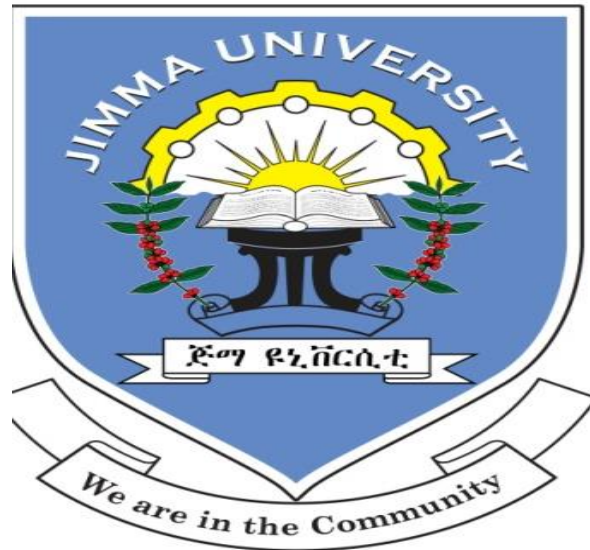# PREVALENCE OF IRON DEFICIENCY AND ITS PREDICTORS AMONG NON PREGNANT WOMEN IN REPRODUCTIVE AGE (15-49) IN ETHIOPIA



BY

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**THESIS SUBMITTED TO JIMMA UNIVERSITY COLLEGE OF PUBLIC HEALTH AND MEDICAL SCIENCES DEPARTEMENT OF POPULATION AND FAMILY HEALTH, HUMAN NUTRITION UNIT IN PARTIAL FULFILLEMENTS FOR THE DEGREE OF MASTERS SCIENCE (MSc) IN HUMAN NUTRITION**

June, 2016

Jimma, Ethiopia

# PREVALENCE OF IRON DEFICIENCY AND ITS PREDICTORS FACTORS AMONG NON PREGNANT WOMEN IN REPRODUCTIVE AGE (15-49) IN ETHIOPIA

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June, 2016

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# Abstract

**Background:** *Micronutrient deficiencies are a major global health problem. More than 2 billion people in the world today are estimated to be deficient in key vitamins and minerals.*

**Objective:** *The aim of this study, was to determine the prevalence of iron deficiency among non-pregnant women in reproductive age,*

**Methods:** *A population based national survey of iron deficiency was carried out from March 2015 to July 2015 among Ethiopian women. A simple random sampling was used to select 1924 women aged 15 to 49 years from eleven accessible regions of Ethiopia. Demographic and health data were collected using structured questionnaire using tablets. Biological samples were collected by laboratory technologists. Analysis was done by STATA software version 13. Descriptive statistics, binary and multivariable logistic regressions were done to identify factors associated with iron deficiency. P <0.05 were used to declare statistical significance.*

**Result:** *The overall prevalence of Iron deficiency was 9.9%. Somalia (69.8%), Harari (44.1%) and Diredewa (40.2%) were found to be higher in Iron deficiency in descending order. The median serum ferritin concentration was 57mg/dl. Having Anemia (AOR= 3.4, [95%CI 2.5, 4.7[), having raised AGP(α -1 acid glycoprotein) (AOR= 2.1, [95%CI:1.39,3.01]), safe drinking water (AOR= 0.63, [95%CI: 0.46,0.86]), overweight/obese (AOR =2.3, [95%CI :1.6,3.4]), not eating animal source food in last 24hr (AOR=1.46, [95%CI:1.1,1.8]) and having family size >5 (AOR=1.48, [95%CI: 1.1,1.9]) were independent predictors of Iron deficiency.*

**Conclusion and Recommendation:** *The prevalence of Iron deficiency among non-pregnant women in reproductive age group in Ethiopia is mild public health importance. Family size >5, being anemic, lack of animal source food in the last 24hour preceding the survey,  and being overweight/obese were independent predictor of iron deficiency.*

*Health professionals and concerned body should give nutrition education for women in reproductive age group about the cause of Iron deficiency to prevent its consequences.*

# Acknowledgements

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| | |
|---|---|
| AGP | α 1 acid glycoprotein |
| BMI | Body Mass Index |
| CRP | C-reactive protein |
| CI | Confidence Interval |
| DDS | Dietary diversity score |
| DEFF | Design effect |
| DHS | Demographic and Health Survey |
| DOS | Department of Statistics |
| EA | Enumeration Area |
| ENMS | Ethiopian nutrient micronutrient survey |
| EPHI | Ethiopian public health institution |
| Hb | Hemoglobin |
| HH | Household |
| IDA | Iron deficiency anemia |
| MoH | Ministry of Health |
| MND | Micro nutrient deficiency |
| PI | Principal Investigator |
| PPS | Probability proportional to size |
| QC | Quality control |
| SD | Standard deviation |
| SF | Serum ferritin |
| SRS | Simple Random Selection |
| WHO | World health organization |
| WRA | Women in reproductive a |

# CHAPTER ONE: INTRODUCTION

## 1.1  BACKGROUND

The health and vitality of human beings depends on diets that include adequate amounts of vitamins and minerals to promote effective physiological processes including reproduction, immune response, brain and other neural functions, and energy metabolism. Vitamins are necessary in small amounts to facilitate growth, maintenance of health, and reproduction. Minerals are elements which do not originate in animal or plant rather from the earth's crust. Although minerals make up only a small portion of body tissues, they are essential for normal growth and functioning (1).

Iron is a trace mineral that is vital for growth and development. It plays a key role as a co-factor for enzymes involved in oxidation-reduction reactions, which occur in all cells during metabolism. Iron is also necessary as the component of hemoglobin that allows red blood cells to carry oxygen needed throughout the body. It is also essential for proper production and catabolism of several neuro-transmitters, but most importantly iron is essential for normal neurodevelopment during fetal and early childhood (2)

Approximately 73 percent of the body's iron is normally incorporated into hemoglobin and 12% in the storage complexes ferritin and hemosiderin. A very important 15% of the mineral, however, is incorporated into a variety of other iron-containing compounds essential to cell function (3).

Iron stores in the body exist primarily in the form of ferritin. The ferritin molecule is an intracellular hollow protein shell composed of 24 subunits surrounding an iron core that may contain as many as 4000-4500 iron atoms. In the body, small amounts of ferritin are secreted into the plasma. The concentration of this plasma (or serum) ferritin is positively correlated with the size of the total body iron stores in the absence of inflammation. A low serum ferritin value reflects depleted iron stores, but not necessarily the severity of the depletion as it progresses. (4)

Many different measures of iron status are available, and different measures are useful at different stages of iron depletion. Measures of serum ferritin can be used to identify iron depletion at an early stage. (5)

Normal ferritin concentrations vary by age and sex. Concentrations are high at birth, rise during the first two months of life, and then fall throughout later infancy (1). At about one year of age, concentrations begin to rise again and continue to increase into adulthood (2). Beginning in adolescence, however, males have higher values than females; a trend that persists into late adulthood. Values among men peak between 30–39 years of age and then tend to remain constant until about 70 years of age. Among women, serum ferritin values remain relatively low until menopause and then rise (6)

In areas of widespread infection or inflammation, defining iron deficiency using serum ferritin is Difficult. If infectious diseases are seasonal, then the survey should be done in the season of lowest transmission; if they are permanent, then the concurrent measurement of two acute phase response proteins, C-reactive protein (CRP) and α1- acid-glycoprotein (AGP), can aid in the interpretation of serum ferritin values indicating acute and chronic infection or inflammation respectively. One method to account for the increase in ferritin values caused by inflammation is to raise the cut-off that defines deficiency, often to 30 µg/l (5). Another method is to exclude individuals with elevated concentrations of CRP or AGP from prevalence calculations based on ferritin. However, increase and age groups where inflammation is nearly universal, such exclusion could artificially depress estimates of the prevalence of iron deficiency based on serum ferritin calculations. Ferritin is typically assessed in serum or plasma with enzyme-linked immunosorbent assays (ELISA) or enzyme immunoassays after venous blood collection.(6)

## 1.2   STATEMENT OF THE PROBLEM

Micronutrients are essential to sustain life and for optimal physiological function. Widespread global micronutrient deficiencies (MNDs) exist, with pregnant women and their children under 5 years at the highest risk. Iron, iodine, folate, vitamin A, and zinc deficiencies are the most widespread MNDs, and all these MNDs are common contributors to poor growth, intellectual

impairments, perinatal complications, and increased risk of morbidity and mortality. Iron deficiency is the most common MND worldwide and leads to microcytic anemia, decreased capacity for work, as well as impaired immune and endocrine function .(8)

Iron deficiency occurs in two main forms: absolute or functional. Absolute iron deficiency arises when total body iron stores are low or exhausted; functional iron deficiency is a disorder in which total body iron stores are normal or increased, but the iron supply to the bone marrow is inadequate. Absolute and functional deficiencies can coexist. Functional iron deficiency can be present in many acute and chronic inflammatory states, and hepcidin—the master regulator of iron homoeostasis has a key role in pathogenesis. (9)

According to WHO the relative extent of Iron store on the basis of serum ferritin is for the age group >5 in female is <15μg/l. whereas; the cut-off value set by WHO; Iron deficiency is not considered as a public health problem if its prevalence is when its <20% without inflammation; if there inflammation and <20% iron deficiency is prevalent and when iron deficiency is >20 iron depletion prevalent. (10)

Recognition of nutrient deficiencies in women of reproductive age is important not only because nutritional status affects women's health and wellbeing, but also because deficiencies are associated with adverse pregnancy outcomes. Deficiencies in micronutrients such as folate, vitamin B12, iron and trace elements can have adverse consequences on infant mortality and morbidity. (11)

The worldwide prevalence of iron deficiency is among women in child bearing age is estimated to be 50% and estimated prevalence in developing countries rage from 40% to 88% (12).
A cross sectional across 10 Europeans cities showed that the prevalence of Iron deficiency in women is 21 %.(13)
Although the magnitude of ID in Ethiopia has not yet been well documented nationwide, limited data is available; on the prevalence rate of ID among in nine administrative regions of Ethiopia the prevalence of Iron deficiency was 50.1% (14) but this study doesn't adjust serum ferritin for inflammation and includes pregnant mothers.

In the first nationwide survey of IDA carried out among women of reproductive age including pregnant women Ethiopia. The study shows that mild to moderate anemia (30.4%), iron deficiency (49.7%) and iron deficiency anemia (17%) are common with distinct regional variations. Furthermore, the study revealed the predominant age groups affected to be 15 - 24 and >35years) (15).

The overall prevalence rate of iron deficiency determined by serum ferritin was 49.7%. The highest prevalence rate was observed in Afar with 65.1% (95) followed by Dire-Dawa (63.9%), Harari (61.8%), Oromia (55.0%), Tigray (44.2%), SNNP (42.5%) and Amhara (37.5%) regions in descending order. About 9.6% of the women exhibited the severe form of ID (SF<12ng/ml) while 40% were moderately iron deficient (12-49ng/ml). The majority of iron deficient cases fell into the category of moderate deficiency (14).

Therefore this study aiming at determining the health related associated risk factors, maternal characteristics associated factors, diet and nutrition related associated factors and environmental related factors of iron deficiency in reproductive age of non-pregnant women in Ethiopia using serum ferritin as biomarkers of Iron deficiency adjusting it to inflammation.

# CHAPTER TWO: LITERATURE REVIEW

## 2.1 Prevalence of iron deficiency

There are so many studies about iron deficiency as proxy indictor for iron deficiency anemia; there are few studies about iron deficiency among non-pregnant women and associated factors.

A cross sectional study among Cambodian non-pregnant women in reproductive age was 8.1%.(16) Similarly a cross sectional study among Nepal women reproductive age showed that the prevalence of iron deficiency was 20.0%.(21)

Another cross-sectional study conducted in nine administrative regions of Ethiopia showed that the prevalence of Iron deficiency was 50.1%. In this study there is regional variation was also associated with the prevalence of Iron deficiency. The higher prevalence is found in Afar with 65.1% followed by Dire-Dawa (63.9%), Harari (61.8%), Oromia (55.0%), Tigray (44.2%), SNNP (42.5%) and Amhara (37.5%) regions in descending order. But this study includes pregnant women. (14)

## 2.2 Factor associated with iron deficiency

1. Socio economic and demographic factors

Study conducted in Cambodian women showed that iron deficiency was better in women living in urban areas as compared to rural areas which was also statically significant .(16)

Another cross sectional study conducted in Saudi showed that there is no association between the prevalence of Iron deficiency and socio demographic and economic variable; residence, region income. But this study showed that there is an association between having a large family size and Iron deficiency. (17)

A community based crossectional study In Ethiopia revealed that prevalence of Iron deficiency was higher; the age groups between 31-49 years was higher than the age group 15-20 and 21-30 years in descending orders and it's also statically significant with Iron deficiency. (14).

The above study also showed that Women who had no formal education and who did not use contraceptives were negatively associated with Iron deficiency.

Another Cross-sectional survey among child bearing age women in Riyadh, Saudi Arabia showed that No significant association was found between Iron deficiency and age, age at menarche, educational level and contraceptive use. And also this study found that no association between Juices, egg, vegetables, tea or coffee consumption. And Iron deficiency. (18)

## 3. Parasite, illness and Environmental factor iron deficiency

A cross sectional study among women in reproductive age in Vietnam showed that intensity of hookworm infection. were associated with indices of iron deficiency. (19)

A crosssectional study in Malesia distribution of iron status indicator was also analyzed according to intensity of STH infections. The Hb, SF and SI levels declined from light infection to heavy infection for T. trichiura, A. lumbricoides and hookworm infections. Although the prevalence of anemia, ID and IDA increased with increasing worm burden, it was not statistically correlated with any of the worm burden thresholds (20)

Women having children above two, using open field as toilet, suffered from chronic illnesses, and had intestinal parasites were positively associated with iron deficiency in the study conducted in Ethiopia women in reproductive age. (14).

## 4. Diet and nutritional related factors associated iron deficiency

A cross sectional study conducted in Mali west Africa to assess the relation between overweight and Iron Deficiency Showed that among the overweight and obese women, only SF was used as an Fe biomarker in the model. The prevalence Odds of ID (SF <12 mg/l) in the overweight group was, 1 and non-significant. Conversely, the CED (chronic energy deficiency); group was at a significantly higher risk of ID than the normal weight group. But this study doesn't adjust serum ferritin value to inflammation (CRP) instead it exclude subjects with raised CRP and serum ferritin value. (21)

Another study in Vietnam showed that meat consumption were associated with indices of iron deficiency in a multiple regression model.(19)

Study conducted in Saudi showed that increasing BMI was associated with Iron deficiency. Having children greater than 2 was associated with Iron deficiency. (18)

Another cross-sectional study in Cambodian women showed that consumption of specific food groups was not associated with low iron stores. Nonetheless markers iron deficiency at abnormal levels were more common in women who were overweight or obese. (16)

Another study conducted in Israel to asses that whether overweight children and adolescents, who often have poor dietary habits, are at increased risk of iron deficiency showed that overweight is associate with iron deficiency; overweight children and adolescent are at high rist of Iron deficiency. (ID)(27)

## 5. Anemia and Iron deficiency

Iron deficiency was assumed to be the major cause of Anemia but different study doesn't show this.

Study conducted in Nepal showed that the prevalence of anemia and IDA were 12 and 6% respectively, indicating that only about half of the anemia was owing to ID. The high prevalence of anemia among women without depleted iron stores (47%) suggests that other anemia-causing nutritional deficiencies may be prevalent in this population (21)

## 2.1 Conceptual Framework

There are multiple determinants of micronutrient deficiency. It is not a simple problem with a single and simple solution. Multiple and interrelated factors involved for its development. The conceptual frame work below shows this factors associated with Iron deficiency. For this study, some of the factors are grouped in classes; namely women's characteristics related factors, nutrition and diet related factors, sociodemographic and economic and health related factors. Each of the factors with their constructs are linked with Iron deficiency as well some of them are related with each other as seen by the direction of linkage.
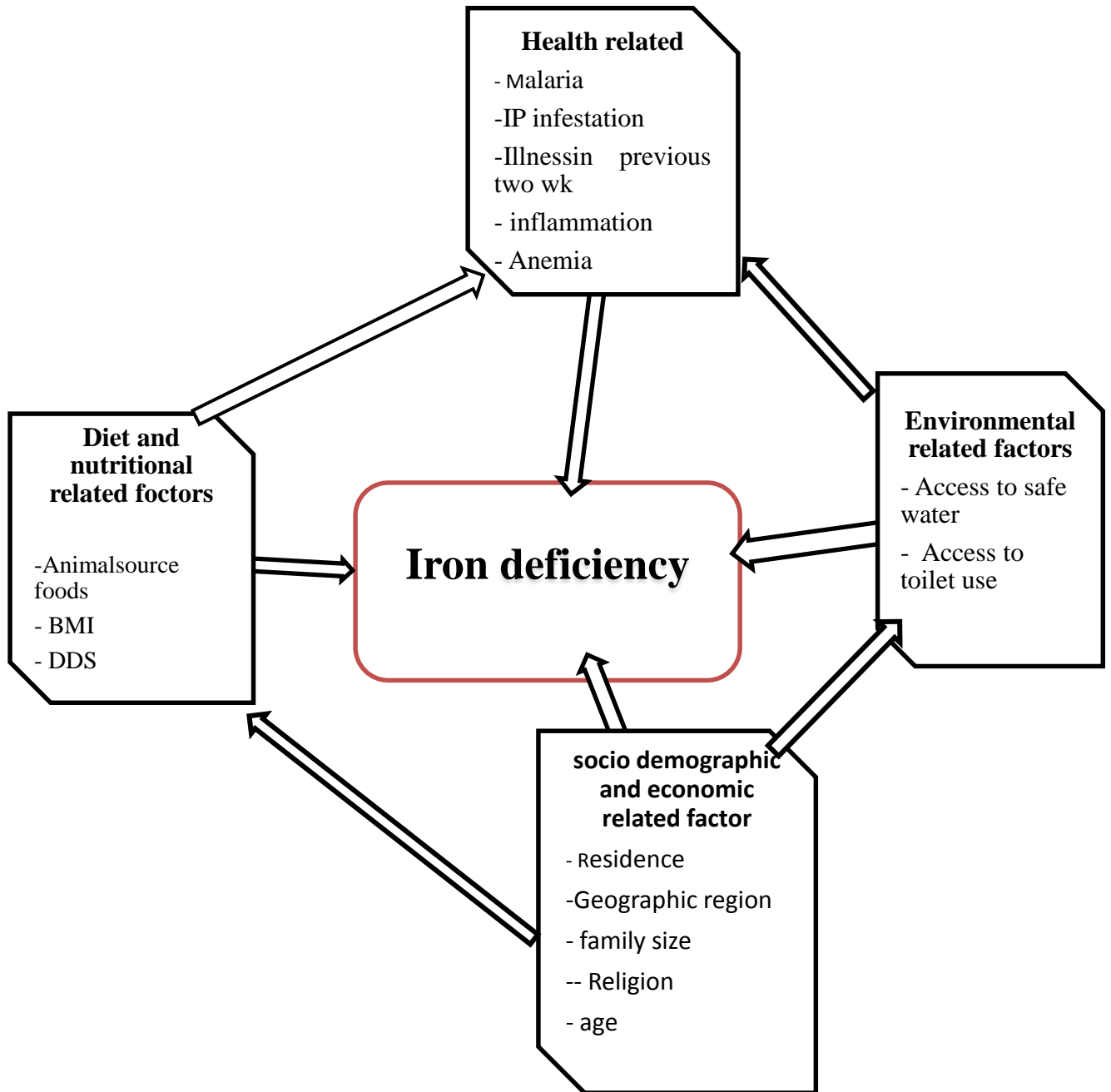
Figure 1. Conceptual framework showing association of dependent and independent variable developed from after viewing different literatures

## 2.2 Significance of the study

In Ethiopia, the prevalence of iron deficiency is high and also of the importance of the increased worldwide concern about the consequences of its deficiencies, the interrelationship between their metabolisms and the sustained deterioration of life quality of the Ethiopian population. The prevalence of Iron deficiency in Ethiopia studied as an indicator for anemia; there was no studies on the Iron deficiency and its associated factors among non-pregnant women in reproductive age. Studying iron deficiency among non-pregnant women contributes health to the group and economically to the country because this is the group that come to be pregnant and supplement iron so if iron status is adequate before pregnancy it helps in so many perspective.

This study will contribute for the better understanding of the public health significance of iron deficiencies and possible underlying causes of iron deficiency.

The study result can be used as baseline information for planning, monitoring and evaluation of micronutrient related programs in the country.

# CHAPTER THREE: OBJECTIVE

## 3.3 General Objective

To assess the prevalence of iron deficiency and associated factors among on pregnant women in reproductive age group in Ethiopia, from June 2015

## 3.4 Specific Objectives

1) To determine prevalence of iron deficiency among women in reproductive age (15 – 49) in Ethiopia.
2) To identify factor associated with iron deficiency among women of reproductive age group in Ethiopia.

# CHAPTER FOUR: MATERIALS AND METHODS

## 4.1 Study area and period

Ethiopia is situated in the horn of Africa between 3 and 5 degrees north latitude and 33 and 48 degrees east longitude. The total area of the country is about 1.1 million sq. km (2007 PHC of Ethiopia). The topographic features range from the highest peak, Ras Dashen, (4,550 meters above sea level), in the north east down to the Afar Depression at 110 meters below sea level in the east. The climatic condition of the country varies with the topography, ranging from 47°C (116.6°F) to 10°C (50°F). According to projection by CSA census the total population of the country is 90,078,002 from which male and female population accounts 45,250,993 and 44,827,012, respectively. Among this females in reproductive age group is 17,223,456.

The National survey was carried out from March 2015 to July 2015.
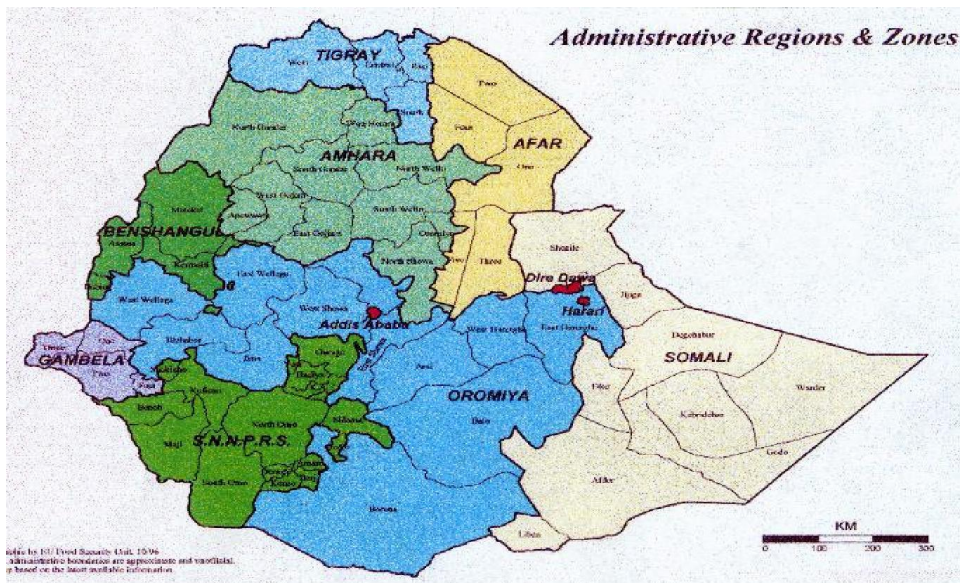


Figure 1 Demographic map of Ethiopia

## 4.2 Study design

Population based cross sectional survey design was used.

## 4.3 Source population

All women of reproductive age group in Ethiopia.

## 4.4 Study population

All selected women in reproductive age group in Ethiopia.

## 4.5 Eligibility criteria

### 4.5.1 Inclusion Criteria of participants

✓  All women of reproductive age group in Ethiopia.

### 4.5.2 Exclusion Criteria of participants

✓ Pregnant and serious ill women were excluded from the study.

## 4.6 Sample size determination

The sample size required for women in reproductive age group was calculated using a single population proportion formula, based on the estimated prevalence for Iron deficiency, an assumed design effect of 2 with a response rate of 85% at the individual level. The national level precision would be about 4%.

$$N = \frac{Z^2{}_{\alpha/2}\, p(1-P)}{d^2} * DEFF + 15\%(n)$$

Where;

n $\qquad$ = Sample size = 1380

$Z_{\alpha/2}$ $\quad$ = Standard errors from mean corresponding to the 95% confidence level

P $\qquad$ = Prevalence = 0.5 because no national survey was done on the prevalence of Iron deficiency among non-pregnant women.

D $\qquad$ = Allowable error = 0.04

DEFF  = Design effect = 2

RR      = Response rate (%) = 85%

The above calculated sample size is the minimum requirement for this stud since it was national survey we use an opportunities to increase our sample size to 1924 and  1624 women are available with complete data so we took this for analysis who had a complete data.

## 4.7 Sampling

## 4.7.1 Sampling procedure

Nine regional and two city administration in Ethiopia were treated as separate strata, allowing for national parameter estimates, as well as estimates by region because there may be regional differences in micronutrient deficiencies. The sampling frame for ENMS was from the list of Central Statistics Agency (CSA) enumeration areas (EAs) during the 2007 Ethiopia Population and housing census. A total of about 80,000 EAs were created at national level. The EAs were developed through a cartographic mapping exercise conducted between 2005 and 2007 and were used for the 2007 census enumeration. EAs were created containing 150 to 200 households in rural and urban houses as a measure of household in the 2007 sampling frame was provide the basis for the selection of primary sampling unit (PSU) for the Ethiopian national micronutrient survey.

## 4.8.1 Household and EA Listing

For the first stage of sampling in each region or city administration, EAs were randomly selected using standard probability proportional to size (PPS).

Prior to the actual survey, each selected EA's were visited and all households were listed within the boundary. At the second stage of sampling EAs were further segmented in to locally known smaller geographic units of 40-60households or less and one segment was randomly selected for the survey. In each household, a census of the people living in the household was conducted. The data from this exercise was used for random selection of 7 households. Therefore, as the third stage of sampling, 7 households were selected from each EA using simple random selection (SRS).

### 4.8.2 Selection of Respondents

Within each EA, 7 households were randomly selected for women participation. This information was preloaded to the data collocation smart phones prior to the survey. When eligible occupants of a house were not present, two continuative return visits with written appointment to the household was made. In the vent where no eligible respondent was available, selected household was recorded as refusal or any reason given for the unavailability of eligible respondent without any replacement of respondents.
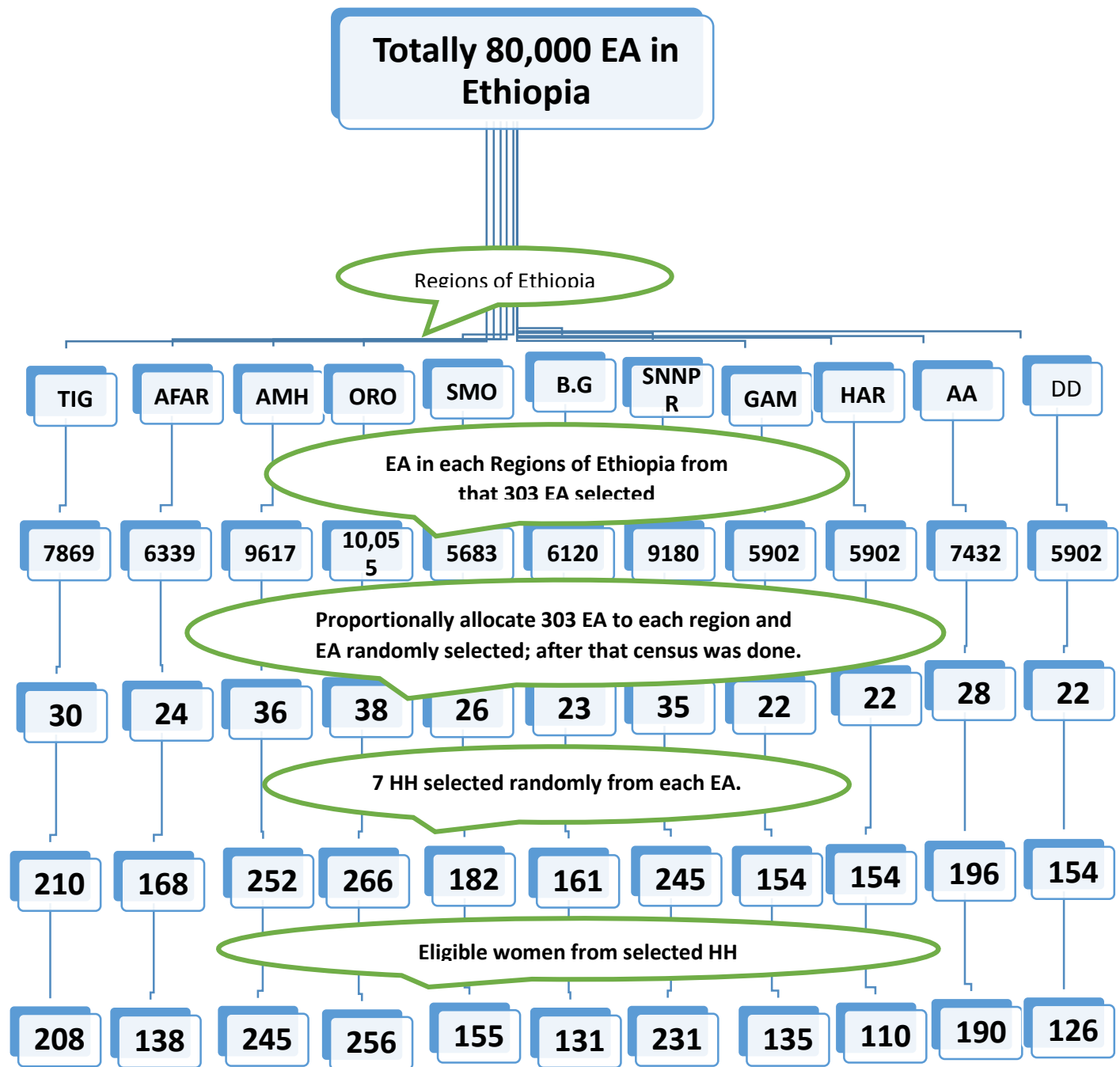
Figure 2 Schematic presentation of sampling procedure

## 4.9 VARIABLES

- Iron deficiency(Serum ferritin <15μg/l)

## 4.9.2 Explanatory variable

- **Health related**

  o Intestinal parasitic Infection

  o infection within 2 week(cough, diarrhea, fever)

  o Malaria

  o Inflammation

  o Anemia

- **Diet related and nutrition related factors**

  - consumption of animal source food

  - BMI

  - DDS

**Sociodemographic and economic characteristics**

  - Residence

  - Age

  - Family size

  - Geographic region

  - Wealth index

**Environmental factor**

  - Access to safe water utilization

  - Access to toilet use

## 4.10 Data collection tools

The survey data collection tool has modules:

**Module: 1 (Interview Questions)** the questions related to demographic, 24hr dieter recall and health status of non-pregnant women aged 15-49 years.

**Module: 2 (Target Population Assessments and Sample Collection)** Biochemical specimens collection format from Non-pregnant women aged 15-49 years old (blood, stool) and anthropetric measurement living in the selected household.

## 4.11 Data quality Assurance

### Training for supervisors and data collectors

The questionnaires and survey tools were pretested prior to the survey training. A three-week training was given for the data collectors, supervisors and overall quality control followed by field pilot testing in a separate cluster (not selected for the survey) with feedback and practice over a period of up to one week.

### Sensitization

The Ethiopian Public Health Institute (EPHI) and the Federal Ministry of Health (FMoH) conducted regional level sensitization meetings with key political and health leaders prior to the survey implementation. They also provided national public service announcements through local and national media to publicize the survey well in advance of survey implementation. Sensitization and an explanation of the survey was also provided during the household listing exercise.

### Field Implementation

Eighteen teams conducted the survey in the field. Each team consist of one Enumeration Sub-Team, one Laboratory (biological sample collection) Sub-Team, one overall Quality Control Supervisor, and a driver. Each team was responsible for completing 18 - 22 clusters. Each sub-team was allocated one car.

1) The Enumeration Sub-Team consisted of 1 Enumeration Supervisor and 2 enumerators (interviewers). This team conducted interviews.

2) The Laboratory Sub-Team consisted of 1 Lab Supervisor and 2 lab technicians. This team collected all the biological samples collection. The Laboratory Sub-Team was responsible

for maintaining the cold chain and processing biological specimens in the field as necessary.

3) The Quality Control Supervisor was conduct community sensitization and oversee the quality of the data, specimen collection and cold chain in each cluster. This person moved to the next cluster first.

**Day 1** On the first day of a visit to an EA, the Quality Control Supervisor conducted mobilization or remind the EA leaders and health officials to provide necessary support for the survey. The Quality Control Supervisor and Enumeration Supervisor worked with the community leaders and local health office to ensure that people are available for biological sample collection by visiting each of the 7 interviewed households. The enumeration supervisor also coordinated the enumeration team for interview logistics and transportation between households. The Lab Supervisor oversaw setting up the field lab and coordinated laboratory activities with the Lab Sub-Team.

**Day 1 and 2**: The Enumeration Sub-Team explained the survey purpose within each household adults 18 years of age or older respondent was asked separately for their voluntary consent. To conduct interview, to provide blood and stool sample for micronutrient status, helminthic infection and testing and then pass out specimen collection vials and stool samples with the subject ID. The Lab Sub-Team visited each household after the enumeration sub-team completes their interviews and collected biological specimens.

**Day 3:** On day 3 the enumeration sub-team and laboratory sub-team continued to conduct interviews and biological sample collection at the households that were not completed on day 1 and 2 before leaving for the next cluster.

The overall quality control supervisor in each team was responsible for completeness and for proper field administration of questionnaires. The quality control supervisor also insured the biological samples are collected and properly labeled, stored for spot testing and the specimens were shipped to EPHI. Any data enumerators or field staff who don't comply with the quality standards that they were oriented during the training before the field work, received oral reprimand and refreshed on what they need to improve on. If there was a second instance of low quality output, a written reprimand was given and if there was a third instance, the staff person was

replaced.  The Regional Coordinators checked 10% of all questionnaires that they received in the field.
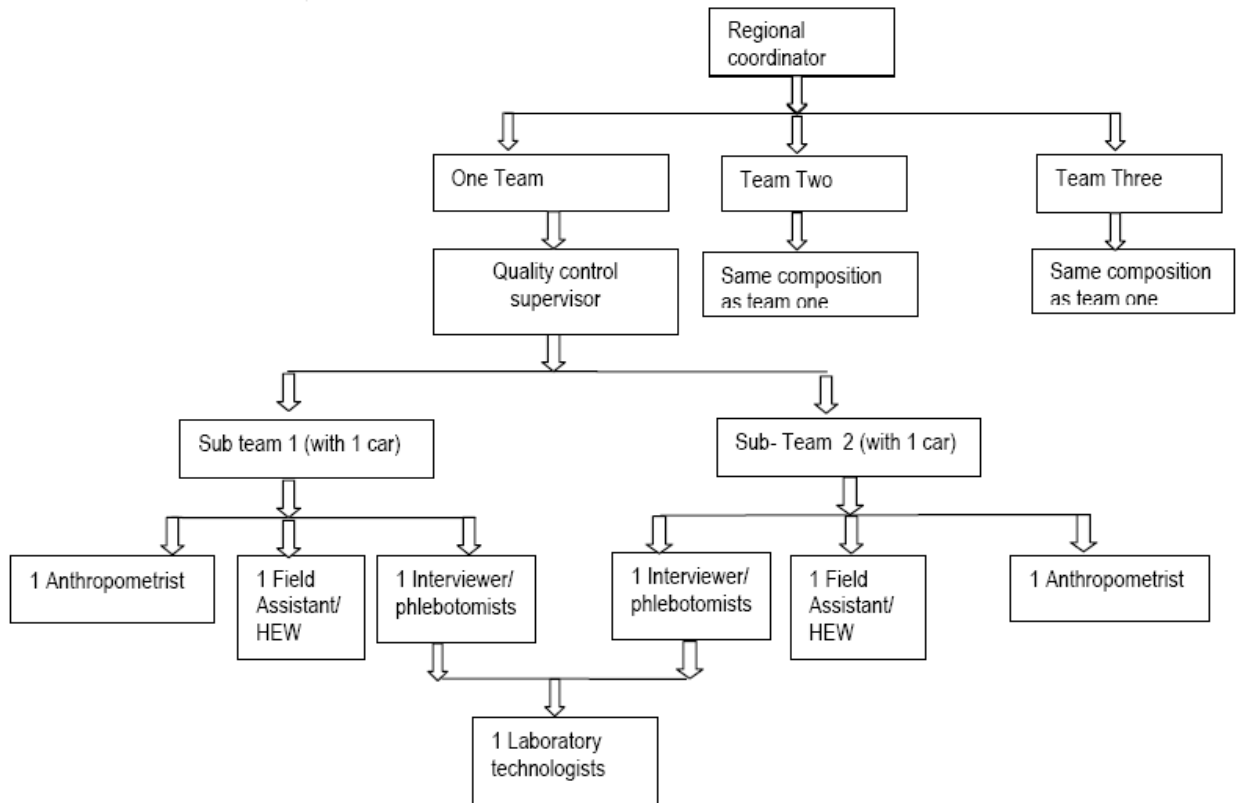


Figure 3 Quality control member and data collector during Ethiopian micronutrient survey data collection.


**Labeling of Questionnaires and Sample**

A unique barcode and ID number was assigned to each respondent consenting to sample collection. At the time of the sample collection, the unique ID number with each respondent's ID was affixed to the questionnaire, vacutainer and stool.

**Questionnaires**

A household questionnaire was completed for each selected household from each cluster. If there was no response from the household, the reason for non-response was documented. Questionnaires captured demographic, socioeconomic, health, dietary diversification information on general demographics of the household was collected. The household questionnaire included questions of family size, number of rooms in the house, livelihood, socio-economic status, and education level. Food and clean drinking water availability, availability of latrines, and other health related questions were asked. The questionnaire was written in English and translated into languages accordingly and back translated into English. The questionnaire was be field tested and revised based on pretesting.

**Anthropometry**

Height and weight were taken from non-pregnant women 15-49 years in Ethiopia. Height is measured in standing position to the nearest 0.1cm. The head should be in Frankfurt plane during measurement, knees should be straight and heels buttock and shoulder blades, should touch the vertical surface of the stadiometer. Weight is measured using beam balance to the nearest 0.1kg.

**Blood collection**

Experienced phlebotomists collected blood from an arm by venipuncture using trace metal free evacuated tube collection system, it was necessary to collect whole blood into 3Vacutainers for quantity needed per analyte and for blood collection protocols). A vacutainer (red top) was used for ferritin, AGP and CRP analysis. Remaining specimens were stored in repository.

**Transportation of blood from household to temporary field lab:**

The samples was transported as soon as possible after collection at the household in cold boxes containing frozen gel packs (<8ºC) by local guides hired specifically to assist each lab technician in rapidly carrying the samples to the centralised temporary field lab site.

Each team vehicle had maintained a self-contained field lab that included a portable centrifuge to allow for immediate centrifugation and aliquoting serum in cryovials. This vehicle also had a -

20ºC freezer that can be powered either by battery or electrical mains power for fast freezing of serum samples in the field. This freezer was used to maintain frozen gel packs for distributing in each cool box that goes to the field during sample collection. In each EA (cluster) a temporary field lab was set up in a central site such as a school, pharmacy, health centre or other location for the technologist to immediately centrifuge the samples brought in from the field and aliquot the serum into appropriate cryovials. When electricity was not available in the EA, the field lab was set up in the vehicle. All samples was processed within <2 hours of collection.

**Collection of stool sample**

Stool sample was collected from non-pregnant women aged 15-49 years. The respondent was asked to provide a stool sample. The stool was placed in a plastic cup with a tight fitting lid, containing 15 mL of 10% formalin for preservation. About 20 – 40 grams of well-formed stool or 5-6 tablespoons full of watery stool was suffice for a routine examination.

**Transportation of stool samples**

The formalin-preserved stool specimens were transported in Ziploc bags stored in a cool box to the EPHI Central Lab.

## 4.12 Laboratory Analyses

An appropriate amount of serum sample were dispensed into cryovialsm and properly stored at <-20 celsius and in the vehicle. All samples were processed within <2 hours of collection.

**Micronutrient analyses**

Iron status were assessed of iron deficiency using an automated electro-chemiluminescence immunoassay (ECLIA) and immuno-turbidimetry technique. Serum ferritin has been recommended by the World Health Organization for population based surveys because it is responsive to iron interventions overtime. However, a major drawback is that serum ferritin is

elevated in the presence of infection because it is an acute phase protein. A secondary analyses were performed on serum ferritin results taking into account the presence of inflammation.

**Infection**

Acute Phase Proteins: Alpha-1-acid-glycoprotein (AGP) and C-reactive protein (CRP) were measured to control for inflammation when interpreting biomarkers. These measures were assessed using the immune-turbidimetry assay.

**Stool: Indicators for intestinal Helminthes**

The examination of fasces for parasitological diagnosis were done to detect adult worms, cysts, Ova and larvae. The formalin-preserved stool specimens were examined in the laboratory using the formol-ether concentration technique for intestinal helminthes infections.

## 4.13 Data Management and Analysis

### 4.13.1 Data Collection and storage

Cluster forms were used to list all household questionnaire responses and follow-up visits to ensure that the appropriate numbers of follow-up visits were conducted and to help calculate response rates in the field. During the survey, the cluster forms were collected from each team after the 7 household follow-up visits have been exhausted and all eligible respondent interviews were completed. After the data were reviewed by the enumeration supervisor, each team's quality control supervisor verify the forms for completeness and errors in the field were corrected prior to departing the cluster. Data from each cluster was compiled and stored in a water proof zip lock plastic bag. The regional coordinators reviewed 10% of data in the field and every 2-3 weeks data were shipped to EPHI for protected storage. The data were kept in locked storage at EPHI for up to five years locked storage at EPHI for up to five years.

Confidentiality was strictly maintained. Only the top staff accessed to these files. Data entry included only numeric identifiers for each participant which has no meaning to any outside observer. All electronic files were password protected. Data were released only in summary form, and the identity of participants was not made public.

**4.113.2 Data Analysis**

Data collection was conducted using Samsung tablet 4 and sent to the EPHI server every night up on completion of every household and reviewed by supervisors. , then the data were imported to STATA version 13 for analysis.

Serum ferritin was adjusted for each respondent who had inflammation by CRP and AGP; using linear regression after checking assumption.

> ➢ Serum ferritin = Original value SF - $\beta$(AGP/CRP)*( AGP/CRP value)

Hemoglobin also adjusted for altitude >1000meter in each respective altitude measurement. (21) Descriptive statistics was done to describe the sample. The results of the descriptive statistic expressed as percentage and frequency. Associations between independent variables and dependent variables was analyzed first using bivariate analysis (binary logistic regression) to identify factors which are significantly associated with iron deficiency. Logistic regression was applied using backward step-wise regression method with $p < 0.25$ and $p > 0.1$ criteria to enter and exit from the model, respectively. Logistic regression model was used to fit the data to identify factors associated with iron deficiency. All explanatory variables that were associated with the outcome variable in bivariate analysis with p-value of 0.25 or less were included in the initial logistic models of multivariable analysis. Multi collinearity between independent variable was also checked. The crude and adjusted odds ratio together with their corresponding 95% confidence intervals were computed. P-value $< 0.05$ was considered to declare a result as statistically significant in this study. The results were presented in text, tables and graphs based on the types of data. PCA analysis was done after cheaking assumption for wealth index and divided into tertials.

## 14.4 Operational definition

- **Iron deficiency**; when serum ferritin level is less than 15µg/l.

- **Anaemia**: when serum hemoglobin value is less than 11mg/dl.

- **Enumeration area:** as were created containing 150 to 200 households in rural and urban houses as a measure of size.

- **Women reproductive age**: age between 15 – 49.

- **Raised AGP;** defined as α 1 acid glycoprotein >1g/l.

- **Raised CRP;** defined as C-reactive protein >5mg/l.

- **Household**; is defined as a group of people who share a common cooking pot.

- **Good DDS**: a woman consume > 5 food group according to FANTA 9 food group classification.

- **Poor DDS**: a woman consume < =5 food group according to FANTA 9 food group classification.

- **Access to toilet facility**: a woman had an access of one of the following; piped sewer system, flush to septic tank, flush to pit latrine, flush to somewhere else, ventilated improved pit, pit latrine with slab and flush don't know where.

- **Access to safe water**: a woman was using one of the following for drinking purpose; piped into dwelling, piped to compound, public tap or stand pipe, tube well or borehole, bottled water.

- **Animal source food intake = Yes**; if a woman consume at least one of the following in the last 24 hour; sea food (fish), eggs, organ meat, beef/pork/lamb/goat, milk and milk products.

- **Animal source food intake =No**; if a woman don't consume at least one of the following in the last 24 hour; sea food (fish), eggs, organ meat, beef/pork/lamb/goat, milk and milk products.

- **Wealth index:** classified into three categories, after adding the house holds asset.

## 14.4. Dissemination plan

Dissemination of survey results will include presentations to JU, FMoH and reports will be distributed to regional offices, other stakeholders and international organizations.  Summary reports will be disseminated to other relevant stakeholders. There will be a workshop to discuss final results and develop programmatic recommendations. Survey findings will also be disseminated and presented at regional conferences, meetings and workshops.

## 14.5. ETHICAL CLEARANCE

Verbal informed consent was obtained from all households before data collection begins.  The Ethiopian National Micronutrient survey ensures that the principles to protect respondents and prevent from unnecessary risk to survey respondents, the proposal was reviewed by EPHI and the national scientific and ethical review committee for ethical approval. If respondents found to have malaria was referred to the nearest health centre.

# CHAPTER FIVE: RESULTS

## 5.1 Socio-demographic characteristics of participant

Of the total 1924 women planned to be included in the study, 1624 non-pregnant women in reproductive age actually participated in the study giving response rate of 84.4%.

The mean age of study participant was 28.1±8.5 of this, 35.3% were in the age group 15–24, 44.7% were in the age group 25- 35 whereas 19.9% were in the 36 -49 age group. The majority of the respondents live in rural areas (64.4%). Among the study participant, 46.3% were Orthodox Christian by religion followed by Muslim (35.9%) and protestant (16.5%). (Table 1)

The mean family size of the respondents was 5.4 and 1217 (56.7%) of the participants had a family size <=5 whereas 927 (43.3%) were had family size >5.

Nearly half of the respondents (47.1%) did not attend formal education whereas 29.8% attended primary, 16.4% secondary  and 6.7% of them attend vocational, college and above. (Table 1)

*Table1. Socio-Demographic Characteristics of non-pregnant WRA (15 – 49) in Ethiopia, June2015 (n=1924)*

| Variables | | N | Percent (%) |
|---|---|---|---|
| Age | 15-24 | 680 | 35.3 |
| | 25-35 | 860 | 44.7 |
| | 36-49 | 384 | 19.9 |
| Residence | Urban | 685 | 35.6 |
| | Rural | 1239 | 64.4 |
| Religion | Orthodox | 891 | 46.3 |
| | Protestant | 317 | 16.5 |
| | Muslim | 691 | 35.9 |
| | Others | 25 | 1.3 |
| Family size (mean (5.4) | <=5 | 1091 | 56.7 |
| | >5 | 833 | 43.3 |
| Educational level | Not attended | 906 | 47.1 |
| | Primary | 573 | 29.8 |
| | Secondary | 316 | 16.4 |
| | Technical/University | 129 | 6.7 |

## 5.2 Environmental Characteristics of non-pregnant WRA in Ethiopia

From study participant 27.5% off them had access to toilet whereas 72.5% of them doesn't had the access. Similarly 67.7% of the respondent had access to safe water whereas 32.3% doesn't had the access. (Table 2)

*Table2.Enviromental Characteristics of non-pregnant WRA (15 – 49) in Ethiopia, June2015 (n=1924)*

| Variable | | Frequency | Percent (%) |
|---|---|---|---|
| Access to toilet use | Yes | 529 | 27.5 |
| | No | 1395 | 72.5 |
| Access to safe water utilization | Yes | 1303 | 67.7 |
| | No | 621 | 32.3 |

## 5.3 Diet and Nutritional characteristics of study participant

Majority of the respondents had normal BMI 1274(66.8%) whereas 400 (20.8%) and 250(13.0%) had thin and overweight/obese BMI, respectively. Among the respondents, 39.9% were consumed animal source food at least one within 24 hours prior to data collection whereas, 60.1% of did not ate animal source foods. (Table 3).

*Table 3. Diet and nutrition related Characteristics among non-pregnant WRA (15-49) in Ethiopia, June 2015(n=1924)*

| Variables | | Frequency (n) | Percent (%) |
|---|---|---|---|
| BMI | Chronic energy deficiency(CED) | 400 | 20.8 |
| | Normal | 1274 | 66.2 |
| | Overweight/ Obesity | 250 | 13.0 |
| DDS | Poor (<=5food group) | 1603 | 83.3 |
| | Poor (>5food group) | 321 | 16.7 |
| Animal source food Consumption in last 24hr. | Yes | 773 | 40.2 |
| | No | 1151 | 59.8 |

***Remark; Animal source food (fish, beef/lamb, organ meat, egg and milk and milk products)***

## 5.4 Morbidity status of non-pregnant WRA in Ethiopia

Of the 1634 of the respondents, 45% had history of fever before two weeks prior to the data collection, while 6% had diarrhea and 11% had history of cough.

Among the respondents, whose blood was investigated for malaria 3.4% of them were positive for malaria rapid diagnostic test and 29% of the respondents were infested with intestinal parasites. (Table 4)

*Table 4. Morbidity status of women among non-pregnant WRA (15-49) in Ethiopia 2015. (n=1924)*

| Variables | | Frequency (n) | Percent (%) |
|---|---|---|---|
| Intestinal parasite | No | 1168 | 71.0 |
| | yes | 478 | 29.0 |
| Illness in the past 2 week(diarrhea) | No | 1529 | 94.0 |
| | Yes | 96 | 6.0 |
| Illness in the past 2 week(fever) | No | 890 | 54.8 |
| | Yes | 735 | 45.2 |
| Illness in the past 2 weeks(cough) | No | 1450 | 89.2 |
| | Yes | 176 | 10.8 |

## 5.5 The concentration of biomarkers

The median concentration of Serum ferritin was 58µg/L with 32.4µg/L and 94.8µg/L the first and the third quantile respectively [95% CI: 55.9 ,61.8]; When it's adjusted with inflammation the median concertation was 57.5 mg/dl with q1 (32.4µg/L) and q3 (92.3µg/L) with CI [95%CI: ,55.4, 60.6]; the median concentration of the unadjusted one was 58 mg/dl with q1 (32.4 mg/dl) and q3(94.8 mg/dl) with confidence interval[95%CI:55.9 , 61.8].

The median concertation of hemoglobin of the respondent was 14.0mg/dl with the first and the third quantile value of 13 mg/dl and 14.9 mg/dl respectively [95%CI: 13.9, 14.0]. After adjusting hemoglobin to altitude median concentration was 13.4mg/dl with the first and the third quantile value of 13 mg/dl and 14.9 mg/dl, respectively with the CI of [95%CI: 13.3, 13.5].

Whereas median concentration inflammation indicator AGP was 0.76 g/l with the first and the third quantile value and 0.6g/l and 0.99 g/l, respectively with CI value [95%CI: 0.75, 0.78] and

CRP median concertation value 0.68 mg/l the first and the third quantile value 0.64 mg/l and 0.73 mg/dl, respectively with CI [95%CI: 0.32, 2.12]. (Table 5)

*Table 5. Median concentration of serum ferritin, hemoglobin, AGP and CRP values among non-pregnant WRA in Ethiopia, June 2015*

| Variable | | No of ob. | Median | Q1 | Q3 | 95%CI |
|---|---|---|---|---|---|---|
| Ferritin | Unadjusted | 1700 | 58 | 33.2 | 94.8 | [55.9, 61.8] |
| | Adjusted* | 1695 | 57.5 | 32.4 | 92.3 | [55.4, 60.6] |
| Haemoglobin | Unadjusted | 1881 | 14.0 | 13 | 14.9 | [13.9,14.2] |
| | Adjusted** | 1881 | 13.4 | 12.5 | 14.2 | [13.3,13.5] |
| AGP*** | | 1723 | 0.76 | 0.6 | 0.99 | [0.75,0.78] |
| CRP**** | | 1721 | 0.68 | 0.32 | 2.12 | [0.64,0.73] |

**Remark * Ferritin adjusted with inflammation (CRP and AGP) **Hemoglobin adjusted with Altitude >1000M ***AGP - α-1-acid glycoprotein ****CRP - C– reactive protein.**

## 5.6 Prevalence of Iron deficiency among non-pregnant WRA in Ethiopia

This study found that the prevalence of iron deficiency among non-pregnant women in reproductive age in Ethiopia without adjusting it to inflammation was 9.4%; when it's adjusted to inflammation the prevalence was 9.9 %.( Figure4).

Similarly the prevalence of anemia without adjusting hemoglobin to altitude it was 10.4% and when adjusted it was 16.8%. Similarly prevalence of Iron deficiency anemia without adjusting it to both for altitude and infection it was 3.4% and when it's adjusted it was 4.3%.altitude and

infection
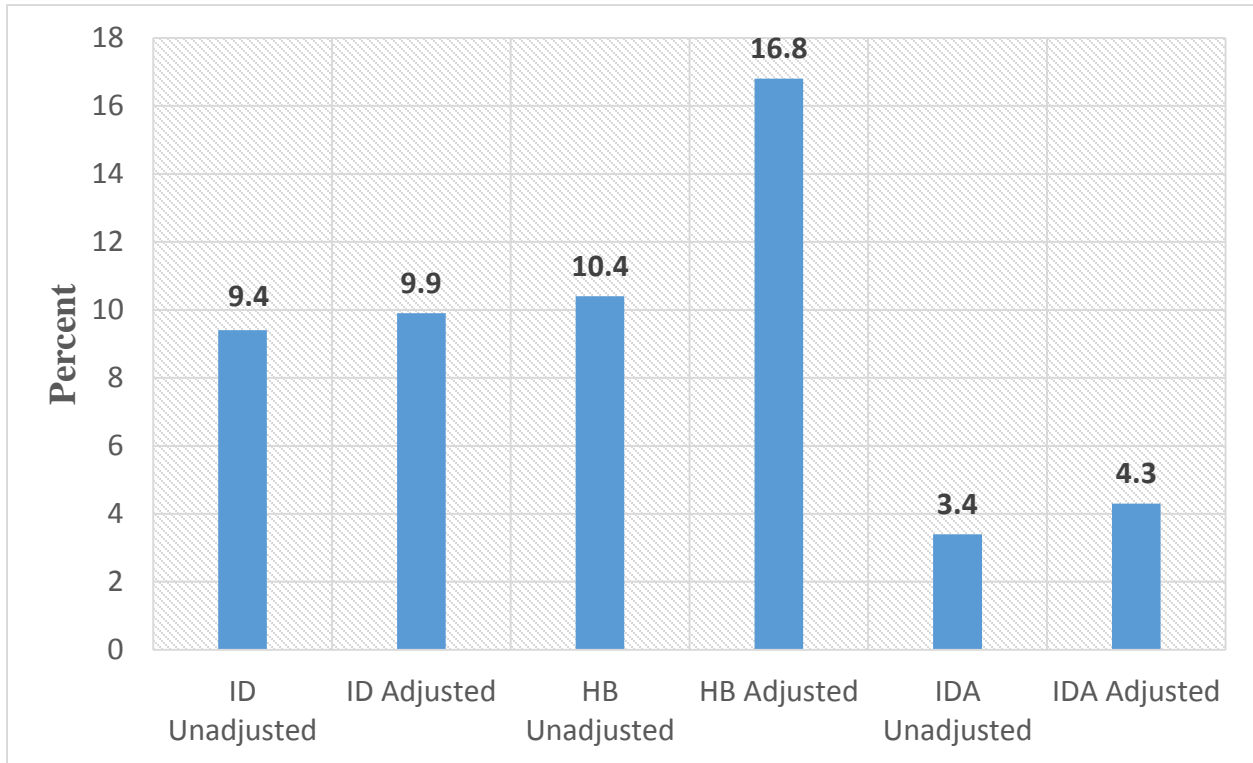


*Figure 4. Prevalence Iron deficiency, Anemia and iron deficiency anemia with adjustment in non-pregnant WRA in Ethiopia, June 2015*

**\*Iron deficiency adjusted with Ferritin and inflammation (CRP and AGP).**
**\*\*Anemia adjusted with hemoglobin and altitude.**
**\*Iron deficiency anemia adjusted with (hemoglobin, altitude, CRP, AGP and ferritin).**

## 5.7. Prevalence of Iron deficiency among non-pregnant WRA by region of Ethiopia

The higher prevalence of iron deficiency was observed in the eastern part of the country, which was 69.8% in Somalia followed by 44.1% in Harari and 40.2% in Diredewa. Whereas the minimum prevalence was in Tigray (13.8%), Amhara (9.1%) and B.Gumuz (6.6%) in descending order.

*Figure 5. Iron deficiency in non-pregnant women in reproductive (15-49) age in Regions of Ethiopia June 2015*

### 5.8 Predictors of iron deficiency

In the bivariate analysis factor associated with iron deficiency were (at $P \leq 0.25$) residence, family size, educational status, wealth index, access toilet facility, access to safe drinking water, animal source food consumption within the last 24hr subclinical infection (AGP), subclinical infection (CRP), illness in the past two week (Diarrhea), illness in the past two week (cough), anemia and BMI (overweight/obese).(Table 6)

In multivariate analysis the following factors were independent predictors of iron deficiency with p- value $< 0.05$. (Table 7)

Women who had raised AGP had 2.1 times higher odds off having Iron deficiency than their counter part (AOR= 2.1: 95%CI [1.39, 3.01]), respondents who had family size >5 had 1.48 times more likely to be iron deficient as compared to who had family size <=5 (AOR= 1.48, [95%CI 1.1, 1.91, 1.8]). This study revealed that anemic women's were 3.4 more likely to be iron deficient

than those who doesn't had Anemia (AOR= 3.4, [95%CI 2.5, 4.7]). This study also revealed that women who were obese/overweight were 2.3 times more likely to be iron deficient than their counter parts (AOR =2.3, 95%CI [1.6, 3.4)]). Women who had access to safe water utilization for drinking had 0.63 lower odds of iron deficiency than their counterpart (AOR= 0.63, [96%CI; 0.46, 0.86]). This study also showed that women who doesn't ate animal source foods at least one in the last 24hr had 1.46 higher odds of being Iron deficient than their counter part. (AOR= 1.46, [95% CI (1.1- 1.8]).

*Table 6: Results of bivariable analyses on iron deficiency among non-pregnant WRA in Ethiopia, June 2015(n=1624)*

| Variables | | Iron Status | | | | COR | p |
|---|---|---|---|---|---|---|---|
| | | Deficient | | Normal | | | |
| | | No. | % | No. | % | | |
| Residence | Urban | 58 | 9.9 | 525 | 90.1 | 1 | |
| | Rural | 102 | 9.8 | 939 | 90.2 | 1.2 | 0.045 |
| Family size (Mean 5.4) | <=5 | 71 | 7.9 | 854 | 92.1 | 1 | |
| | >5 | 84 | 12.3 | 615 | 87.7 | 1.6 | 0.00 |
| Educational status | Not attended (Illiterate) | 89 | 11.9 | 660 | 88.1 | 1 | |
| | Primary | 35 | 7.2 | 464 | 92.8 | 0.6 | 0.23 |
| | Secondary | 24 | 8.9 | 254 | 91.1 | 0.7 | 0.81 |
| | Vocational, college and above | 9 | 10 | 89 | 90 | 0.7 | 0.49 |
| Wealth Index | Middle | 95 | 60.8 | 466 | 83.2 | 1 | |
| | Poorest | 130 | 24.2 | 406 | 75.8 | 1.57 | 0.13 |
| | Highest | 153 | 28.2 | 390 | 71.8 | 1.9 | 0.02 |
| Access to toilet facility | Yes | 131 | 30.1 | 308 | 61.9 | 1.7 | 0.00 |
| | No | 239 | 22.3 | 946 | 79.7 | 1 | |
| Access to safe drinking water | Yes | 236 | 21.2 | 888 | 78.8 | 0.7 | 0.09 |
| | No | 134 | 27.1 | 366 | 72.9 | 1 | |
| BMI | Normal | 98 | 9.25 | 962 | 90.8 | 1 | |
| | CED | 35 | 10.1 | 321 | 89.9 | 1.1 | 0.79 |
| | Overweight/Obesity | 26 | 12.5 | 182 | 87.5 | 1.4 | 0.00 |
| | Yes | 270 | 41.3 | 384 | 58.7 | 1 | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Animal source food consumption in the last 24hr | No | 428 | 44.1 | 542 | 55.9 | 1.12 | 0.00 |
| Subclinical infection,AGP | Raised | 179 | 48.0 | 194 | 52.0 | 5.5 | 0.00 |
| | Normal | 177 | 15.1 | 1075 | 84.9 | 1 | |
| Subclinical infection,CRP | Raised | 180 | 81.5 | 41 | 18.5 | 24.9 | 0.00 |
| | Normal | 210 | 14.3 | 1193 | 85.7 | 1 | |
| Illness over two weeks (Diarrhea) | Yes | 28 | 31.8 | 64 | 68.8 | 1.5 | 0.05 |
| | NO | 342 | 22.5 | 1189 | 77.5 | 1 | |
| Illness over two weeks (Cough) | Yes | 48 | 28.4 | 124 | 71.6 | 1.4 | 0.07 |
| | No | 324 | 22.3 | 1131 | 77.7 | 1 | |
| Anaemia | Yes | 101 | 46.2 | 117 | 53.8 | 3.5 | 0.00 |
| | No | 279 | 19.6 | 1126 | 80.4 | 1 | |

*Table:7 Multivariable logistic regression analysis on Iron deficiency among non-pregnant women in reproductive age in Ethiopia; June 2015 (n=1624)*

| Variables | | Iron Status | | | | COR | AOR |
|---|---|---|---|---|---|---|---|
| | | Deficient | | Normal | | | |
| | | No. | % | No. | % | | |
| Family size (Mean 5.4) | <=5 | 71 | 7.95 | 854 | 92.05 | | 1 |
| | >5 | 84 | 12.34 | 615 | 87.66 | 1.6 | **1.5(1.13,1.92)** |
| Wealth Index | Middle | 95 | 60.8 | 466 | 83.2 | | 1 |
| | Poorest | 130 | 24.2 | 406 | 75.8 | 1.57 | 1.3(0.75,2.27) |
| | Highest | 153 | 28.2 | 390 | 71.8 | 1.9 | 0.7(0.05,0.52) |
| Access to toilet facility | Yes | 131 | 30.1 | 308 | 61.9 | 1.7 | 1.9(0.92,1.93) |
| | No | 239 | 22.3 | 946 | 79.7 | | |
| Access to safe drinking water | Yes | 236 | 21.2 | 888 | 78.8 | 0.7 | **0.6(0.46,0.86)** |
| | No | 134 | 27.1 | 366 | 72.9 | | 1 |
| BMI | Normal | 98 | 9.25 | 962 | 90.8 | | 1 |
| | CED | 35 | 10.09 | 321 | 89.91 | 1.1 | 0.9(0.67,1.31) |
| | Overweight/Obesity | 26 | 12.5 | 182 | 87.5 | 1.4 | **2.3(1.62,3.38)** |
| Animal source food consumption in the last 24hr | Yes | 270 | 41.3 | 384 | 58.7 | | |
| | No | 428 | 44.1 | 542 | 55.9 | 1.12 | **1.5(1.12,1.89)** |
| Subclinical infection,AGP | Raised | 179 | 48.0 | 194 | 52.0 | 5.5 | **2.1(1.39,3.01)** |
| | Normal | 177 | 15.1 | 1075 | 84.9 | | 1 |
| Illness over two weeks (Cough) | Yes | 48 | 28.4 | 124 | 71.6 | 1.4 | 1.3(0.93,2.05) |
| | No | 324 | 22.3 | 1131 | 77.7 | | 1 |
| Anaemia | Yes | 101 | 46.2 | 117 | 53.8 | 3.5 | **3.4(2.5,4.75)** |
| | No | 279 | 19.6 | 1126 | 80.4 | | 1 |

# CHAPTER SIX: DISSCUSION

This study aimed at determining prevalence Iron deficiency and its predictors among non-pregnant women in reproductive age (15-49 years) group in Ethiopia. From the total of 1624 respondents, 9.9% were Iron deficient. Based on WHO criteria, this finding indicates that iron deficiency is a mild public health problem. Prevalence of anemia was 16.8% whereas the prevalence of iron deficiency anemia only 4.3%. Having raised AGP, having family size >5, having access to safe water for drinking, being overweight/obese, being anemic and animal source food consumption in the last 24hr were independent predictor of Iron deficiency.

Serum ferritin; a positive acute phase response protein and its concentrations increase during inflammation confounding the size of the iron store. This makes the interpretation of normal or high serum ferritin values difficult in areas of widespread infection or inflammation. (9) Therefore, the observed 9.9% Iron deficiency was adjusted for inflammation. The prevalence of Iron deficiency before adjusting it to inflammation was (9.4%) showing the underestimation of the prevalence of Iron deficiency that could result from inflammation.

The finding Iron deficiency prevalence(9.9%) is nearly consistent with study finding in Cambodian women in reproductive age (8.1%) which also exclude pregnant women and adjust for inflammation(16); but lower as compare to study findings among women in reproductive age group in Vietnam (23.1%) (19) and lower than the study finding in nine administrative regions of Ethiopia (50.1%) (15). The difference between this study and both studies is might be due both includes pregnant women's in the study while this study exclude pregnant women because the physiologic change due to pregnancy can cause Iron deficiency. (15, 16, 19) The finding prevalence of iron deficiency is low this might be due to the strategies that has been implemented to reduce micronutrient deficiency like health education, supplementation and other contribute to this finding but it needs further work to eradicate this problem.

In this study, the highest prevalence of iron deficiency in regions of Ethiopia; Somalia region (69.8%), followed by Harari (44.1%), Dire Dewa (40.2%) and Afar (28.4%) and whereas the lowest prevalence seen in Amhara and B.Gumuz 9.1% and 6.6% respectively. This implies that

there is regional variation on Iron deficiency. This finding is supported with the study finding in nine administrative regions of Ethiopia showing that there is regional variation in prevalence of Iron deficiency (15). The higher prevalence found in eastern part of the country might be due to high chewing khat habit in this part of the contrary. Khat contains a substantial amount of tannin, which reduces the bioavailability of iron and the loss of appetite may contribute to this finding; in addition, people use nuts extensively during khat chewing which may also interfere with iron absorption due to high oxalate content. (26)

In this study the prevalence of anemia after adjusting for altitude was 16.8% (unadjusted prevalence was 10.4%). This finding implies that estimation of anemia prevalence must be adjusted for altitude as it can be significantly underestimated. This finding was consistent with EDHS 2011 result in Ethiopia 17% in non-pregnant women (28). The finding result according to WHO classification the prevalence of Anemia is in a mild public health problem. Women with anemia were nearly 3.6 times higher odds of being Iron deficiencient than their counter parts

The prevalence of Iron deficiency anemia was 4.3% implying that iron deficiency contributes only to 4.3% of 16.8% anemic cases. So, other factors contribute much of the deficiency of anemia than Iron deficiency, which should also be taken into account in designing intervention programs. This finding is consistent with the report of a study in Nepal which showed that of 16% anemic cases only 6% had iron deficiency. (23)

Respondents who had raised AGP were 2.1 more likely to be iron deficient than their counterpart. This can be explained by this might be due to chronic inflammation limits the iron availability. The synthesis of hepcidin is greatly stimulated by inflammation hepcidin is the predominant negative regulator of iron absorption in the small intestine.(27) and consistent with the study conducted in nine administrative regions of Ethiopia in which chronic inflammation associated with iron deficiency.(15)

Having family size >5 was also the significant independent predicator of Iron deficiency. Households with a family size of greater than five had 1.48 times higher odds of being Iron deficient than with lower family size. This might be due to when the family size increase sharing of diet when the the family size increase the portion of women decrease and nutrient intake also

decrease. This finding is supported by study done in Saudi (17) but differ from the study finding in nine administrative regions of Ethiopia (15).

In this study not eating animal source food in the last 24hr was associated with 1.5 time's higher odds of having Iron deficiency compared with women who consumed animal source foods in the last 24hr. Since the best source of Iron in diet is animal source food with the best bioavailability (2), not eating animal source food will expose to Iron deficiency. This finding is supported by the study conducted in Vietnam different from the finding of a study in Nepal (23).

This study also revealed that drinking safe water was associated with 47% less likelihood of having Iron deficiency. It has been reported that drinking safe water protects from intestinal parasite and other infection.

Other predictor of Iron deficiency in this study were women in reproductive age; it was observed that obese/overweight women were 2.34 times higher odds of having Iron deficiency than their counterparts. The etiology appears to be multifactorial, and may include inadequate bioavailable iron relative to body weight, as well as diminished intestinal absorption and decreased iron bioavailability induced by inflammatory adipokines found in those with excessive adiposity. (26) This finding consistent with the study conducted in Israel adolescent women(27) and also supported by comparative study conducted in USA between obese and non-obese subjects showed that hypoferremia of obesity appears to be explained both by true iron deficiency and by inflammatory-mediated functional iron deficiency.(29). This finding also consistent with the study conducted in Saudi (18) and Cambodia (16) in which overweight/obesity associated with iron deficiency.

The strength of this study is the fact that it was conducted in all regions of Ethiopia with a stratified multistage random sampling method based on population distribution, so the sample was a representative of the Ethiopian women and the results from the study also reflected the status of iron deficiency among Ethiopian women to some extent. In Ethiopia, a number of strategies and programs have been implemented to reduce maternal and child mortality and morbidity. Women in reproductive age group and children's are at high risk of developing micronutrient malnutrition. In this age group, different physiological, economic and social factors increase the vulnerability to

be micronutrient deficiency. Accordingly, this study aimed in identifying the prevalence iron deficiency and associated factors among women in reproductive age group in Ethiopia.

We acknowledge the following limitation in this study. In this study we try to adjust serum ferritin value with inflammation. Because inflammation had an effect on the serum ferritin value that may underestimate the prevalence of Iron deficiency. WHO recommend to use both serum ferritin and soluble transferrin and their index in inflammation prevalent area, so as to see real effect inflammation. Another limitation of this study is 24hr dietary recall cannot show the usual dieter intake women. Recall biases and social desirability biases was also the limitation of this study.

# CHAPTER SEVEN: CONCULUSION AND RECOMMENDATION

## 7.1 Conclusion

In this study, the prevalence of Iron deficiency according to WHO classification was mild 9.9%. The highest prevalence of Iron deficiency was found in Somalia (69.8), Harari44.1% and Diredewa 40.2% regions.

Only about 4.3% women with anemia also had Iron deficiency, suggesting that other causes of anemia and other micronutrient (folate and VB12) may be prevalent in this population.

AGP, Anemia, BMI, safe drinking water, animal source food and family size >5 were also independent predictors of Iron deficiency among non-pregnant women in reproductive age (15 – 49) in Ethiopia.

## 7.2 Recommendation

The finding indicates that of iron deficiency is labeled as mild public health importance; if not intervention taken it can increase any time. So based on the above finding we recommend:

 - ➢ The policy makers and other stake holder should consider in the development of programs that address micronutrient deficiency in Ethiopia.
 - ➢ In regions with high prevalence of Iron deficiency; FMOH should give attention.
 - ➢ Regional health bureau and other stakeholders who work on the area should also give attention to prevention of Iron deficiency.
 - ➢ We also suggest researchers to do research on the cause of the higher Iron deficiency prevalence in this part of the eastern regions of the country.
 - ➢ Health professionals and concerned body should give nutrition education for women in reproductive age about the cause of Iron deficiency to prevent its consequences.

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**ANNEX**

Annex 1.

**Household ID** ☐☐☐☐☐

EA (3 digit)  HH(2digit)

## WOMEN OF REPRODUCTIVE AGE 15-49 YEAR OLDS
## ETHIOPIAN NATIONAL MICRONUTRIENT SURVEY 2015

### Ethiopian Federal Ministry of Health, Ethiopian Public Health Institute
### Enrolment Informed Consent for Females 15-49 years old

Hello. My name is _____ and I am working with the Ethiopian Public Health Institute (EPHI).  We are conducting a national Micronutrient survey. We would very much appreciate your participation in this survey. This information will help the government to plan health and nutrition services. The survey usually takes about 30 minutes to complete.

First, I would like to sit down and ask you some questions about what you eat, and we would also like to collect a small sample of your blood, stool and urine.  We will also examine your neck for goiter and your eye for spots. We will also measure your mid upper arm circumference, height, and weight and ask questions related to what you are eating and your health habits.

The benefit to you for taking part in this survey is that you will get your results for height, weight, mid upper arm circumference, malaria, blood in urine and anemia. The other information you give us will not benefit you in a direct way. However, we will add the information you give us to that of other houses in Ethiopia, and will create a report.  The report will contribute to the good of your community.  What you say is important and valuable, and will help the Ministry of Health to improve their health and nutrition programs.

If you are not interested, you do not have to take part in this survey.  If I ask you any question you don't want to answer, just let me know and I will go on to the next question.  You may choose to stop the interview at any time.  Refusing to answer will not affect your family's access to health services.

All of the answers you give will be confidential and will not be shared with anyone other than members of our survey team. This form with your answers will be stored under lock and key.  You don't have to be in the survey, but we hope you will agree to answer the questions since your views are important.

| No. | QUESTION | CODING CATEGORIES | | SKIP |
|-----|----------|-------------------|---|------|
| W01 | **How old are you?** (VERIFY THAT THE AGE IS THE SAME AGE AS WRITTEN ON THE HOUSEHOLD LISTING) | ☐☐ Years | | |
| W02 | **Have you ever attended school?** | No………………………... Yes ……….…………… | 00 01 | 00→W04 |
| W03 | **What is the highest level of school you attended?** | Primary …………….....……………… Secondary ……………………….. Technical / vocational certificate…… Higher / university/ college …………. Don't know …………….................. . | 01 02 03 04 88 | 02-04 →W05 |
| W04 | **Now I would like you to read this sentence to me.** *SHOW CARD TO RESPONDENT.* *IF RESPONDENT CANNOT READ WHOLE SENTENCE, PROBE:* ***Can you read any part of this sentence to me?*** | Cannot read at all …………………….. Able to read only parts of sentence ... Able to read whole sentence………. Blind/visually impaired……………… | 01 02 03 04 | |
| Now I would like to ask you some questions about your health. We will first ask about the last 6 months. | | | | |
| W05 | **Did you take any drugs for intenstinal worms in the past six months?** | No……………………………...............…. Yes …….…..……………………………… Don't know…………………………..…… | 00 01 88 | |
| **Now I would like to ask you about your health in the last 2 weeks.** | | | | |
| W07 | **Have you been ill with diarrhoea in the past 2 weeks**? *DEFINED AS 3 OR MORE LOOSE OR WATERY STOOLS IN A 24-HOUR PERIOD* | No……………………………...............…. Yes …….…..……………………………… Don't know……………………………… | 00 01 88 | |
| W08 | **Have you been ill with a cough or breathing problems in the past 2 weeks?** | No……………………………...............…. Yes …….…..……………………………… Don't know…………………………….… | 00 01 88 | 00→W11 88→W11 |
| W09 | **When you had an illness with a cough, did you breathe faster than usual with short, rapid breaths or have difficulty breathing?** | No……………………………...............…. Yes …….…..……………………………… Don't know……………………………… | 00 01 88 | 00→W11 88→W11 |
| W10 | **Was the fast or difficult breathing due to a problem in the chest or to a blocked or runny nose?** | Chest only …..……...........………………. Blocked or runny nose only. …….......…... Both …….…..……………......…………. Other (specify)_____ Don't know …………….….……… | 01 02 03 77 88 | |
| W11 | **Have you been ill with a fever in the past 2 weeks?** | No……………………………...............…. Yes …….…..……………………………… Don't know……………………………… | 00 01 88 | |
| W12 | **Have you been ill with malaria in the past 2 weeks?** | No……………………………...............…. Yes …….…..……………………………… Don't know……………………………… | 00 01 88 | |

| W12 | What is the religion of the head of the HH? | Orthodox.………………………………………………………… | 01 | |
| | | Roman catholic …………………………………….............. Protestant/other | 02 | |
| | | Christian……………………….......................... | 03 | |
| | | Muslim...…………………………………………………… No | 04 | |
| | | religion…………………………………………………… Other (specify) | 05 | |
| | | _____ Don't | 77 | |
| | | know…………………………………………………….. | 88 | |
| W13 | What is the main source of drinking water for members of your household? | <u>PIPED WATER</u> | | 0 |
| | | PIPED INTO DWELLING……………………………………. | 01 | 1 |
| | | PIPED TO COMPOUND/PLOT……………………………........ | 02 | → |
| | | PUBLIC TAP/STANDPIPE…………………………….. | 03 | H |
| | | TUBE WELL OR BOREHOLE…………………………………… | 04 | 7 |
| | | <u>DUG WELL</u> | | 0 |
| | | PROTECTED WELL……………………….................... | 05 | 2 |
| | | UNPROTECTED WELL……………………………………. | 06 | → |
| | | <u>WATER FROM SPRING</u> | | H |
| | | PROTECTED SPRING…………………………………… | 07 | 7 |
| | | UNPROTECTED SPRING……………………………….. | 08 | |
| | (CIRCLE ONE ONLY) | RAINWATER……………………………………………….. | 09 | |
| | | TANKER TRUCK……………………………………………….. | 10 | |
| | | CART WITH SMALL TANK…………………………………… | 11 | |
| | | <u>SURFACE WATER</u> RIVER/DAM/LAKE/POND/STREAM/CANAL/IRRIGATION | | |
| | | CHANNEL……………………………………………… | | |
| | | BOTTLED WATER………………………………………….. | 12 | |
| | | OTHER *(SPECIFY)*_____ | 13 | |
| | | Don't know…………………………………………………….. | 77 | |
| | | | 88 | |
| W14 | What kind of latrine/toilet facility do members of your household usually use? | Flush to piped sewer system…………………........................ Flush to | 01 | |
| | | septic tank…………………………………………………… Flush to pit latrine | 02 | |
| | | …………………………………………… Flush to somewhere else | 03 | |
| | | ………………………………………… Flush, don't know | 04 | |
| | | where………………………………………… | 05 | |
| | (Observation**)** | Ventilated improved pit latrine (vip)…..................................... | 06 | |
| | | Pit latrine with slab…………………………………………….. | 07 | |
| | | Pit latrine without slab/open pit …………………………….... | 08 | |

| | | | | |
|---|---|---|---|---|
| | | Bucket toilet …………………………………….......................... | 09 | |
| | | No facility/bush/field …………………………………………….. | 10 | |
| | | Other *(specify)*_____ | 77 | |
| **W15** | **What is the main material of the house floor?** (OBSERVATION ONLY) | Natural floor                    Earth/sand………………………………………… Dung……………………………………………....   Rudimentary floor wood planks……………………………………….           Palm/bamboo …………………………………………….   Finished floor parquet or polished wood……………………........           Vinyl or asphalt strips ……………………………           ceramic tiles…………………………………………. cement…………………………………………………. Carpet……………………………………………………           Other *(specify)*_____ | 01 02 03 04 05 06 07 08 09 77 | |
| **W16** | **What is the main material of the roof of the house:** (OBSERVATION ONLY) | Natural roofing                                        No roof………………………………………………………           Grass / thatch ……………………………………..           Dung / mud …………………………………………………           Rudimentary roofing Rustic mat/plastic sheets……………………..................  Reed/bamboo ……………………………………………..           Wood …………………………………………………………. Cardboard ……………………………….…………………           Finished roofing Corrugated iron………….. ……….....................................           Wood planks ……………………………………….......................... Asbestos sheet ……………………………………….………           Cement concrete……………………………........................ Tiles……………………………………………………………… other *(specify)*_____ | 00 01 02 03 04 05 06 07 08 09 10 12 77 | |
| **W17** | **Main material of the (inside) walls of the house:** (OBSERVATION ONLY) | Natural walls                                        No walls…………………………………………………………. Cane/palm/trunks/ bamboo………………….………………….. Dirt/mud/dung………………………………………….………  rudimentary walls                          Stone with mud…………………………………….……………….………  Wood/ bamboo with mud | 00 01 02 03 | |

| | | | | |
|---|---|---|---|---|
| | | …………………………………………… Uncovered | 04 | |
| | | adobe….…………………………………………… | 05 | |
| | | Plywood…………………………………………….…..... | 06 | |
| | | Cardboard………………………………………… Reused | 07 | |
| | | wood……………………………………………finished walls | 08 | |
| | | cement………………………………………. Stone with | | |
| | | lime/cement…………………………………… | 09 | |
| | | Bricks……………………………………….. Cement | 10 | |
| | | blocks……………….…......…………… Covered | 11 | |
| | | adobe……………………………..... Wood | 12 | |
| | | planks/shingles……………………………… | 13 | |
| | | Other_____ *(specify)* | 14 | |
| | | | 77 | |

| | | | No | Yes | |
|---|---|---|---|---|---|
| **W18** | **Does your household have:** | Clock/watch …………………………………………… | 00 | 01 | |
| | | Electricity ……………………………………………… | 00 | 01 | |
| | | Radio ……………………………………………….. | 00 | 01 | |
| | (ASK FOR EACH ITEM) | Television ………………………………………… | 00 | 01 | |
| | | Mobile telephone ………………………………………… | 00 | 01 | |
| | | Fixed telephone ……… ………………………………… | 00 | 01 | |
| | | Refrigerator …………………………………… Solar | 00 | 01 | |
| | | panel………………………………………………… | 00 | 01 | |

| | | | No | Yes | |
|---|---|---|---|---|---|
| | **Does any member of this household own:** | | | | |
| | | BICYCLE………………………………………… | 00 | 01 | |
| **W19** | | MOTORCYCLE/SCOOTER………………………………… | 00 | 01 | |
| | (ASK FOR EACH ITEM) | ANIMAL-DRAWN CART………………………………… | 00 | 01 | |
| | | CAR/TRUCK………………………………..……………… | 00 | 01 | |
| | | BOAT WITH MOTOR……………………………................ | 00 | 01 | |

| | | | | |
|---|---|---|---|---|
| **W20** | **Where is the cooking usually done for this Household?** | In the house ………………………………………………….. In a | 01 | **0 2 → H 2 5 0 3 → H 2 5** |
| | | separate building………………………………………… Outdoors… | 02 | |
| | | ……………………….…..………………………………… | 03 | |

| W16 | Do you have a separate room which is used as a kitchen? | No ……………………………………………………… 00<br>Yes …………………………………………………… 01 | |
|------|------|------|------|
| W17 | What type of fuel does your household mainly use for cooking? (CHECK ONE ONLY) | Electricity. …………………………………………………….. 01<br>LPG/natural gas……………………………………………….... 02<br>Biogas………………………………………………………….. 03<br>Kerosene…………………………………................................ 04<br>Charcoal……………….....……………………………………... 05<br>Wood………………..…...………………………………………… 06<br>Straw/shrubs/grass. ………………………………………….. 07<br>Animal dung…………………………………………………….. 08<br>No food cooked in household…………………………………….. 09<br>Other, (SPECIFY) _____ 77 | |
| W18 | How many rooms in this household are used for sleeping? | Rooms          ☐☐ | |
| W19 | Does your household own this structure (house, flat, shack), do you rent it, or do you live here without pay? | Owns……………………………………………………… pays 01<br>rent/lease…………………………………………… no 02<br>rent, with consent of owner…………………………… no rent, 03<br>squatting………………………………………………… Don't 04<br>know………………………………………………….. 88 | |
| W20 | Does any member of this household own any agricultural land? | No …………………………………………………… 00<br>Yes …………………………………………………… 01 | **0→**<br>**H30** |
| W21 | How many Hectares of land (altogether) are owned by the members of this family? | Number (in local Unit of Measurement)    ☐☐☐☐<br>                   ..<br>Specify the name of measurement     _____<br><br>Number of Hectares …………………………………………<br>(Calculate Hectares if answer given is in local unit of measurement)<br>If ≥1000 record 999.9             ☐☐☐☐<br>Unknown …………………………………………… 888.8 | |
| W22 | Does this household own any livestock herds? | No ……………………………………………………………………………… 00<br>Yes ………………………………………………… 01<br>……………………… | **0→**<br>**H33** |

| | | |
|---|---|---|
| **W23** | **If yes, how many animals?**<br><br>(IF NONE, WRITE 000, IF MORE THAN 1,OOO, WRITE 999) | Number of animals<br><br>1   Milk cows or ox<br>2   Horse/donkey/mule<br>3   Goats<br>4   Sheep<br>5   Poultry<br>6   Camels<br>7   Pigs<br>77  Other_____ |
| **W24** | **Does your household have a separate room outside the house for the livestock (any of the animals listed above)? (observation)** | No ……………………………………………………………………… 00<br>Yes………………………………………………………………… 01 |

| | **Food groups with Examples** | | | |
|---|---|---|---|---|
| **W25** | Are you currently fasting? | No ………………………… <br> Yes ……………………….. | 00 <br> 01 | |
| **W26** | Did you eat foods made out of any of the following cereals, such as bread, pasta, thick-grained porridge, injera or kita? <br><br> **( Read each food type from the list)** <br><br> Multiple response is allowed | No …………………….. <br> Teff…………………………. <br> Maize………………………. <br> Wheat …………………….. <br> Barley …………………….. <br> Sorghum …………………. <br> Millet …………………….. <br> Oat …………………………. <br> Other (specify) _____ | 00 <br> 01 <br> 02 <br> 03 <br> 04 <br> 05 <br> 06 <br> 07 <br> 77 | |
| **W27** | Pumpkin, yellow yams, butternut, carrot, squash or sweet potatoes that are yellow or orange inside? | No ………………………… <br> Yes ……………………….. <br> Don't know ………………. | 00 <br> 01 <br> 88 | |
| **W28** | Any other food made from roots or tubers, like white potatoes, taro root, white yams, cassava or any other food made from roots? | No ………………………… <br> Yes ……………………….. <br> Don't know ………………. | 00 <br> 01 <br> 88 | |
| **W29** | Any dark green leafy vegetables? | No ………………………… <br> Yes ……………………….. <br> Don't know ………………. | 00 <br> 01 <br> 88 | |
| **W30** | Ripe mango, pawpaw, guavas? | No ………………………… <br> Yes ……………………….. <br> Don't know ………………. | 00 <br> 01 <br> 88 | |
| **W31** | Any other fruits like bananas, apples, avocados…..? | No ………………………… <br> Yes ……………………….. <br> Don't know ………………. | 00 <br> 01 <br> 88 | |

| W32 | Any other vegetables like green beans, tomatoes……? | No …………………………… | 00 | |
| | | Yes ……………………….. | 01 | |
| | | Don't know ……………… | 88 | |
| W33 | Liver, kidney, heart and other organ meats (offals)? | No …………………………… | 00 | |
| | | Yes ……………………….. | 01 | |
| | | Don't know ……………… | 88 | |
| W34 | Any meat such as beef, pork, lamb, goat, chicken or? | No …………………………… | 00 | |
| | | Yes ……………………….. | 01 | |
| | | Don't know ……………… | 88 | |
| W35 | Egg? | No …………………………… | 00 | |
| | | Yes ……………………….. | 01 | |
| | | Don't know ……………… | 88 | |
| W36 | Fresh or dried fish, shell fish or other seafood? | No …………………………… | 00 | |
| | | Yes ……………………….. | 01 | |
| | | Don't know ……………… | 88 | |
| W37 | Any food made from beans, peas or lentils? | No …………………………… | 00 | |
| | | Yes ……………………….. | 01 | |
| | | Don't know ……………… | 88 | |
| W38 | Milk, cheese, yoghurt or other food made from milk? | No …………………………… | 00 | |
| | | Yes ……………………….. | 01 | |
| | | Don't know ……………… | 88 | |
| W39 | Oil, fats or butter added to food or used for cooking | No …………………………… | 00 | |
| | | Yes ……………………….. | 01 | |
| | | Don't know ……………… | 88 | |
| W40 | Sugar, honey, sweetened soda or sugary foods such as chocolates, candies, cookies and cakes | No …………………………… | 00 | |
| | | Yes ……………………….. | 01 | |
| | | Don't know ……………… | 88 | |
| W41 | Spices(black pepper, salt) | No …………………………… | 00 | |
| | | Yes ……………………….. | 01 | |
| | | Don't know ……………… | 88 | |
| W42 | Condiments (berbere, hot sauce, other examples), | No …………………………… | 00 | |
| | | Yes ……………………….. | 01 | |

| | | Don't know ……………… | 88 | |
|---|---|---|---|---|
| **W43** | Alcoholic beverages OR local alcohol Example Tela, Areke, Borde… | No ………………………… <br> Yes ……………………….. <br> Don't know ……………… | 00 <br> 01 <br> 88 | |
| **W44** | Any food made from nuts? | No ………………………… <br> Yes ……………………… <br> Don't know ……………… | 00 <br> 01 <br> 88 | |

**CONSENT STATEMENT FOR ANTHROPOMETRY AND BIOCHEMICAL SAMPLE COLLECTION**

As part of this survey, we are asking people all over the country to take an anemia and malaria test.  We would also like to assess the vitamins and minerals in your body.  Anemia is a serious health problem that usually results from poor nutrition, infection, or chronic disease. This survey will assist the government to develop programs to prevent and treat anemia.

We would like to measure your height, weight,  and eyes for spots and we would also take a sample of your blood, and stool. The tests are safe.  Some tests may cause you slight discomfort, such as a needle prick to take a blood sample.  For the blood sample, the blood is taken from a vein in the arm with a needle. The equipment used in taking the blood is clean and completely safe. It has never been used before and will be thrown away after your test. We would also like you to collect a sample of your urine and stool in a cup. By giving stool to test, you will help the Ministry of Health learn more about parasites that make people sick in Ethiopia.

We will also test your blood for anemia and malaria immediately, and tell you your results.  We will also provide information on your weight and height.

The benefit to you for taking part in this survey is that you will get results for weight, height, malaria, anemia and  referral to the nearby health facility if needed. The other information you give us will not benefit you in a direct way. However, we will add the information you give us to that of other houses in Ethiopia, and will create a report. The report will contribute to the good of your community.  What you say is important and valuable, and will help the Ministry of Health to improve their health and nutrition programs.

The results will be kept strictly confidential and will not be shared with anyone outside our survey team.

We will refer you to the clinic if you have malaria, or severe anemia.

You can say yes to any of these tests, or you can say no. It is up to you to decide. Do you have any questions?

May we take your weight and  height

Will you provide a small amount of blood and stool?

**If the women is pregnant do not collect venous blood**

| Consent given for: 0= No or 1= Yes | **WL01**  Blood | **WL03**  Stool | **WL04**  Anthro/goiter | |
|---|---|---|---|---|
| | ☐ | ☐ | ☐ | ☐ |

| **WL05** Anthropometrist  Code: | ☐☐ |
|---|---|
| **WL06** Nurse/Phlebotomist Code | ☐☐ |
| **WL07** WEIGHT IN KILOGRAMS  Refused = 777.7  Not measured = 000.0 | KG  ☐☐☐ . ☐ |
| **WL08** HEIGHT IN CENTIMETERS  Refused = 777.7  Not measured = 000.0 | CM  ☐☐☐ . ☐ |

| | |
|---|---|
| **WL11** BLUE TOP TUBE (METAL FREE)<br>**Did not work =00.0**<br>**Refused = 77.7**<br>**Pregnant = 99.9** | ML. ☐☐ • ☐ |
| **WL12** PURPLE TOP TUBE (EDTA)<br>**Did not work =00.0**<br>**Refused = 77.7**<br>**Pregnant = 99.9** | ML. ☐☐ • ☐ |
| **WL13** REDTOP TUBE (EDTA)<br>**Did not work =00.0**<br>**Refused = 77.7**<br>**Pregnant = 99.9** | ML. ☐☐ • ☐ |
| **WL14** Date blood sample taken (Ethiopian calendar) | Date: _____/_____/_____<br>Day / Month / Year |
| **WL15** TIME BLOOD DRAW (Ethiopian time) | Blood draw ___ ___ : ___ ___<br>Hour Minute |
| **WL16 When did you eat your most recent meal (food)?**<br>(Ethiopian date and time) | _____/_____/_____ __ ___ : ___ ___<br>Date /Month/ Year Hour Minute |
| **WL 17 Finger prick or venous sample taken** | 01 Finger prick<br>02 Venous |
| **WL18** MALARIA RESULTS (RDT) | NEGATIVE………………………………………..... 00<br>POSITIVE P *falciparum* .………………………….... 01<br>POSITIVE P *vivax* …...…………………………..... 02<br>POSITIVE FOR BOTH P *falciparum and* P *vivax* ............... *03*<br>INVALID .……………………………………………. 04 |
| **WL19** HEMOGLOBIN RESULTS | g/dL ☐☐ • ☐ |
| **In order to determine if you have blood in the or worms we would like to collect stool sample. If you can provide this now, we appreciate it. If not now, we can come back to pick up the sample at a later time.**<br>*INSTRUCTIONS IF UNABLE TO PRODUCE AT WILL*:<br>**For stool**: We will return tomorrow to pick up your stool. We would like the freshest stool you can give us. Please use one cup to collect the first stool you pass. | |
| **WL22** Stool collected? | No………………............................................00<br>yes ………..…........................................01 |

| | |
|---|---|
| **WL23** Date stool sample taken (Ethiopian calendar) | Date: _____/_____/_____ <br><br> Day / Month / Year |
| **WL24** Time when stool passed by the respondent (as recorded on cup) (Ethiopian time) | <br> ___ ___ : ___ ___ <br> Hour        Minute |
| **WL25** Time when stool collected from the respondent (Ethiopian time) | <br> ___ ___ : ___ ___ <br> Hour        Minute |
| **WL26** TIME BLOOD centrifuged (Ethiopian time) | <br> ___ ___ : ___ ___ <br> Hour        Minute |
| | |

Annex 3.

| Ethiopian National Micronutrient Survey (ENMS) | | |
|---|---|---|
| **Referral for Health Care** | | |
| *Please refer all participants in the ENMS who are found to have:*<br><br>• *Hb< 8.0*<br><br>• *Malaria (positive rapid diagnostic test)*<br><br>*Participants should be referred to the nearest government health facility.* | | Referral given<br><br>(Circle<br><br>Yes or No) |
| Name of participant:<br><br>_____ | Village:<br><br>_____ | N/A |
| Signature of team Lab Supervisor:<br><br>_____ | Date: _____/_____/ 2013 | N/A |
| HEMOGLOBIN RESULTS | g/dl ☐☐. ☐ | N/A |
| ANAEMIA RESULTS | | Yes       No |
| MALARIA POSITIVE and<br><br>FEVER IN PREVIOUS 24 HOURS | Malaria Positive   or/and<br><br>Had fever in previous 24 hours | Yes       No |
| Name of Interviewer | Date: ___/____/_____ | |
| Questions? Contact study Principal Investigator Dilnesaw  Zerfu at 0911421720. | | |

**Annex 4: Biochemical sample volumes for analyses in all target groups**

| Indicator | Sample Type | Min. Vol. needed for single Analysis | Lab |
|---|---|---|---|
| Malaria | Whole Blood (WB) | 2 drops | Field |
| Ferritin | Serum | 250 µL | Lab (ECLIA) |
| CRP | Serum | | |
| AGP | Serum | | |
| Stool (intestinal parasites) | Stool | 30 g | Lab |

## Annex 6: Sample Collection

### A. Universal Precautions

1. Universal Precautions are defined by CDC as a set of precautions designed to prevent transmission of human immunodeficiency virus (HIV), Hepatitis B virus (HBV), and other blood-borne pathogens.
2. Blood and other patients' body fluids are considered potentially infectious for HIV, HBV, and other blood-borne pathogens.
3. Therefore health-care workers who handle body fluids such as blood, mucus, sputum, urine, stool etc. should observe the following precautions:
   - Prevent skin and mucous-membrane exposure when handling blood or other blood-borne pathogens.
   - Use personal protection barriers (e.g. gloves, lab coats and eye glasses).

- Wash hands after removing the gloves.
- Clean laboratory benches before and after procedures with an appropriate disinfectant.
- Dispose lancets in sharps containers to prevent injuries.
- Dispose cuvettes and all other used materials in biohazard bags for incineration or appropriate disposal.
- Immediately report all accidents or injuries to your supervisor and follow the below precautionary measures:
  - In case of injury, it is necessary to squeeze the blood out of the injury, thoroughly wash the injury with soap and running water, cleanse the skin with 70% alcohol.
  - In case of contamination of hands with blood, immediately wash the hands with warm water and soap.
  - In case blood gets to face, it should be thoroughly washed with warm water and soap.
  - Test the specimen of the source individual for HIV and hepatitis as early as possible (within 24 hours of exposure).
  - Document the following data, related to the nature of exposed, status of source individual & status of exposed health worker
    - Name and data of the source individual.
    - Time & date of exposure.
    - Nature of exposure.
    - Body site exposed.
    - Infective status of the source.
    - Previous testing & Immune status of the exposed health worker.
  - Seek medical assistance as soon as possible

### B. <u>Venous Blood collection</u>

1) Obtain informed consent for blood collection and make sure participant is sitting comfortably.

2) Locate a suitable flat surface area for the blood collection procedure and lay out all blood collection supplies onto a disposable absorbent pad (including vacutainers, tourniquets, butterfly needle, gauze, alcohol swabs, etc.)

3) Select a serum (red top), EDTA (purple top) and trace element free (blue top) vacutainers and label as appropriate with ID and date of collection.

4) Assemble needle into Vacutainer holder being sure that it is firmly seated into threads. Loosely place Vacutainer tube into holder, but do *not* puncture top. Assemble and open supplies needed for collection.

5) Examine both arms to find the best vein.  Locate the puncture site.   Apply tourniquet (not too tightly).

6) Wipe the area with an alcohol swabin a circular motion making sure the area is thoroughly clean. Dry with gauze.

7) If it is necessary to feel the vein again, do so.  After you feel it, cleanse the area again with an alcohol swab.  Dry with gauze.

8) Fix the vein by pressing down on the vein about 1 inch below the proposed point of entry into the skin and pull the skin taut.

9) Remove needle shield.

10) Approach the vein in the same direction the vein is running. Hold the needle so that it is at an approximately 15° angle with the participant's arm.

11) Push the needle with bevel facing up firmly and deliberately into the vein. Activate the vacuum collection tube by pushing the tube onto the needle and puncturing the tube top.  If the needle is in the vein, blood will flow freely into the tube.  If no blood enters the tube, probe once or twice for the vein until entry is indicated by blood flowing into the tube Start with the serum tube (Red Top), EDTA tube (Purple Top) then Zinc tube (blue top).

12) For collection, loosen the tourniquet immediately after blood flow is established and release entirely as the last tube fills.

13) Withdraw the tube, then the needle.  Heavy pressure as the needle is being withdrawn should be avoided.

14) When the needle is out of the arm, press gauze firmly on the puncture.

15) Have the participant raise his arm (not bend it) and continue to hold the gauze in place for several minutes. This will help prevent hematomas.

16) Place a bandage on the participant's arm.

17) Report to the supervisor any unusual reaction experienced by the participant during the venipuncture procedure.

18) Label all tubes with the pre-printed labels provided. Use a permanent marker to add the date collected to the label (if a date or date range is not already printed). If the label contains a barcode, the barcode needs to be vertical like a ladder when placed on the tube. If the barcode is not vertical, the laboratory will not be able to read the label with the barcode reader. Place the label over the existing tube label so that it can be read from left to right starting from the cap end.

19) You may re-sheath the needle, but only with proper technique. With the needle top on the absorbent pad, slowly slide the needle into the needle top.

20) The needle should be discarded into a Sharps container. Place all labelled tubes in a cool box, and discard waste into appropriate biohazard container.

21) Dispose of biological waste according to the waste disposal guidelines.

22) Document the time of blood draw

23) Ask the participant the last time they had a meal (including juice) and write the time on the lab/anthro form.

24) Transport whole blood samples to laboratory for analysis.

25) Record: time of arrival to laboratory, and information from temperature recorders

26) Thank the participant for their participation

*Stool Collection*

The respondent was asked to pass the stool sample directly into a plastic cup, with a tight fitting lid, containing ~15 mL of 10% formalin for preservation. About 20 – 40 grams of well-formed stool or 5-6 tablespoons full of watery stool was suffice for a routine examination. Attention was given to ingestion of some medicines 1-2 weeks prior to collection of fecal sample that may interfere with the detection of parasites. All specimens was properly labeled with the respondent's name, age, sex, and date of collection. The formalin-preserved stool specimens was be transported and examined in the laboratory using the formol-ether concentration technique for intestinal

helminthes infections. The examination of faeces for parasitological diagnosis will be done to detect adult worms, cysts, ova and larvae.

## Annex 6: Field laboratory processing and transportation

```
                    ┌─────────────────┐      ┌──────────────────┐      ┌──────────────────┐      ┌──────────────────┐
                    │ Collect 5mL     │      │ Store the        │      │ At thefield      │      │ Store cryovials  │
                    │ of blood in a   │ ──▶  │ Vacutainer       │ ──▶  │ lab:Transfer     │ ──▶  │ at -20°...       │
                    │ serumVacutai    │      │ immediately in a │      │ the serum into   │      │                  │
                    │ ner (Red top)   │      │ cool box         │      │ two(2) labeled   │      │ Store the packed │
                    │                 │      │ and centrifuge   │      │ cryovials (1.8   │      │ cells at -20°C   │
                    │                 │      │ to separate      │      │                  │      │                  │
                    │                 │      │ the serum        │      │                  │      │                  │
```

**Non-pregnant women aged 15-49 years**

```
┌─────────────────┐   ┌──────────────────────────┐   ┌──────────────────┐   ┌──────────────────┐
│ Collect         │   │ 1) measure haemoglobin   │   │ At thefield      │   │ Transfer         │
│ 2mLof blood     │   │                          │   │ lab:             │   │ plasma into a    │
│ in EDTA         │ ─▶│ 2) Test for malaria      │ ─▶│                  │ ─▶│ 1.8ml labeled    │
│ Vacutainer      │   │    using RDT             │   │ Transfer 100 ul  │   │ cryovial         │
│ (Purple         │   │                          │   │ of blood into    │   │ (backup vial)    │
│                 │   │ 3)  Store the remaining  │   │ one labeled      │   │                  │
│                 │   │     samples at <8°C      │   │ cryovial and     │   │                  │
│                 │   │                          │   │ lyses with 1.0   │   │                  │
│                 │   │                          │   │ mL of 1 %        │   │                  │
│                 │   │                          │   │ Ascorbic acid.   │   │                  │
│                 │   │                          │   │                  │   │                  │
│                 │   │                          │   │ Mix gently by    │   │                  │
│                 │   │                          │   │ inverting the    │   │                  │
│                 │   │                          │   │ tube 5-6 times   │   │                  │
```

```
┌─────────────────┐   ┌──────────────────────────┐   ┌──────────────────┐   ┌──────────────────┐
│ Collect 6mL     │   │ Store the Vacutainer     │   │ At thefield      │   │ Store the cry... │
│ of blood in     │   │ immediately in a cold    │   │ lab:Transfer     │   │ 20°C             │
│ Vacutainer      │ ─▶│ box(<8°C) and centrifuge │ ─▶│ serum into four  │ ─▶│                  │
│ (Blue top)*     │   │ within 1-2 hours to      │   │ (4) labeled      │   │                  │
│                 │   │ separate the serum       │   │ cryovial (1.8    │   │                  │
│                 │   │                          │   │ mL)              │   │                  │
```

```
                ┌──────────────────────────┐   ┌──────────────────────────┐   ┌──────────────────────────┐
                │ A.  Collect ~20mLof      │   │ 1)  Test urine specimens │   │ Transport all cryovials  │
                │     urine in a plastic   │   │     for hematuria using  │   │ EHNRI Lab for urinary    │
                │     cup                  │ ─▶│     urine dipsticks      │ ─▶│ iodine (UI) analysis.    │
                │ B.  Collect 20-40g of    │   │ 2)  Make two aliquots of │   │                          │
                │     stool in a plastic   │   │     urine: vial #1 for   │   │                          │
                │     cup with 10%         │   │     analysis and vial #2 │   │                          │
                │     formalin             │   │     for backup           │   │                          │
```

## Annex 7: Micronutrient analysis

*Roche Immunoassay kits for analysis of Iron status indicators (ferritin),*

Serum ferritin has been recommended by the World Health Organization for population based surveys because it is responsive to iron interventions overtime. However, a major drawback is that serum ferritin is elevated in the presence of infection because it is an acute phase protein. Since serum ferritin is an acute phase reactant, levels also increase due to infection, inflammation, liver disease, juvenile rheumatoid arthritis, leukemia, and Hodgkin's disease; this can confound low serum ferritin levels due to iron deficiency.Serum ferritin is a major iron storage protein and is a much more sensitive indicator and it is the first indicator known to drop in iron deficiency. Serum ferritin levels will be assessed by an automated electro-chemiluminescence immunoassay (ECLIA) using the cobas Elecys 411 clinical analyzer and Roche reagent kits. The Roche ferritin assay is a competition principle and fully automated method. The experienced laboratory analyst will refer to the Roche ferritin package insert to determine normal serum ferritin levels range, reportable range and values indicating deficiency (in ng/mL). Values will be used to assess the extent of iron deficiency in the population using the manufacturer recommended cut off points for men and women.

Anemia will also be assessed by measuring hemoglobin in red blood cells, using a Hemo Cue (Hb-201) instrument.

*Serum CRP and AGP*

C-reactive protein (CRP) is an acute phase protein synthesized by the liver in response to inflammatory stimuli. It is used to adjust or eliminate the results of some nutritional tests (retinol, retinol binding protein (RBP) and ferritin) because these indicators are temporarily reduced at the same time that CRP is elevated during acute infections. The method principle for measurement of CRP will be immuno-turbidimetry using Roche immunoassay kits. Latex particles coated with anti-CRP (mouse) agglutinate with human CRP in the sample and forms insoluble antigen-antibody complexes. The turbidity formed is proportional to the CRP concentration. The resulting change in turbidity of the solution, proportional to the CRP

concentration, will be measured on the modular P eCobas Integra 400 clinical analyzer. The experienced laboratory analyst will refer to the Roche CRP package insert to determine normal serum CRP measurement range, reportable range and values indicating inflammation (in mg/L).

AGP, another protein indicative of inflammation, will also be assayed and measured with the immune-turbidimetry method using Roche immunoassay kits and the P-module of the Roche eCobas Integra 400 clinical analyzer.

## **Annex 8: Malaria Rapid Diagnostic Kit**

### **BACKGROUND**

Malaria is a serious, sometimes fatal parasitic disease characterized by fever, chills and anemia and is caused by a parasite that is transmitted from one human to another by the bite of infected Anopheles mosquitoes.  Four kinds of malaria species that can infect humans: *Plasmodium falciparum, P. vivax, P. ovale* and *P. malariae*.

The mainstay of malaria diagnosis has always been clinical diagnosis and malaria microscopy. However in recent years, rapid diagnostic tests have gained increasing importance in for use in the field. The rapid malaria antigen (HRP2/pLDH) combo test kit for *Plasmodium falciparum* and *P. vivax*.  The test contains a membrane pre-coated with two monoclonal antibodies as two separate lines across the test strip: one monoclonal antibody for *P. falciparum* and one monoclonal antibody for *P. vivax*.

### **SUPPLIES NEEDED**

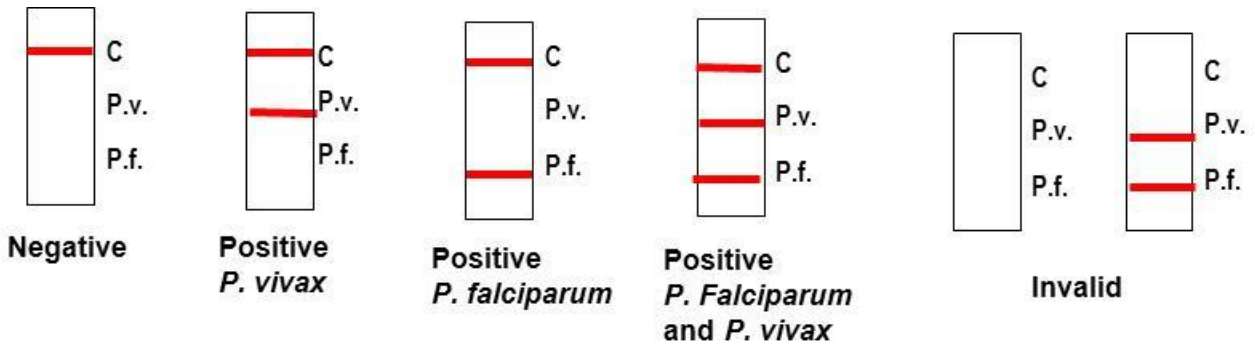Malaria rapid test kit, gloves, absorbent pad, correct form and pencil

### *Procedure:*

1) Set the timer to 20 minutes (do not start the timer at this point).

2) Lay out all items needed for testing malaria, including specimen pipette, assay buffer, test card, and purple top vacutainer containing blood.

3) Carefully remove the cap from the vacutainer and using the specimen pipette provided immerse it into the vacutainer containing blood and collect 5µL of blood. Gently release the pressure on the bulb of the specimen pipette to draw blood into the specimen pipette up to pipette guideline. DO NO COLLECT TOO MUCH BLOOD FROM THE VACUTAINER.

4) Add the 5 µL of blood into the "Sample Well" by squeezing the pipette.

5) Add 2 drops (60 µL) of assay buffer into the "Buffer Well".

6) Start the timer, wait for 20 minutes and read the test result.

**Test Procedure**
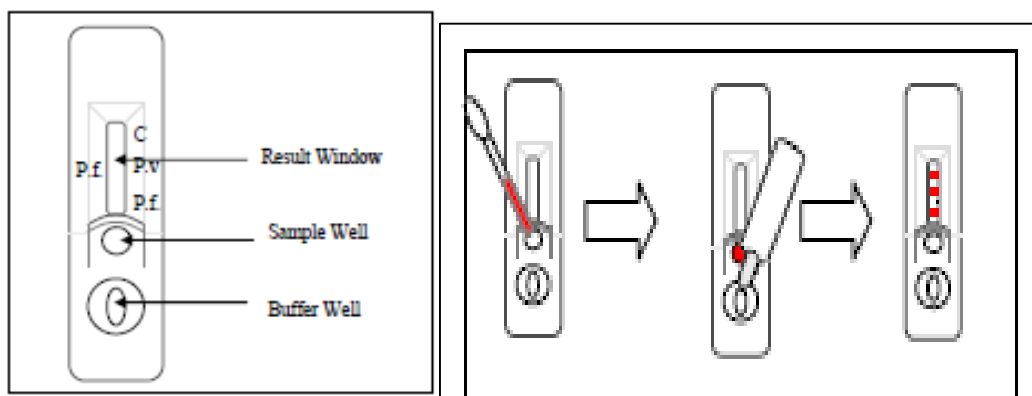


**Interpretation of the Test**

Negative | Positive P. vivax | Positive P. falciparum | Positive P. Falciparum and P. vivax | Invalid

1. <u>Positive reaction</u>: The presence of three color bands indicates a positive result for *P. falciparum* and *P. vivax*. The presence of two color bands at C and *P.v* or C and *P.f* indicates a positive test for *P. vivax* or *P. falciparum*, respectively.

2. <u>Negative reaction</u>: The presence of only one band within the result window indicates a negative result.

3. <u>Invalid</u>: The test is invalid if the C line does not appear. The test is also invalid if no lines appear. If either of these occurs, the test should be repeated using a new test.

**Annex 9: Criteria to define hematological, biochemical variables and nutritional status**

| Indicator | Level | |
|---|---|---|
| Haemoglobin(Hb) | < 12 g/dl | |
| Itestinal parasite | Ova/larva | |
| BMI | Underweight <br> <18.5 <br> Normal weight <br> 18.5-24.9 <br> Overweight | |

| | 25-29.9 | |
| | Obesity | |
| | ≥30.0 | |
| | **Serrumferritin<** <15 µg/L | |

# ANNEX 10 ; HEMOCUE PROCEDURE

**Hemoglobin testing from a purple top Microtainer® or Vacutainer® using HemoCue (Hb-201) photometer**

1. Hb-201HemoCue instrument does not have a control cuvette or liquid controls. When the instrument is turned "ON", it automatically performs self-test.
2. Turn ON the HemoCue Hb-201 photometer. **As this instrument has self-test, it does not have a control cuvette and does not need any liquid controls**
3. In about 30 seconds three lines show up the photometer screen (- - -).
4. Collect blood from the labeled Microtainer or Vacutainer Gently invert the Microtainer or Vacutainer about 10 times to prevent from forming clots.
   Fill the HemoCue cuvette by holding the Microtainer tube or Vacutainerin a horizontal position and <u>carefully</u> tapping the blood forward to the edge of the Microtaineror Vacutainer. Place the pointed tip of the HemoCue cuvette into the blood drop. The cuvette will fill automatically by capillary action. Never try to top off the cuvette after the initial filling.
5. Clean any excess blood from the cuvette using a Kimwipe or tissue paper. Do not touch the open end of the cuvette with the wipe as this will suck out the blood out. Inspect the cuvette for any air bubbles and if any air bubbles are seen, discard the cuvette and use a fresh cuvette.
6. Place the cuvette in its holder and gently close the holder into the photometer. The results will be displayed in approximately 15-45 seconds
7. Record the hemoglobin results on the "ENMS Hemoglobin Status and Malaria Referral Form" and give to participant's mother or caretaker. Explain the result to the mother/caretaker. Dispose of the cuvette in sharps container. Refer participant to the local clinic if hemoglobin level is <11 g/dL.
8. Dispose of the cuvette in the sharps container. Properly discard of all other materials in the biohazard bag.