, including wild ones + <i>locally available vitamin A rich leaves such as amaranth, cassava leaves, kale, spinach etc.</i><br><br>diQ arNæd_ Qe®ì aTKLÄ{ (bë&R A³ ba·²b^W<br>yi@gi,, b<Yªì@N ybl;g& Qe®ì aTKLÄ{ lM±l_<br>l^ál^à¤ y·±< QeL¤ ·l_¤ ÆmN¤ ÖS½ A³<br>ym±slfT)                                                   |                                 |
| 5                         | Other vegetables<br>l_É{ aTKLÄ{                                            | Other vegetables (e.g. tomato, onion, eggplant), including wild vegetables<br><br>t#ìt#M¤}Nk&RT¤ ²z^NjN A³ yë&R aTKLÄ{                                                                                                                                                                                                                                |                                 |

|    |                                                                                 |                                                                                                                                                                                                                                                                                                                                                                  |  |
|----|---------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--|
| 6  | Vitamin A rich fruits<br>b<Y <sup>a</sup> i@N a_ ybl;g&<br>F <sup>TM</sup> r_ç{ | Ripe mangoes, cantaloupe, apricots (fresh or dries), ripe papaya, dried peaches + <i>other locally available vitamin A rich fruits</i><br><br>ybsl ìNÆ <sup>a</sup> g^}½ <sup>a</sup> ×}M (y <sup>2</sup> UR ×K) (Tk&S wYM ydrq) <sup>a</sup> ybsl °°¶ <sup>a</sup> drQ ×K A <sup>3</sup> ba·bb^W yi@gi,,<br>b<Y <sup>a</sup> i@N a_ ybl;g& F <sup>TM</sup> r_ç{ |  |
| 7  | Other fruits<br>l_É{ F <sup>TM</sup> r_ç{                                       | Other fruits, including wild fruits<br><br>yë&R F <sup>TM</sup> r_ç{N ¶ešL@L                                                                                                                                                                                                                                                                                     |  |
| 8  | Organ meat<br>ysWnT KFL S¬                                                      | Liver, kidney, heart or other organ meats or blood-based foods<br><br>g&bT <sup>a</sup> k&@l^T <sup>a</sup> LB A <sup>3</sup> l_É{ ysWnT a·L S¬ç{<br>wYM dM-t×R MGİ{                                                                                                                                                                                             |  |
| 9  | Flesh meats<br>lmBL yi@ÒN S¬                                                    | Beef, pork, lamb, goat, rabbit, wild game, chicken, duck, or other birds<br><br>ybr_ S¬ <sup>a</sup> ya±ì S¬ <sup>a</sup> bG <sup>a</sup> FyL <sup>a</sup> EN[L <sup>a</sup> yë&R Ans± <sup>a</sup> İÅ <sup>a</sup> ëKâ wYM L@ ywF ZR¶ç{                                                                                                                         |  |
| 10 | Eggs<br>Anq>@L                                                                  | Chicken, duck, guinea fowl or any other egg<br><br>yİÅ <sup>a</sup> yëKâ <sup>a</sup> JG <sup>TM</sup> wYM ìN¼WM Å@ Anq>@L                                                                                                                                                                                                                                       |  |
| 11 | Fish<br>a±                                                                      | Fresh or dried fish or shelfish<br><br>Tk&S wYM ydrq a± <sup>a</sup> qND aW½ç{                                                                                                                                                                                                                                                                                   |  |
| 12 | Legumes, nuts and seeds<br>E <sup>TM</sup> Er_ç{ <sup>3</sup> lWZ               | Beans, peas, lentils, nuts, seeds or foods made from these<br><br>²q†@ç{ <sup>a</sup> atR <sup>a</sup> MSR <sup>a</sup> lWZ wYM kAnz^U ytsÝ MGİ{                                                                                                                                                                                                                 |  |
| 13 | Milk and milk products<br>wtT <sup>3</sup> ywtT t\$ :ç                          | Mik, cheese, yoghurt, or other milk products<br><br>wtT <sup>a</sup> aYB <sup>a</sup> ARE wYM l_É{ ywtT We_Ä{                                                                                                                                                                                                                                                    |  |



## Annex 4: Standard calibration curves

### For Zinc

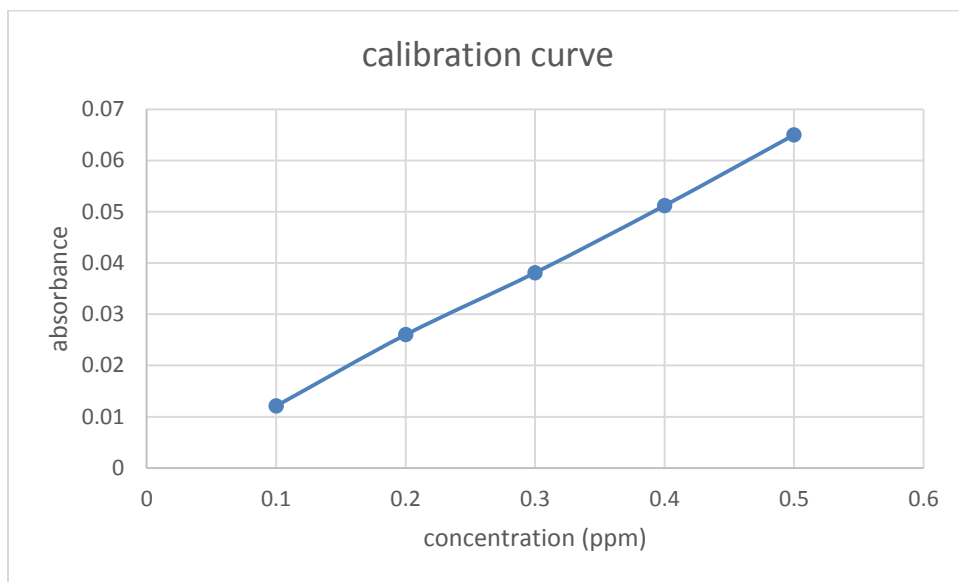


$$\text{Abs.} = 0.56122\text{conc.}$$

$$r = 0.9996$$

| Concentration (ppm) | Absorbance (Abs.) |
|---------------------|-------------------|
| 0.1000              | 0.0611            |
| 0.2000              | 0.1119            |
| 0.3000              | 0.1685            |
| 0.4000              | 0.2158            |
| 0.5000              | 0.2676            |

### For Copper



$$\text{Abs} = 0.12876\text{conc}$$

$$r = 0.9998$$

| Concentration (ppm) | Absorbance |
|---------------------|------------|
| 0.1000              | 0.0121     |
| 0.2000              | 0.026      |
| 0.3000              | 0.0381     |
| 0.4000              | 0.0512     |
| 0.5000              | 0.065      |