Micronutrient survey manual
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Acknowledgements

The Micronutrient Survey Manual (2020) content and website are an update of the 2007 publication *Indicators and methods for cross-sectional surveys of vitamin and mineral status of populations* \(^1\) and its associated online Survey toolkit. This document is part of an updated interactive website developed to meet the demand of countries interested in assessing the micronutrient status of their populations. Appreciation is extended to the numerous individuals who led this revision. Specifically, we would like to thank Ms Katie Tripp, Dr Jacqueline Knowles and Dr Anne Williams for drafting and coordinating all content or finalizing all revisions. The following individuals contributed to selected modules and content or provided critical review: Dr O Yaw Addo, Dr Krista Crider, Dr Elisa Dominguez, Dr Deborah Galuska, Dr Annette Imohe, Dr Maria Elena Jefferds, Ms Julia Krasevec, Dr Roland Kupka, Ms Carine Mapango, Dr Zuguo Mei, Dr Juan Pablo Peña-Rosas, Dr Christine Pfeiffer, Dr Lisa Rogers, Mr Laird Ruth, Dr Mary Serdula, Dr Rosemary Schleicher and Dr Ralph (Donnie) Whitehead. The multi-year effort was carried out under the careful watch of core advisory committee members: Dr Maria Elena Jefferds (CDC), Dr Anne Williams (McKing Consulting Corporation as a contractor for CDC), Dr Luz Maria De Regil and Dr Sara Wuehler (Nutrition International), Dr Roland Kupka (UNICEF) and Dr Lisa Rogers (WHO).

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Introduction

Micronutrients, commonly known as vitamins and minerals, are essential in small amounts for proper growth and development. Micronutrient deficiencies can lead to impaired physiological development and socioeconomic achievement. Preventing deficiencies requires that people have access to enough food and an appropriately diverse diet. Certain population groups, especially women and children, are at greater risk of deficiencies. Populations that are deficient in one micronutrient are often vulnerable to deficiencies in others, thus interventions to address different micronutrient deficiencies frequently use the same delivery mechanism.

Surveys to assess micronutrient status provide a basis for policy makers and programme implementers to understand variations among different population groups, to assess where micronutrient deficiencies exist, to evaluate the impact of interventions to improve micronutrient status, and to gather the evidence needed to improve programming.

The manual

This manual contains modules covering all aspects of a cross-sectional micronutrient survey, from planning through implementation to analysing, reporting, disseminating and using the data. The main audience for the manual is programme managers responsible for the design and implementation of a micronutrient survey. Others involved in specific aspects of survey planning and implementation should also find certain procedures and tools useful.

The manual focuses on cross-sectional cluster surveys, which are designed to provide estimates of the prevalence or population status of selected micronutrients. The surveys also provide information on relevant health indicators and estimates of intervention coverage. The manual has limited information about simple random sample surveys, since that method usually only applies to small-scale micronutrient surveys in concentrated populations, for example refugee camps.

Designing and implementing large, population-based micronutrient surveys is a complex and costly activity. Because there is often a programmatic need to study the status of several micronutrients within the same population group, and because most of the costs are incurred during field work (transportation, accommodation, allowances, maintenance of a cold chain for specimens, and other logistical issues) it is common practice to include multiple micronutrients in one single survey. The present manual is written from that perspective.

This manual emphasizes the use of indicators recommended by the World Health Organization (WHO) and other internationally recognized agencies for assessing vitamin and mineral status, for classifying deficiencies at the individual and population levels, for defining public health problems and for monitoring progress toward preventing and eliminating micronutrient deficiencies. Other indicators that may be useful for specific research studies but that are not suitable for large cross-sectional surveys are not included in this manual. It is important to recognize that recommended methods to assess the micronutrient status of

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Introduction

a population change frequently. The information contained in this manual is current at the time of publication and will be updated when appropriate to reflect changes.

Where possible, this manual has drawn from existing survey resources, including the Multiple Indicator Cluster Surveys (MICS) and the Demographic and Health Surveys (DHS). The manual is organized as a series of stand-alone modules for each of the key aspects to be considered when planning and implementing a micronutrient survey, and when carrying out data management, analysis and reporting. The modules are available individually on line and have been assembled in this manual. The manual may be used in its entirety for planning a micronutrient survey, or individual modules may be referred to as needed. Each module discusses specific considerations and is designed to guide the user through these at every step in the process.

The manual is complemented by an online toolkit that provides additional resources, including standardized tools and examples of how they have been used in the field. Links to relevant tools are provided throughout this manual.

The following table provides a short description of each module. Programme managers, responsible for designing and implementing micronutrient surveys, should read and become familiar with every module. In addition, as suggested on the table, certain specialists will find specific modules most useful.

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## Micronutrient survey manual modules at a glance

<table>
<thead>
<tr>
<th>Module</th>
<th>Title</th>
<th>Description</th>
<th>Personnel targeted for use</th>
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<tbody>
<tr>
<td>1</td>
<td>Planning and designing a micronutrient survey</td>
<td>Describes key considerations necessary for planning and implementing a micronutrient survey. Specifically, it describes defining the scope and objectives for the survey, and navigating the next steps of protocol development and ethical approval.</td>
<td>Programme manager, Survey coordinator, Steering committee, Technical committee</td>
</tr>
<tr>
<td>2</td>
<td>Indicators of programme coverage, specimen selection, management and analysis</td>
<td>Describes how to select biological specimens for different population groups and food samples for surveillance and evaluation of fortification programmes.</td>
<td>Programme manager, Technical committee, Data coordinator, central data team, field, laboratory and management staff</td>
</tr>
<tr>
<td>3</td>
<td>Biomarker selection and specimen handling</td>
<td>Describes the specific biomarkers, the type of specimen to collect, and analytical methods required for various micronutrients and related health indicators. There is ample information on laboratory methods and the correct interpretation of micronutrient biomarkers.</td>
<td>Programme manager, laboratory coordinator, Regional laboratory supervisor field, laboratory and management staff</td>
</tr>
<tr>
<td>4</td>
<td>Survey design</td>
<td>Focuses on how various design factors would complement certain survey objectives. For example, is it necessary to have national or sub-national estimates for the chosen indicators? Could the survey objectives be met if the data collection was nested within another ongoing survey? These factors, along with periodicity of data collection and mode of data collection are discussed.</td>
<td>Programme manager, Technical committee, Survey statistician</td>
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### Introduction

Describes how to calculate appropriate sample sizes for key indicators among population groups of interest with adequate precision. The survey objectives must be taken into consideration and balanced with the available budget. There are examples of sample size calculations that account for stratification, clustering, and non-response.

“Clusters” is the most common term used to describe the primary sampling units (PSUs) that are selected for sampling in micronutrient surveys. This module explains methods for and provides examples of selecting clusters, using probability proportional to size (PPS), simple random sampling (SRS), and systematic sampling (SS).

Reviews the general principles of household mapping and listing, and outlines the responsibilities of listing and mapping personnel. There are examples on how to (1) map clusters and households, (2) segment primary sampling units, (3) select households in a cluster and (4) identify and select eligible individuals in selected households.

Outlines survey oversight and staffing. This module describes the personnel needs for micronutrient surveys, from the overarching organizational structure to the field team composition. There are suggestions for field team recruitment as well as for the roles of the subcommittees overseeing the coordination of the survey.

Provides detailed information and links to tools to help plan for specific survey supply and equipment needs. Since equipment and supplies may not be readily available and can take a while to procure, it is important to determine the

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<td>5</td>
<td>Sample size</td>
<td>Describes how to calculate appropriate sample sizes for key indicators among population groups of interest with adequate precision. The survey objectives must be taken into consideration and balanced with the available budget. There are examples of sample size calculations that account for stratification, clustering, and non-response.</td>
<td>Programme manager, Technical committee, Survey statistician</td>
</tr>
<tr>
<td>6</td>
<td>Selecting clusters</td>
<td>“Clusters” is the most common term used to describe the primary sampling units (PSUs) that are selected for sampling in micronutrient surveys. This module explains methods for and provides examples of selecting clusters, using probability proportional to size (PPS), simple random sampling (SRS), and systematic sampling (SS).</td>
<td>Programme manager, Technical committee, Survey statistician</td>
</tr>
<tr>
<td>7</td>
<td>Selecting households and participants</td>
<td>Reviews the general principles of household mapping and listing, and outlines the responsibilities of listing and mapping personnel. There are examples on how to (1) map clusters and households, (2) segment primary sampling units, (3) select households in a cluster and (4) identify and select eligible individuals in selected households.</td>
<td>Programme manager, Survey statistician</td>
</tr>
<tr>
<td>8</td>
<td>Survey supervision and personnel</td>
<td>Outlines survey oversight and staffing. This module describes the personnel needs for micronutrient surveys, from the overarching organizational structure to the field team composition. There are suggestions for field team recruitment as well as for the roles of the subcommittees overseeing the coordination of the survey.</td>
<td>Programme manager, Survey coordinator, household listing and mapping team, Field coordinator, regional supervisors</td>
</tr>
<tr>
<td>9</td>
<td>Survey equipment and supplies</td>
<td>Provides detailed information and links to tools to help plan for specific survey supply and equipment needs. Since equipment and supplies may not be readily available and can take a while to procure, it is important to determine the</td>
<td>Programme manager, Survey coordinator, Steering committee, Technical committee, Field</td>
</tr>
<tr>
<td>10</td>
<td>Budget and timeline</td>
<td>Provides an example of micronutrient survey budgets and timelines.</td>
<td>Programme manager, Survey coordinator, Technical committee, Laboratory coordinator, Field coordinator</td>
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<td>11</td>
<td>Data collection tools, field manual, and database</td>
<td>Describes the development of data collection tools such as questionnaires, and discusses the survey database. Early planning on database development can enable file checking throughout data collection, which would lead to higher quality survey data. This module also covers informed consent and accurate determination of a child’s age.</td>
<td>Programme manager, Survey coordinator, Steering committee, Technical committee, budget and finance subcommittee, Laboratory coordinator</td>
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<tr>
<td>12</td>
<td>Training and pilot testing</td>
<td>Discusses how to develop training agendas and conduct the pilot testing for micronutrient surveys.</td>
<td>Programme manager, Technical committee, Field coordinator, Data coordinator, central data team, regional supervisors</td>
</tr>
<tr>
<td>13</td>
<td>Field logistics</td>
<td>Outlines the complexities of data collection in the field and provides suggestions for effective communication, efficient movement of supplies, staff and specimens throughout the survey. If the survey requires a cold chain then proper mapping, planning and budgeting prior to data collection is necessary. The requirements for cold chain can be underestimated.</td>
<td>Programme manager, Technical committee, Deputy coordinator, Field coordinator, Laboratory coordinator, regional supervisors</td>
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<td>14</td>
<td>Data entry and cleaning</td>
<td>Describes the steps necessary after data collection to come to a complete database for the survey. It also discusses outlier variables and dichotomizing continuous variables for reporting prevalence estimates in the final report.</td>
<td>Programme manager, Technical committee, Deputy coordinator, Field coordinator, Data coordinator, central data team, Laboratory coordinator, regional supervisors</td>
</tr>
<tr>
<td>15</td>
<td>Data processing and analysis</td>
<td>Focuses on appropriate reporting of estimates collected by the survey. Specifically, it addresses the use of complex survey design parameters such as cluster, strata and weighting variables.</td>
<td>Programme manager, Data coordinator, central data team, regional supervisors</td>
</tr>
<tr>
<td>16</td>
<td>Survey reports and dissemination</td>
<td>Provides considerations for disseminating the new information generated from the micronutrient survey. Examples include an executive summary or key indicator report, the full survey report, protocol papers or scientific manuscripts.</td>
<td>Programme manager, Technical committee, Data coordinator, Database managers and data processing staff</td>
</tr>
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Module 1. Planning and designing a micronutrient survey

In this module, we will discuss:

- Oversight committees for planning and implementation: Steering committee and Technical committee
- Developing overall objectives of the survey
- Defining the stratification and scope of the survey
- Using anthropometry
- Factors in micronutrient survey design and protocol development
- Obtaining ethical approval
Planning is an iterative process that ensures that changes to one aspect are accounted for in other aspects of the survey, including objectives, sample size and field logistics, and ensures that any revisions result in a plan that still fits within the budget. Planning and designing a micronutrient survey needs to take into consideration that the initial plan is likely to evolve depending on such factors as stakeholder priorities, sample size, budget, estimated costs, laboratory assays and field logistics. The following sections provide brief descriptions of points to consider when planning.

Box 1.1 presents a list of decisions to make when planning a micronutrient survey. The “Initial planning checklist” online tool contains additional helpful information.

**Oversight committees for planning and implementation: Steering committee and Technical committee**

Two principal committees, the Steering committee and the Technical committee, provide consultation and feedback during survey planning, in particular related to objectives, design, and implementation. The survey plan usually requires considerable discussion, amendment, and compromise before it is sure to meet stakeholders’ most important needs while remaining within the constraints of resources and time. Detailed information about the roles, responsibilities, and composition of these committees is presented in Module 8: Survey supervision and personnel, and in the “Survey tasks and roles for survey personnel” online tool.

**Steering committee:** Survey results need to be accepted, endorsed, and used by relevant public and private sector organizations. To ensure that this is done, it is critical that stakeholders who have identified a need for, or who will use, the survey data are engaged in the planning process. One common method of getting the necessary buy-in is to establish a Steering committee that includes high-level personnel from stakeholder organizations. This committee will provide oversight, help guide decisions and foster a sense of common ownership of the survey. The committee will be involved in

- Defining the need for and scope of a micronutrient survey;
- Planning and implementing the survey, including securing approvals and permissions;
- Analysing, disseminating and guiding the use of survey data.

It is good practice to conduct a survey only when there is a clear gap in timely data and when the Steering committee agrees on how the results will be used to inform and improve national or subnational programmes.

**Technical committee:** In addition to the Steering committee, it is important to establish a Technical committee to promote best practices and ensure the quality of all survey-related processes. The composition of this committee can vary but in general includes members of government agencies working in nutrition, the national statistics agency, and other organizations implementing or supporting nutrition-related activities in the country. These may be international and local nongovernmental organizations (NGOs), United Nations agencies and academic institutions. The Technical committee helps ensure that the survey coordinator, who manages the day-to-day activities, has all the technical information needed. To this end, the survey Laboratory coordinator, a Logician, and a Data manager should also be part of the committee.
Module 1. Planning and designing a micronutrient survey

Box 1.1. Decisions to make when planning a survey

Decisions concerning survey objectives:
1. The rationale for the survey, informed by the Steering committee and Technical committee
2. The population groups to include for micronutrient and nutritional status assessment
3. Which micronutrients are critical to assess and are of programmatic interest
4. Whether to undertake anthropometric measurements to assess nutritional status
5. The existence of relevant national nutrition programmes that may be monitored, such as fortified food product coverage or quality

Decisions concerning survey protocol, budget, and timeline:
6. The desired precision of estimates for the main survey indicators within each stratum
7. Sample size calculations and sampling frame
8. The method of collecting data (paper-based or electronic)
9. Biomarkers* and clinical indicators to assess micronutrient status, and methods for their analysis
10. Supplies and equipment required to conduct the training, fieldwork and laboratory analyses
11. Logistics for implementing fieldwork and for transporting and storing samples and specimens
12. Development of the survey tool modules, including questions on programme/process, output and outcome indicators
13. The protocol for managing data
14. Plans for fieldwork training and pilot testing
15. Compensation for participants if applicable (money, food products or other)
16. The ethical approval process
17. Report writing
18. Dissemination to ensure use of the data

*In this context, a biomarker is a biological indicator of micronutrient or related health status.

Developing overall objectives of the survey

Overall objectives usually describe some or all of the following:
- Population group(s) to be covered
- Micronutrient(s) and related health indicators to assess
- Methods for assessing nutritional status (such as anthropometry)
- Relevant indicators of ongoing programmes or interventions, for example:
  - Coverage of large-scale nutrition-specific interventions, including fortifiable or fortified foods, fortified products, and/or supplements
  - Specific practices for infant and young child feeding (IYCF)
  - Dietary diversity, and/or frequency of consuming specific (fortifiable or fortified) foods
  - Knowledge and practice in relation to large-scale nutrition-specific interventions
- Level of stratification for which representative data will be available for each population group.
Module 1. Planning and designing a micronutrient survey

The overall survey objectives and key aspects of the survey design, for example the population groups and the planned level of precision for estimates of micronutrient status, should take into consideration the data that stakeholders need for programme planning, monitoring and evaluation. The objectives will influence subsequent aspects of survey planning, such as sample size, laboratory assays, questionnaire design, field logistics and budget requirements. They also need to consider technical feasibility and cost.

At the planning stage, it is essential to weigh essential data needs against optional data interests. For example, stakeholders may determine that iron deficiency among children 6–59 months of age is the primary indicator for the survey. Based on this, the sample size calculations should ensure a level of precision for estimates of iron deficiency in this population group that will be useful for effective programme management and decision-making. This sample size is then the basis for estimating much of the proposed cost of fieldwork. The available budget and other needs should then determine whether assessing additional micronutrients and other indicators of interest can be included as survey objectives, with or without adjustments to the desired sample size.

Examples of survey objectives can be found online in the “Malawi micronutrient survey protocol” as well as in the national survey reports from Kyrgyzstan, Malawi, Nepal, the United Republic of Tanzania, and Zambia (online tools accompanying Module 16: Survey reports and dissemination).

Defining the stratification and scope of the survey

**Stratification:** The Steering committee should decide on the survey scope and level of stratification. For a survey of national scope, some level of stratification will be useful to obtain representative data for different geographical, administrative or programme-related areas. For example, it may be known that populations in different parts of the country have different vulnerabilities to certain micronutrient deficiencies, or different levels of access to a specific intervention. In that case, it would be useful to have data for each situation so that associated factors and appropriate follow-up can be determined. In countries with decentralized governments, it may be particularly important to consider stratification based on policy and administrative areas where decisions are made.

If the survey aims to assess the impact of an intervention that is implemented in only a single region of the country, then all survey planning decisions and expected outcomes need to relate to that one region. In that case, there is usually no, or only a small degree of stratification, perhaps limited to urban and rural strata.

The overall sample size, fieldwork, and logistical costs are greatly influenced by the number of strata, and it is usually necessary to find a balance between the desired number of strata and the available budget. More information about factors to consider with regard to stratification can be found in Module 4: Survey design.

**Micronutrients to assess:** The micronutrient deficiencies with the largest known public health burdens concern iron, vitamin A, iodine, zinc, vitamin D, vitamin B12 and folate.

Here are some key issues to consider when deciding which micronutrients and associated indicators to assess in a survey:

1. Which populations groups are of interest? Usually women and children are the most highly affected populations.
2. Which nutrients and associated indicators should be assessed, and what is the feasibility of their assessment? (Questions 1 and 2 are discussed in more detail in the section below on Population groups to assess).

3. What relevant interventions are currently in place or planned?
Consider which micronutrient status data are needed at what level of precision to understand impact and accountability, and to adjust advocacy or policies. For example:
   a. Can intervention coverage be used instead of a biomarker, where an intervention has previously shown impact?
   b. What level of confidence in (the precision of) an estimate is needed to show a “real” change after implementation of an intervention?

4. Are there other available sources of data? What data on micronutrient status or effective implementation of proven interventions are already available, how recent are they, what is the representativeness of the data, and what is the quality of the data? If recent, representative, high-quality data on the status of a specific micronutrient among the population group of interest are available from a different source, there may be no need to include this indicator in the planned micronutrient survey.

5. What additional data would be useful to understand and improve implementation?
Even if data are available from other sources, there might still be a need to assess a particular micronutrient if, for example:
   • The status of a different population group is required;
   • Existing data do not include programme-related indicators which, together with micronutrient status data would shed light on the relationship between status and implementation of a specific intervention.
Likewise, if recent indicators of intervention coverage are available, it may not be necessary to include the same indicators in the survey unless, as above, it is important to examine the relationship between status and intervention.

6. Are there other surveys planned that include information about micronutrient status? For example, Demographic and Health Surveys and Malaria Indicator Surveys often collect data on anaemia and may be able to include coverage of micronutrient supplements or fortified foods.
If a micronutrient survey intends to assess only one or two micronutrients, serious consideration should be given to incorporating this into another planned survey. Resources should be invested in a separate micronutrient survey only if there are strong arguments about why the required data cannot be obtained through other channels.

More detail on all these factors can be found in Modules 2–16.

Population groups to assess: Priority should be given to those groups that are most vulnerable to one or more micronutrient deficiencies or for whom deficiencies may lead to measurable negative health consequences. Consideration should also be given to a specific population group that is the main intended beneficiary of an existing or planned micronutrient intervention. Children 6–59 months of age and women of reproductive age are the groups most frequently assessed in micronutrient surveys. This is due to their vulnerability to deficiencies and to the common design of interventions to address the needs of these groups.
A survey will be more efficient when it covers population groups vulnerable to multiple micronutrient deficiencies.

When defining the population groups to assess, ensure that:

- There are recommended cutoff values to define different categories of status (such as deficient, insufficient and normal) for the micronutrients of interest. For example, for ferritin, there is no internationally recognized cutoff value to define deficiency among pregnant women, thus pregnant women would not be a suitable group in which to assess this indicator.
- A sufficient sample size can be accessed without having to visit a large number of households. For example, the population percentage of women of reproductive age who are pregnant may be around 6%. The number of households you would have to visit to obtain a representative sample of pregnant women for each survey stratum may be too high to be feasible. At the same time, if the scope of the survey includes data on pregnant and lactating women, an adequate sample size must be ensured. Sometimes specimens are collected from all consenting pregnant and lactating women in all survey households, with the expectation that the final number of samples may be sufficient to give a reasonable estimate of status at the national level.

The following sections provide considerations for including different population groups.

**Children under 6 months of age** are often included as the focus for questions that address breastfeeding practices. Members of this age group are not usually included for collection of biological specimens, because refusal rates are likely to be high. In addition, there are no internationally recognized cutoff values in this age group to define categories of micronutrient status for many micronutrients.

**Preschool-age children (6–59 months)** are particularly vulnerable to the consequences of micronutrient deficiency and are therefore frequently included for assessing multiple micronutrients. Priority indicators for this age group in low- and middle-income countries include the status of iron, vitamin A and anaemia, along with markers of inflammation that may affect interpretation of the results. This group is also vulnerable to deficiencies of zinc and vitamins B12 and D, however, these micronutrients are less frequently assessed.

**School-age children and adolescents**: The exact definition of school-age varies from country to country but is most commonly defined as from 5 years up to but not including 15 years of age (thus 5.0–14.9 years of age). If this group is included in a household-based survey, the survey should take place when children are more likely to be at home, such as during school holidays, on weekends, or after school hours. Alternatively, school-age children can be assessed in a school-based survey, however, the rate of school attendance should be considered, to account for any potential bias in the results. A school attendance rate of around 75% or higher is usually required to obtain what may be considered as representative data through these means. Micronutrient and related health status indicators most often assessed among this group are iodine, zinc, iron, and anaemia, along with markers of inflammation that may affect interpretation of the results. The category of adolescents is usually defined as from 10 to 19 years of age. The focus for this group tends to be females, who are most vulnerable to anaemia due to menstruation and reproductive health risks including early pregnancy.

**Women of reproductive age** are also vulnerable to vitamin and mineral deficiencies. They are usually divided into three subgroups: non-pregnant/non-lactating, pregnant, and lactating. The survey protocol should clearly indicate whether to collect specimens from women who are of unknown pregnancy or lactating status, and how to record these women in the household record. Micronutrient and related health status assessments for
Module 1. Planning and designing a micronutrient survey

Pregnant and lactating women usually include iodine status (urinary iodine concentration [UIC] and anaemia [haemoglobin]). Pregnant women comprise a priority group for assessing iodine status, even if only a nationally representative sample is possible.

Because their nutritional status can influence the developing fetus, women of reproductive age are often the focus of nutrition-related interventions and are frequently included in micronutrient surveys. Micronutrient and related health status indicators generally assessed among this group include iron, vitamin A, folate, iodine, vitamin B12, and anaemia, along with markers of inflammation that may affect interpretation of the results. Module 3: Biomarker selection and specimen handling provides additional details and considerations for assessing vitamin A status and iron status.

Men can also have inadequate micronutrient status. Because they tend to be less severely affected than children 6–59 months of age and women of reproductive age, they are less frequently included for assessment of micronutrient and related health status biomarkers. In some cases, men may be selected from a subsample of households, in order to provide sufficient numbers to understand the overall micronutrient status among this group.

In some settings, men are included in the assessment of haemoglobin concentration to help understand the role that iron deficiency plays in anaemia among women and children. If the prevalence of anaemia is high among men, as well as among women and children, then causes of anaemia such as malaria or other parasitic infections are likely to be prevalent.

Even if micronutrient status biomarkers are not collected, men are sometimes included in the interview process to assess their level of knowledge, attitudes, and practices concerning how to prevent micronutrient deficiencies among household members.

Additional information on the selection of micronutrients by population group, choice of biomarker, type of sample required, and related biomarker cutoff values for different population groups can be found in Module 2: Indicators of programme coverage, specimen selection, management and analysis and Module 3: Biomarker selection and specimen handling.

Using anthropometry

Micronutrient surveys commonly include the assessment of nutritional status using anthropometry. Variables most often measured among children 0-59 months are: date of birth and date of visit (to calculate age), weight (in kilograms), and height/length 1 (in centimetres). These measurements are used to calculate weight-for-age (to assess underweight), height-for-age (to assess stunting), and weight-for-height (to assess wasting and overweight). The resulting z-scores 2 are compared to international standard reference ranges to assess an individual’s nutritional status. It is generally recommended to use the WHO Child Growth Standards as the

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1 Height is measured among all population groups except children under 24 months of age, where length is measured.
2 z-score, or standard deviation-score = [observed value - median value of the reference population] / standard deviation value of reference population
Module 1. Planning and designing a micronutrient survey

Reference ranges. In some contexts, mid-upper arm circumference (MUAC) is measured in children 6–59 months of age, in addition to height/length and weight, to assess wasting. 

Among (non-pregnant) adult population groups, anthropometric measurements usually include height (in centimetres) and weight (in kilograms) to assess body mass index (BMI) and identify short stature. In addition to height and weight, MUAC is sometimes used as an indicator of nutritional status, however, height and weight are preferred since there are no global standards available for MUAC. In programmes or clinical settings, MUAC is mainly used for identifying wasting among pregnant women.

More information on anthropometry assessment methods and the interpretation of results, along with associated tools, are discussed in Module 11: Data collection tools, field manual, and database; Module 12: Training and pilot testing; and Module 14: Data entry and cleaning.

Factors in micronutrient survey design and protocol development

The survey protocol, budget and timeline should be drafted after the overall objectives of the survey have been agreed and defined. The protocol needs to include decisions on the following aspects of the survey, details of which are presented in subsequent modules of this manual.

Precision of estimates for the main survey indicators within each stratum: The required precision of estimates at the stratum and national levels is determined somewhat by the expected programmatic use. For example, a relatively precise estimate may be required to determine whether an intervention implemented for the past two years has had an impact, that is, whether a change from the baseline estimate is real. Module 4: Survey design describes factors to consider in these decisions in more detail.

Sample size calculation and sampling design: Agreement must be reached on the calculated sample size, the level of stratification and the balance between the number of clusters and the number of samples targeted for collection from each cluster. For these decisions, the overall budget and time available need to be taken into consideration. Module 5: Sample size presents detailed guidance on calculating sample size and designing the survey sample for different contexts.

Method of collecting data: An important decision to make during the design phase is how data will be collected and managed. In the past, most surveys used paper-based questionnaires with manual data entry. Currently, digital data collection using methods such as computer-assisted personal interviews (CAPIs) or Open

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4 BMI is calculated as weight in kg / height in m²
5 Short stature: < 145 cm among women of reproductive age
Module 1. Planning and designing a micronutrient survey

Data Kit (ODK) are increasingly the default option, using either small computer tablets or large-screen smartphones. The advantages and disadvantages to each method are described further in Module 4: Survey design.

Selecting biomarkers, food sample nutrients, and clinical indicators: Because there may be multiple biomarkers for a particular micronutrient, decisions need to be made about which are the most appropriate ones for the survey context. Information to guide these decisions can be found in Module 2: Indicators of programme coverage, specimen selection, management and analysis and Module 3: Biomarker selection and specimen handling.

Decisions on the biomarkers and food sample nutrients to analyse will affect such factors as the needed skills and expertise of fieldworkers, the estimated time required per survey participant and survey household, fieldwork supplies and logistics, laboratory supplies, and the overall budget.

Clinical signs and symptoms of micronutrient deficiency or excess are no longer commonly included in large, complex population-based surveys because:

- they typically appear only when a deficiency has reached a severe level, whereas blood and urine biomarkers can assess deficiency and risk of excess at a subclinical, less severe, stage;
- once a micronutrient intervention has been implemented for a period of time, any prior clinical signs among the population may occur infrequently or be an indication of previous deficiency, in which case they are not a reliable indicator of current status. For example, a large nodular goitre from previous iodine deficiency may remain for many years even when a person’s diet becomes sufficient in iodine;
- reliable assessment of clinical micronutrient-related disease signs and symptoms may require specialized equipment and personnel, for example an ophthalmologist to accurately assess xerophthalmia, which occurs with severe vitamin A deficiency; or
- many signs and symptoms of non-optimal micronutrient status, such as anorexia, diarrhoea, fatigue, and weakness, have other possible causes that would be difficult to differentiate.

Supplies, equipment and logistics: The supplies and equipment required for training, pilot testing, fieldwork, sample transport, and laboratory analysis depend on the method of data collection and the selection of biomarkers and food nutrients. Module 9: Survey equipment and supplies provides more detailed information and links to tools to help plan for specific survey supply and equipment needs. The “Equipment and supplies list (single worksheet)” online tool can be used to work out a rough calculation of the supplies, equipment and associated budget needed for the proposed number and type of field and laboratory tests.

Managing the logistics of training and fieldwork, along with the appropriate storage of specimens and samples, is complex and includes determining:

- the number of teams and the team size and composition;
- the training agenda, including details on a pilot field test of all procedures, with proposed location and accommodations;
- the process and training required for mapping and household listing or other sampling procedures;
- transportation of:
  - Field staff and supervisors
  - Field supplies and equipment
- Questionnaires (where paper-based), specimens and samples from survey sites to the appropriate place for management and analysis;
- storage and cold chain facilities for samples and specimens;
Module 1. Planning and designing a micronutrient survey

- maintenance and charging of electronic devices, where used; and
- community mobilization strategies.

More detailed information to guide logistics planning can be found in Module 8: Survey supervision and personnel, Module 9: Survey equipment and supplies, and Module 13: Field logistics.

Survey questionnaires: There are numerous examples of survey questionnaires, some of which can be adapted to meet specific survey objectives. Module 11: Data collection tools, field manual, and database includes detailed guidance on developing and testing the main survey questionnaire. Questionnaires also need to collect information to meet the needs of secondary data requirements such as:

- Indicators of household wealth/vulnerability to poverty
- Intervention-related factors, for example, household access to a fortifiable or fortified product
- Cultural or environmental factors that may affect micronutrient status, for example, specific dietary choices or the use of insecticide-treated nets to protect against malaria infection.

Data management, reporting and dissemination: The data management section of the survey protocol should include plans for initial data entry (if paper-based data collection), data processing, data weighting, analysis and presentation of results. A Data manager is a necessary and important member of the survey team and Technical committee. Module 14: Data entry and cleaning, and Module 15: Data processing and analysis provide detailed guidance on the process of and personnel requirements for data management and analysis.

Reporting and dissemination are critical components of the overall survey. Plans for report preparation, printing and distribution, and for dissemination events, should be defined early on to ensure that the end product will meet the objectives and procedures agreed by stakeholders and to inform the budget and timeline. The report formats and an outline of their content should also be agreed. Module 16: Survey reports and dissemination provides details on aspects to consider in planning these activities.

Budget and timeline: The budget and timeline are key to guiding survey implementation. They must be realistic and allow for unexpected costs and delays. The Survey manager and the Technical committee need to consider all the issues and factors that might affect them, including timing (for example, any holidays or periods during which much of the population may be working away from their usual place of residence) and budget (for example, an increase in fuel prices).

Module 10: Budget and timeline provides detailed guidance on planning survey budgets and timelines. The “Generic budget” and “Generic timeline” online tools may be useful to develop a rough calculation of the budget and timelines required for all survey components.

At this point in the planning process, it is useful to develop a brief concept note covering the main points of the survey (objectives, design, and estimated timeline and budget). An example can be found in the “Micronutrient survey concept note” online tool.

You can find examples of survey protocols in the “Protocol-generic nutrition survey” online tool and a specific country example in the “Malawi Micronutrient Survey protocol”.

You can find examples of survey protocols in the “Protocol-generic nutrition survey” online tool and a specific country example in the “Malawi Micronutrient Survey protocol”.
Obtaining ethical approval

The final protocol and draft of the survey questionnaire should be submitted for national ethical review and approval to ensure that participants’ rights and welfare are protected. Ethical clearance procedures vary from country to country but are usually required for a micronutrient survey. If for some reason ethical review is not required in the country, all internationally recognized ethical procedures should be followed.

The review process, relevant ethical approval body or institutional review board should be identified early in the planning process, and time for the review needs to be factored into the timeline. Some ethics committees may meet only infrequently, some national approval processes may require review and approval from multiple committees or groups and, depending on the stakeholders involved in the survey, different types of ethical review may require different approvals.

Questions to answer when developing the request for ethical approval may include: ¹

- How will the survey affect participants?
- Although harm to participants must always be avoided or minimized, it is never possible to eliminate all risk, and risks should be specified during the informed consent process.
- What is the population under study?
  - If participants include vulnerable populations, this needs to be indicated. Vulnerable populations include individuals who are not able to provide full consent or who are particularly susceptible to harm, including minors, prisoners, pregnant women, or people with a mental disability.
- How will the survey results be used?
  - It should be specified whether survey results will be shared or published, whether the survey procedures will be confidential, and if data management will include removal of identifiers.
  - Although household surveys cannot be completely anonymous at the point of data collection, care should be taken during training to ensure that enumerators are fully aware of requirements for ethical conduct and for data and biological specimen collection. Procedures can be designed to minimize the risk of specific details about individuals becoming available and ensuring that data remain confidential.
- Will individual results of some or all biomarker tests be shared with the participants? Who will have access to the raw data and procedures in place to protect this?
  - The greater the number of individuals who have access to identifiable data, the greater the risk that confidentiality will be compromised. The steps that will be taken to minimize this risk should be described.
  - All who do have access to full identifiable data or direct contact with human subjects should have completed certification in an approved human subjects’ research training programme.

The free online training and certification “Protecting human research participants”, available at https://grants.nih.gov/sites/default/files/PHRP_Archived_Course_Materials_English.pdf, covers many of the points above. Issues of particular concern to the ethical review process are discussed in the following sections.

Informed voluntary consent: It is important to document the procedures for securing individual and community-level informed consent and the training that will be ensured for enumerators, supervisors and all

¹ Adapted from https://www.cdc.gov/surveillancepractice/policy.html.
others who will have access to the participants or their data. The survey protocol should describe whether consent will be oral or written, how it will be obtained for those who are not literate and whether assent is required for minors. Procedures usually include informing participants about the purpose of the study, about what participation involves, that participation is voluntary, that consent can be withdrawn at any time, about how participant identity will be protected, and about whom to contact with questions or concerns. Informed consent is a standard expectation of participation in a micronutrient survey, regardless of whether it is required by an ethical committee. When a participant does not consent to participate in the collection of biologic specimens, informed consent for the survey questionnaire is still required.

Confidentiality: The survey protocol should document the training that will be provided to all staff to ensure that confidentiality is maintained at the individual level, including consequences should confidentiality not be maintained. It should also describe the de-identification process for collected data so that all results are confidential, where the data will be securely stored, for how long data will be stored, who will have access to the data, whether and how paper or electronic data will be destroyed, and any plans for public data release.

Identification of a health condition: The survey protocol should describe which results will be provided to survey participants, together with the biomarker cutoff values (international or national) to determine whether a referral is required (for example, haemoglobin <70 g/L or a positive result to a malaria rapid diagnostic test). Referral procedures should always be developed in conjunction with and approved by the ministry of health and detailed in the protocol. Participants should not be referred to the health facility for conditions that would not be treated. Health facility staff in survey areas should be aware of the survey, the tests that will be done, the possibility of referrals, and the process for referral. Participants should be informed about which referrals will be made and how, which individual test results they will and will not receive, and why.
Module 2. Indicators of programme coverage, specimen selection, management and analysis

In this module, we will discuss:

- Assessing indicators of programme coverage
- Collecting specimens for assessing biomarkers
- Location for collecting samples and data
- Selecting laboratories to test specimens
Module 2. Indicators of programme coverage, specimen selection, management and analysis

The overall objectives of the micronutrient survey describe the populations to be included, as well as the micronutrients and programme process indicators to be assessed (see Module 1: Planning and designing a micronutrient survey). The subsequent steps determine the type of samples and specimens required, and how they will be collected, transported, stored and analysed. Development of recommendations concerning most biomarkers and food nutrients requires analysis in a laboratory setting with specialized equipment and training. These are described in more detail in Module 3: Biomarker selection and specimen handling.

Decisions made during the planning process will influence the survey outcome, protocol development, draft budget, and equipment and supplies needed. Some points to consider:

- program coverage indicators will require knowledge of national and large-scale nutrition interventions, and may reflect a national nutrition policy;
- the selection of indicators will be based on the feasibility of collecting required food samples and biological specimens, maintaining a cold chain if required, access to appropriate laboratory capacity either in the country or internationally, and available budget; and
- the survey scope may need to be adjusted, depending on the cost of collecting, transporting, and analysing the samples and specimens required to assess the chosen indicator(s).

Module 3: Biomarker selection and specimen handling describes the options and main factors to consider in making these decisions.

Ordering equipment and supplies is an important determinant of the survey timeline, as described further in Module 9: Survey equipment and supplies and should be done well in advance of the survey. At the same time, if this is done too early there is a risk of exceeding expiration dates on the supplies purchased (for example, cuvettes for a portable photometer).

Assessing indicators of programme coverage

Indicators of large-scale interventions to improve micronutrient status are often included in a micronutrient survey. This allows the investigation of the relationship between micronutrient status and access to interventions. Results can be applied to improve programmes and policies, as well as to understand impact. Nationally relevant intervention indicators should be incorporated and can include questions about the knowledge and use of fortified foods, micronutrient supplements, and point-of-use fortification (for example, micronutrient powders, or MNP) by the intended user group.

Coverage of interventions such as vitamin and mineral supplementation, point-of-use fortification and deworming can be assessed in a micronutrient survey, in a variety of ways. The most common way to obtain information is via self-reporting. Some questions, for example if or when a particular health service was received, can be validated through examination of medical records, child health cards or immunization records. The documentation varies by country, and each type has its limitations. Documents may not be completed reliably to reflect the true coverage or timing of the intervention of interest, especially when the intervention is part of a mass campaign where the priority is to deliver interventions to the largest possible number of eligible participants in a given time period. Self-reporting by adults, and caregiver recall for children, are subject to several biases including socially desirable reporting and potential errors associated

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1 In this context, a biomarker is a measurable indicator of micronutrient or related health status.
Module 2. Indicators of programme coverage, specimen selection, management and analysis

with long recall periods. Recognizing this, coverage data are often collected using different sources within a single survey. These data are then reported separately, compared and triangulated with other data sources.

Coverage of vitamin and mineral supplementation

Two crucial issues to consider when collecting data on the provision or consumption of vitamin and mineral supplements are the potential for confusion between the objective of the supplements (prevention or treatment), and the timing of the survey. When assessing access to and coverage of a national supplementation programme, it is helpful to show the participant an example of the exact supplement.

Supplementation with vitamins and minerals is usually specific to certain population groups and, depending on the programme, the same supplement could be intended for prevention or for treatment. For example, iron-folic acid supplements might be provided periodically to all adolescent girls to prevent iron deficiency. However, the same or a different-looking supplement may be provided as treatment when an individual is clinically diagnosed with anaemia or iron deficiency. Similarly, it is common for children 6–59 months of age to receive high-dose vitamin A capsules every two years, however clinicians may also administer capsules as part of routine care for acute illness. The questionnaire should include questions that differentiate between the use of supplements for prevention and as part of clinical care. Often the interviewer will ask to see the supplement bottle used by the participants, and is trained to record the micronutrients contained or the brand name shown on the label.

Questions requiring recall about supplements should be based on a time period that reflects the particular programme cycle and on evidence-based expectations for reliable recall. For reporting on the use of antenatal iron and folic acid supplementation (in tracking progress towards the Global nutrition target of reducing anaemia among women of reproductive age), it has been suggested to ask women about their consumption of iron-containing supplements during a current or past pregnancy within the last two years.¹ When assessing the coverage of high-dose vitamin A supplementation for children, the Global Alliance for Vitamin A (GAVA) recommends using data from post-event coverage surveys rather than from household surveys.² There is a transient increase in serum retinol following high-dose vitamin A supplementation. Thus, when assessing vitamin A status in a survey, it is important to time the survey so that a sufficient amount of time has passed since the last vitamin A supplementation campaign.

Coverage of point-of-use fortification

In many countries, point-of-use fortification (also called ‘home fortification’) is used to improve the nutritional status of children under 5 years of age or the status of school age children. This is most often done through the use of micronutrient powders (MNP). Depending on the scope of the intervention, the survey may include questions related to the use of MNP. Where MNP are provided in only a limited area of the country, the expected positive responses for their use may be too low to have relevance in the proposed survey.

When the use of MNPs is assessed as part of a micronutrient survey, questions are usually included on both receipt of the product and intake of MNP sachets by the intended population group. Other useful questions might concern the receipt of counselling on correct use of the MNP and the perceived benefits and side effects. An example of a module for MNPs can be found in the “MNP questionnaire template” online tool.

**Coverage and quality of fortified or fortifiable foods**

Many countries implement large-scale food fortification to achieve optimal intake of a range of micronutrients. Fortifiable foods generally refer to commonly consumed food items (usually staple foods or condiments) that can be produced on a large scale, meaning at least 20 metric tons per day, by companies with the technical and financial capacity to add micronutrients in line with voluntary or mandatory fortification regulations and policies. Examples of such foods include flours (usually wheat or corn), cooking oil, sugar, food grade salt and other condiments (such as bouillon, soy sauce and fish sauce). It is common to include indicators of household use of foods that are fortified or fortifiable, as well as indicators of consumption among groups that are more vulnerable to certain deficiencies. There are two categories of foods to consider incorporating into surveys: those mandated to be fortified according to national policies and regulations, and those that are potentially fortifiable and are likely to reach the main population of interest. Relevant issues to consider when selecting programme indicators to collect on large-scale fortifiable foods are shown in Box 2.1.

Many surveys collect a small sample of the fortifiable food from households to test for the presence and concentration of micronutrients. This is most commonly done to assess household coverage of iodized and adequately iodized salt, but it can also be done with edible oil, flour and other products. The planning team may decide to provide a replacement sample or a small monetary contribution to the households. This can be particularly important in settings where not providing a replacement or contribution will affect response rates or may adversely affect the family, for example by leaving them with no oil for cooking for the day and no resources to get more. Food replacements or monetary contributions need to be included in the overall budget and field logistics plan.

The presence of iodine in salt has been routinely tested in the field using specially developed rapid test kits. However, these kits are not quantitative and do not provide reliable estimates of salt iodine concentration. Various methods are being explored for salt and other means that involve collecting and analysing individual and composite samples, with various qualitative and quantitative protocols to assess micronutrient content.

Comprehensive information on testing iodine in salt can be found in the UNICEF document *Guidance on the monitoring of salt iodization programmes and determination of population iodine status*.  

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Box 2.1. Factors to consider when deciding on the type and wording of programme indicators for large-scale fortifiable foods

- Do fortification regulations apply to only a specific type of the fortifiable food, or do typical industry practices mean that certain product types are more likely to be fortified?
  - If fortified wheat flour regulations are applied only to high extraction (“white”) flour or flour used to bake bread, then indicators need to be designed for that product type accordingly.
  - Salt iodization quality can vary widely by salt type. Where a range of salt grain types are consumed, it is necessary to distinguish the main type of salt used in a household. Where a sample is collected for quantitative assessment of iodine, laboratory personnel should also determine and record the salt type according to clear standards.

- Are there labelling or specific logo requirements for fortified products?
  - Observation of, or questions about food labels could be used to assess the use of a fortified product. For example, the national policy may require use of a specific logo in order to support demand and consumer choice of fortified foods. This means that the logo or labeling information will also indicate compliance with national policies. However, not all products will be in their original packaging and some products may be falsely labelled.

- Is there extensive home production of the fortifiable product, or is it mainly commercially produced?
  - Home produced staple foods are generally not fortified, unless there was hammer mill level fortification. Therefore, a question about the source of the product is helpful so only the relevant data about commercially produced foods will be analysed and presented to determine industry compliance.
  - Questions on brand name, manufacturer, and country of origin are also useful for internal programme management, although it is generally not acceptable to present nutrient content analysed by a salt manufacturer in a publicly available report.

- Is (or was) there a communication component to the overall national fortification strategy?
  - If such a component exists or existed in the past, then consider including questions about respondent knowledge and awareness of the fortified product as well as the source of this information. In addition, including a question on whether the respondent looks for and identifies the fortified product at purchase may provide useful information for monitoring the communication component.

- Is the fortifiable staple product used by the food industry to produce processed foods or condiments with a large market across different consumer groups?
  - If so, then questions about consumption of these specific processed foods or condiments could be included in the questionnaire. For example, bouillon is a major source of (potentially iodized) salt in many West African countries, and subsidized Baladi bread is an important source of fortified flour in Egypt.
Module 2. Indicators of programme coverage, specimen selection, management and analysis

Supplies for collecting and storing food samples need to be considered in addition to those for biologic specimens. Generally, simple collection containers can be used—resealable plastic bags for dry food products and containers with screw tops for edible oil. Some foods, such as bread, involve additional logistical considerations because the bread must be weighed to assess water content at the point of collection and at the laboratory, and will become mouldy within a few days or weeks. In general, food samples should be stored and transported to the laboratory in cool conditions and protected from direct sunlight.

Additional details on food sample collection techniques, storage and transport considerations, analytical methods and presentation of resulting data can be found in the “Analysis of food samples” online tool.

Collecting specimens for assessing biomarkers

The subclinical status for most micronutrients is assessed using biomarkers found in biological specimens (blood or urine). It is not current practice to include clinical signs and symptoms of micronutrient deficiencies or excess in large population-based surveys. Module 3: Biomarker selection and specimen handling provides detailed information (see Box 2.2).

Surveys can also include the collection of food samples for assessing specific nutrient levels. Discussions with experts during the planning stage should specify the type of specimens or samples needed for each biomarker and food nutrient, the most appropriate collection method and devices needed, processing, and requirements for transportation and storage. Other specimens may be collected for different purposes, such as stool specimens for assessing intestinal parasites. Detailed discussion of these specimens is beyond the scope of this manual.

Collecting blood specimens

Blood specimens are required for assessing the following common indicators:

- haemoglobin concentration (as an indicator of anaemia)
- micronutrient status of populations: iron, vitamin A, vitamin D, vitamin B12, folate and zinc
- acute phase proteins, \(^1\) primarily C-reactive protein (CRP) and α-1-acid glycoprotein (AGP)
- other factors related to micronutrient status, such as malaria or other infections, and, in countries where these conditions are prevalent, haemoglobinopathies.

Blood specimens can be collected using either capillary or venous sampling, depending on the volume of blood needed for laboratory testing. The collection device and subsequent blood processing, if any, depend on the blood fraction required for the biomarker of interest. Blood fractions include red blood cells, plasma, and serum. Different collection tubes may be required to provide the types of specimen needed for different analyses (see Module 3: Biomarker selection and specimen handling). Collection of specimens as dried blood spots on filter paper is not currently recommended for assessing micronutrient status in cross-sectional surveys. The one exception is folate status assessment with a microbiologic assay.

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\(^1\) Acute phase proteins are markers of subclinical inflammatory response that can affect interpretation of biomarkers, such as for iron and vitamin A status.
Testing for haemoglobin levels (using a portable photometer) and for malaria (using a rapid diagnostic test) can be conducted in the field using small volumes (~30 µL) of capillary whole blood obtained from a finger or heel prick. Otherwise, biological specimens generally require collection, storage, and transport to a laboratory for processing and analysis.

Capillary blood collection: To collect a capillary blood sample from a finger or heel prick, use a disposable, single-use, contact-activated lancet. Lancets are available with different blade widths and depths. The more blood that is needed, the greater the blade depth and/or width is required. The age of the population group also needs to be considered.

Capillary sampling can be used to collect blood into a small, trace element-free, blood collection tube (with or without anticoagulant) for laboratory assessment of a wide range of biomarkers. The maximum collected is usually 500 µL of capillary blood, an amount that generates approximately 200 µL of serum or plasma.

Survey staff with or without phlebotomy experience can be trained to collect capillary blood samples. However, it is essential that experienced trainers conduct the training and ensure the use of standardized techniques. If larger volumes of capillary blood samples are required, particularly from young children, extensive training is needed. Only trainees with demonstrated skills and confidence should be selected for these tasks.

Venous blood collection: Venous blood collection is used when the total blood volume needed exceeds what can be collected by capillary sampling. The volume needed depends on the number of laboratory tests and the types of assays used. For large-scale, population-based surveys, only experienced phlebotomists

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should collect venous blood specimens. It is particularly difficult to successfully collect venous blood from young children and from malnourished and dehydrated individuals. Therefore, in micronutrient surveys, it is best practice to select phlebotomists who perform this procedure on a regular basis, particularly among the selected population groups.

The exact supplies needed for blood collection depend on the tests to be performed. More details are available in Module 3: Biomarker selection and specimen handling and in the “Equipment and supplies list (more complex worksheet)” online tool.

**Blood processing in the field and managing a cold chain when collecting blood specimens:** Capillary or venous blood analysis requires centrifugation, typically within 24 hours of collection. In addition, biological specimens usually require a cold chain that begins the moment a sample is collected and is functional through processing, storage, transport and arrival at the laboratory that will conduct the analysis. This avoids the risk that temperature changes (thawing and refreezing) alter the outcome of the analyses (see Module 3: Biomarker selection and specimen handling). Mobile freezers will be needed if the length of time between specimen collection and storage at a facility with a -20°C freezer is too long.

In some countries, facilities with reliable electrical power, centrifuges and −20°C freezers are available for survey teams to use for processing and storing specimens during fieldwork. However, if this is not the case, analysis teams may need a self-contained field laboratory. Field laboratory equipment usually includes portable centrifuges and −20°C portable freezers. Centrifuges may be powered by a car or motorbike battery and can be used for the timely processing of blood specimens into serum or plasma fractions, for storage in cryovials. Portable freezers may be powered by a car battery, a portable generator, or standard electrical outlets. It is useful to freeze gel packs when storing specimens in a cool box prior to processing and then to freeze the specimens quickly after processing to maintain the cold chain in the field. It is important to consider the space required in the field vehicles for −20°C portable freezers and other equipment and supplies, so that plans and budgets include the appropriate size and number of vehicles.

Some biomarkers require additional special considerations. For example, if assessment of serum or plasma zinc concentration is included in the survey plan, strict precautions must be taken to avoid contamination from exogenous sources, either in a temporary field laboratory or in a facility laboratory. You can find more information on the specific needs for each micronutrient test in Module 3: Biomarker selection and specimen handling.

**Collecting urine specimens**

A spot (single) urine specimen is recommended in cross-sectional surveys. These specimens are analysed in the laboratory for urinary iodine concentration (UIC), which can be used to determine population iodine status. In general, individuals are asked to capture urine in a disposable sterile cup from which the urine is then pipetted into small sterile cryovials. The urine specimens do not need to be kept cool for analytical purposes, but it is convenient to store them in the cool box with other specimens. This will also reduce odour. You can find additional details for urine collection for the assessment of iodine concentration in Module 3: Biomarker selection and specimen handling.

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1 Note that this method does not determine the iodine status of the individual.
Module 2. Indicators of programme coverage, specimen selection, management and analysis

Safety and other issues

When collecting blood and urine specimens there are a number of other issues to consider related to training, personal safety, hygiene, equipment, field processing and storage. These are discussed in the “IATA guidance document on infectious substances” online tool and in WHO guidelines on drawing blood. For example, appropriate sharps and biohazard disposable containers and protocols must be available for the safe disposal of cuvettes, blood tubes, needles and lancets.

Location for collecting samples and data

The survey management team should determine where the interviews, the collection of biologic specimens and food samples and biological laboratory processing will take place. These may be at the same or different locations. With more complex surveys, especially those in settings where households are very far apart or in difficult terrain, it may be best to set up a mobile laboratory in a central location. Sampling could also be done in households or through a clinic or school.

The main advantages of setting up a field laboratory for collecting and processing data include:

- A minimum number of sets of equipment may be required per team, thus reducing costs.
- Data collection in a single place eliminates the time and effort needed to move between households carrying equipment and supplies, including anthropometry equipment, that would need to be set up in each household. It also reduces data collection time, is more convenient for the survey team members, prolongs equipment life and can control the conditions where precautions against contamination are required, such as with blood collection for zinc analysis.

However, it is not always possible to find a convenient central site or it might be inconvenient for participants to travel to the central location.

Conducting interviews at the household rather than in a central location can improve participant comfort and privacy. In addition, household-level data collection may lead to more complete information about programme processes, such as being able to access child health cards and observe the labelling on food products. A household location also helps avoid potential biases in the collection of food samples, for example, a respondent may buy or obtain some of the requested food on their way to the central site.

It may be useful to use both locations, for example asking individual questions and collecting food samples at the household, and collecting biological specimens and anthropometry data at a central location. No matter the setting, care must be taken to match the unique ID labels for questionnaires with associated samples and specimens so that they can be correctly linked during data management and analysis.

All field procedures must be pilot tested. The survey management team needs to be able to change plans based on the most efficient and effective method for high-quality data collection and high response rates. The selected method should be the same for each cluster.

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Selecting laboratories to test specimens

Access to laboratories with high-quality performance and an acceptable cost per specimen is a critical factor in determining the feasibility of including selected vitamin or mineral biomarkers in a micronutrient survey. The laboratories may be national or international. If international, the survey management team needs to plan for the required export permissions and costs of shipping samples or specimens out of the country, as well as for the requirements for them to be accepted in the receiving country. Identification of laboratories to conduct quality-assured analyses should be done very early in the planning process.

Laboratories under consideration for micronutrient biomarker analysis should first be assessed by experts in the field. The assessment should consider the following criteria:

1. The laboratory performs analyses using accepted standard methods (usually the gold standard or an internationally accepted standard);
2. The laboratory staff has the technical skills required for performing the analyses;
3. The analyses are routinely carried out in the laboratory (within the last few months);
4. The laboratory has the ability to analyse the appropriate number of samples daily to provide the necessary throughput for a survey and maintains the instruments in good working condition;
5. Quality assurance measures are in place or can be easily set-up including:
   a. Preparing quality control (QC) materials for analysing survey samples
   b. Using bench QC pools for every run
   c. Using blind QC pools for every run
   d. The laboratory participates and performs well in external quality assurance (EQA) programmes, including the Centers for Disease Control and Prevention’s (CDC’s) Vitamin A Laboratory – External Quality Assurance (VITAL-EQA for vitamin A, B vitamins, vitamin D, iron and CRP) programme, Ensuring the Quality of Iodine Procedures (EQUIP for iodine) programme, the Vitamin D External Quality Assessment Scheme (DEQAS for 25-hydroxyvitamin D), and EQA for the folate microbiological assay (serum and whole blood)
   e. Use of international reference material (where available) to periodically verify the accuracy of other methods used, and acceptable performance for all selected analytes.

It is preferable that a single laboratory analyses all specimens for a specific assay. This will avoid any possible inter-laboratory variation. The “Questionnaire for laboratory evaluation” online form can assist with the selection process.

Key issues to consider when selecting a method include:

- the type of biological specimens and/or food samples required
- the number and volume of specimens to be tested
- the timeline for completing tests (testing large numbers of specimens for certain nutrients may take several months)
- how the specimens should be collected, processed, stored, and transported to the laboratory (this will include any cold chain requirements)
- assay costs
- the requirements and costs of quality assurance processes for each method.
Module 3. Biomarker selection and specimen handling

In this module, we will discuss:

- Factors to consider when selecting biological indicators and specimens
- Micronutrient and related health indicators
- Haemoglobin
- Iron
- [Iron deficiency anaemia]
- Vitamin A
- Iodine
- Vitamin B12
- Folate
- Vitamin D
- Zinc
Factors to consider when selecting biological indicators and specimens

Many factors drive the selection of indicators. Some of these factors relate to participants, for example the acceptability and feasibility of the collection methods. Others concern field work (the ease of specimen collection, processing, storage, and transportation) and laboratory issues such as equipment, training, reagents, and costs. All these factors must be considered during the planning stage of the survey.

This module provides information to guide the selection of appropriate and reliable biomarkers, types of specimens, and methods of collection and analysis applicable to large micronutrient surveys. For each micronutrient there is a discussion of: specimen collection and management, biomarker analysis, an approximate budget, and how to interpret results. Issues of quality control are described where appropriate. The recommended collection containers, processing, storage and transport conditions for different specimen types are summarized in Table 3.11 at the end of the module.

Costs have been calculated based on prices known in 2019. Actual costs for analyses may differ greatly from one laboratory to another.

Advice about the most suitable indicators and methods change periodically. During the planning phase, it is advisable to review the latest recommendations and consult with appropriate experts. Review papers on micronutrient status assessment developed under the Biomarkers of Nutrition for Development (BOND) program are shown in Box 3.1.

Micronutrient and related health indicators

This section describes indicators for selected micronutrients (iron, iodine, folate and vitamins A, B12, and D) and related health issues (anaemia, iron deficiency and iron deficiency anaemia).

Throughout the section, recommended cutoff values for defining deficiency or insufficiency are listed. It is important to note that cutoffs may not be available for all population groups of interest. In addition, inflammation is an area of current research that affects micronutrient measures and new methods to adjust for inflammation are being explored.

Cutoff values for populations groups

Given the higher nutrient demands required for growth and reproduction, young children and women of reproductive age are the most vulnerable population groups and are thus the most common targets of nutrition surveys.

For pregnant women, unique cutoff values are only available for haemoglobin (anaemia), ferritin during the first trimester (iron deficiency) and urinary iodine. No cutoff values specific to this group are available for other micronutrients, and research is not sufficient to determine whether such values are needed. If stable representative estimates for pregnant women are needed in a survey, then this group will need to be oversampled and the results categorized separately.
Inflammation

The acute phase response, triggered by infection and trauma, is a collection of non-specific changes including the production of proteins that promote inflammation and activate, complement, and stimulate phagocytic cells. All of these are inflammatory markers. This cascade of immune response activity is intended to remove harmful molecules and pathogens and to prevent further damage to tissues (7).

Inflammation affects the circulating concentrations of multiple micronutrients. During the acute phase response there is a change in many indicators of micronutrient status, such as retinol and ferritin, leading to an over- or under-estimation of deficiency. The acute phase proteins, C-reactive protein (CRP) and alpha-1-acid glycoprotein (AGP), are commonly used indicators of inflammation (7). The concentration of some acute phase proteins (APPs) in plasma, called positive APPs, will increase in the presence of inflammation. Examples of such APPs include CRP, AGP, and ferritin. Other APPs decrease in the presence of inflammation. These negative APPs include retinol, retinol binding protein (RBP), and albumin.

The BRINDA project (Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia) has been investigating approaches to adjust population estimates of iron, vitamin A, and zinc in the presence of inflammation. The progress for iron and vitamin A has been published, and work on zinc is ongoing. In general, BRINDA described methods that incorporate an internal (country- or survey-specific) regression-correction approach (8). This is a rapidly evolving area and it is important to review the literature for updated information. The BRINDA website also contains useful resources (9).
In many lower income countries, the prevalence of infection and resultant inflammation is high, and many individuals will have elevated APPs even without clinical signs or symptoms of disease. Additionally, chronic conditions without infection, such as diabetes, hypertension and obesity, result in low grade inflammation.

**Measuring inflammation by CRP:** CRP concentration increases rapidly during inflammatory processes, and returns to pre-infection levels over 18-20 hours when the stimuli end, which is more quickly than AGP (7).

*Specimen collection and management:* CRP is typically measured in serum samples obtained by centrifuging whole blood that was collected by venipuncture or capillary sampling. CRP is stable, so whole blood processing can be delayed for 1-2 days. Serum is stable for up to two weeks at 4°C and up to one year at -20°C.

*Biomarker analysis:* CRP and high-sensitivity CRP (hs-CRP) (10, 11) is measured by immunoassays (nephelometry or turbidimetry), either on a fully automated clinical analyser or by using a manual ELISA assay. Commercial assay kits are available for both analytical techniques. The required analysis volume is normally <25 µL; however, a minimum specimen volume of >150 µL may be needed to fill the sample cup for the clinical analyser. The assay product sheet will explain matrix requirements. Not all assays can utilize EDTA (ethylenediaminetetraacetic acid) or heparin plasma. Serum is the preferred matrix.

*Quality control:* Appropriate quality control measures must be followed to ensure high quality results. The assay kits include calibration materials and may include quality control materials. It is nonetheless recommended to establish “in-house” quality control materials that can be tracked over a longer period to verify that the method did not shift over time. The method imprecision is typically ~10%. A human serum international reference material (ERM-DA474/IFCC) is available through the Institute for Reference Materials and Measurements (IRMM) at the European Commission Joint Research Centre. However, not every assay may be able to use this material because the assay performance may differ between patient samples and reference materials that have undergone some processing. Moderate differences between assays can be observed in proficiency testing programmes, such as the Immunology Survey of the US College of American Pathologists (CAP) and the National Institute of Standards and Technology (NIST).

*Approximate budget requirements for analysis:* Instrumentation needed for CRP includes either a clinical analyser (approximately US$ 100 000) or a plate-washer, plate-reader, and various pipettes (approximately US$ 30 000). The cost for materials and supplies is roughly US$ 3 to US$ 5 per sample for a commercial kit assay. Material costs may be slightly lower for locally developed ELISA assays that measure CRP in addition to other micronutrients.

*Interpretation of results:* The suggested CRP cutoff value to define inflammation is >5 mg/L (7, 12).

**Measuring inflammation by AGP:** The concentration of AGP normally increases within 24 to 48 hours following an infection and stays elevated for a longer duration than CRP. Typically, it is elevated for 5 days, from day 2 to day 7 after a single clinical infection (13–15). Hence, AGP captures a unique phase of the acute phase response, compared to CRP (7).

*Specimen collection and management:* Like CRP, AGP is stable, and specimens can be handled using similar conditions.
Module 3. Biomarker selection and specimen handling

Biomarker analysis: The analytical techniques to measure AGP are the same as for CRP; however, there are fewer clinical analysers available that measure AGP. A human serum international reference material (ERM-DA470K/IFCC) is available from the European IRMM. AGP is usually not covered in proficiency testing programmes and it is therefore difficult to compare among assays.

Approximate budget requirements for analysis: The same resources and instrumentation described for CRP are needed for the measurement of AGP.

Interpretation of results: The suggested AGP cutoff value to define inflammation is >1 g/L (7, 12).

Vitamin A

There are multiple indicators for determining vitamin A deficiency. The four most commonly used biological indicators are serum (or plasma) retinol, retinol-binding protein (RBP), and the modified relative dose response (MRDR). Breast milk retinol can be used in some circumstances.

WHO recommends the use of two different criteria for determining the presence and severity of vitamin A deficiency as a public health problem. One is when the population prevalence of at least two biological parameters from a range of functional and biochemical indicators, one of them being serum retinol, exceed the threshold for defining a public health problem (18). The second criterion specifies one biological indicator with a prevalence below the population-level cutoff value and at least four ecologic risk factors for vitamin A deficiency, two of which should be nutrition or diet-related (18). Demographic or ecologic risk factors include nutrition-related and illness-related risk factors. Examples of relevant risk factors are:

Nutrition- and diet-related risk factors:
- <50% prevalence of breastfeeding in infants 6 months of age
- Median dietary vitamin A intake <50% of recommended safe levels of intake among 75% of children 1-6 years of age
- Stunting rate ≥30% and/or wasting rate ≥10% among children under 5 years of age
- Food frequency assessment findings that foods with high vitamin A content consumed <3 times per week by ≥75% of vulnerable groups

Illness-related risk factors:
- Infant mortality rate >75 per 1000 live births and child mortality rate of >100 per 1000 live births
- Full immunization coverage <50% of children between 12-23 months of age
- Two-week prevalence of diarrhoea of >20%
- Measles case fatality rate of ≥1%
- No formal schooling for >50% of women 15-44 years of age
- <50% of households with a safe water source (boiled, treated, filtered, properly stored)
Serum (or plasma) retinol: WHO recommends that serum retinol be used along with either another biological indicator of vitamin A status or with the other risk factors listed above to define the degree of public health significance of vitamin A deficiency at the population (not individual) level and to assess the need for vitamin A interventions (18, 19). Serum (or plasma) retinol can indicate subclinical, or marginal, vitamin A deficiency. Concentrations can change in response to vitamin A interventions when liver stores are low; when liver stores are replete, retinol concentrations may not respond to vitamin A interventions (19). Serum retinol also correlates with the prevalence and severity of xerophthalmia.

Serum retinol concentrations are homeostatically controlled, but inflammation does cause them to decrease. Without an accompanying indicator of inflammation, artificially depressed serum retinol concentrations may lead to an overestimation of vitamin A deficiency prevalence. Inclusion of indicators such as CRP or AGP are recommended in any survey that includes the assessment of serum retinol to identify individuals with inflammation. However, at the time of this writing, the BOND review states that there is no consensus on the need for, or best method to, adjust for identified inflammation (3).

Specimen collection and management: Most commonly, retinol is measured in serum samples that are obtained by centrifugation of whole blood collected by venipuncture or finger prick. Whole blood needs to be refrigerated immediately and centrifuged within a few days of collection. When protected from light, the vitamin A in serum is stable for at least one week at 4°C and for at least one year at -20°C (16). However, procedures such as centrifugation and an adequate cold chain can be difficult to implement in remote field conditions.

Biomarker analysis: The most common and accurate method for measuring serum retinol is high-performance liquid chromatography (HPLC) with UV detection. In an adapted “micromethod,” (20) the required analysis volume is only 25 µL of serum or plasma. The minimum specimen volume is 100 µL to provide enough sample for a repeat analysis if needed. EDTA or heparinized plasma can be used, but serum is the preferred matrix. Commercially available retinol with greater than 95% purity is used as a calibrator (HPLC-grade reagents are preferred). Retinyl acetate, which is also commercially available, is used as an internal standard to correct for variations during the analytical procedure.

Quality control (QC) measures: Analytical method imprecision is typically around 5%. Moderate assay differences can occur with analyses conducted in different laboratories, therefore laboratories should participate in an external quality assurance programme such as CDC’s Vitamin A Laboratory and External Quality Assurance (VITAL-EQA) programme (21), CDC’s Performance Verification Program for Serum Micronutrients (22), which provides a one-time or annual performance report, or the National Institute of Standards and Technology (NIST) Health Assessment Measurements Quality Assurance Programme (HAMQAP). Serum-based certified reference materials (multiple levels of Standard Reference Material® (SRM®) 968) are available from NIST (Gaithersburg, MD, USA) to verify method accuracy. Quality control materials for serum micronutrients including retinol are available from CDC to support in-house quality assurance programmes for laboratories engaged in public health work (23).

Approximate budget requirements for analysis: Instrumentation needed for this method includes an HPLC and UV detector, centrifuge, vortex, and various pipettes (costing approximately US$ 50 000). The cost for materials and supplies is approximately US$ 5 per sample.
Interpretation of results: Low serum retinol is defined as <0.70 µmol/L in children 6–71 months of age. A serum retinol cutoff value of <1.05 µmol/L is sometimes used to indicate vitamin A insufficiency. Interpretation of a population’s prevalence of serum retinol concentrations <0.70 µmol/L for defining a public health problem is presented in Table 3.1. It is important to note that this is based on low serum retinol values that have not been adjusted for inflammation.

Table 3.1. Prevalence of low serum retinol (<0.70 µmol/L),\(^a\) used in conjunction with another indicator,\(^b\) to define a public health problem at the population level and its level of importance among children 6–71 months of age (19)

<table>
<thead>
<tr>
<th>Level of importance as a public health problem</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild</td>
<td>2 to 9%</td>
</tr>
<tr>
<td>Moderate</td>
<td>10 to 19%</td>
</tr>
<tr>
<td>Severe</td>
<td>≥20%</td>
</tr>
</tbody>
</table>

\(^a\) Equivalent to serum retinol <20 µg/dL. Prevalence estimates based on retinol values that have not been adjusted for inflammation.

\(^b\) "Another indicator" pertains to either a second biomarker for vitamin A deficiency, or at least four ecologic indicators for vitamin A deficiency, two of which should be nutrition or diet related.

Retinol Binding Protein (RBP): RBP can be used as a surrogate, or proxy, indicator for retinol (3); however, a 1:1 molar equivalence between retinol and RBP does not usually occur. This means that the serum retinol cutoff value cannot be applied to RBP. The currently recommended approach to calculating an RBP cutoff value, detailed in the BOND review (3) and practiced in survey reports (24, 25), is to measure serum retinol concentrations by HPLC in a subsample of the population where RBP is being used to assess vitamin A deficiency. Within the retinol subsample, given a reasonable correlation between RBP and retinol, a linear regression model is used to calculate an RBP concentration equivalent to retinol of 0.7 µmol/L. Ideally, the same blood draw would be used for measuring retinol and RBP (as opposed to taking a morning collection for RBP and an afternoon collection from the same person for retinol, for example). Outliers should be removed when reviewing the serum retinol-RBP regression plot, using studentized residuals larger than 3 in absolute value (26). A minimum sample size for the retinol subsample is suggested to be 20% of the total survey population or at least 100 observations, selected at random. The field of vitamin A assessment is rapidly evolving; the guidance presented in this manual is based on the best evidence available at the time of publication.

Specimen collection and management: RBP can be measured in serum or plasma. Blood should be collected, processed and stored as noted for the collection and management of specimens for serum (or plasma) retinol. The BOND Expert Panel for Vitamin A assigned the same degree of difficulty to serum RBP and serum retinol for sample collection and sample transportation. The dried blood spot (DBS) methodology for measuring serum retinol and RBP is less reliable than the method based on serum or plasma (27).

Biomarker analysis: Several commercial ELISA methods and laboratory-developed tests are available to measure RBP in serum or plasma, with serum being the preferred specimen matrix. However, no certified reference materials are available to verify RBP method accuracy, and RBP is therefore not included in external quality assurance programmes. Notably, the CDC VITAL-EQA programme (21)
includes quality assurance for retinol, as well as for other nutritional biomarkers. In this programme, RBP is compared to retinol. Similar to using RBP as a proxy for retinol in surveys, laboratories that participate in VITAL-EQA may also compare RBP to retinol. Thus, although there are no external quality assurance programmes for RBP there is the opportunity to measure RBP in serum or plasma in commercial ELISA methods and compare to laboratory-developed tests against retinol as a proxy. Another advantage of these results is sample comparability because both biomarkers will be performed on the same specimen, handled under the same conditions. The specimen should be gently and thoroughly mixed before measuring the retinol and RBP.

Approximate budget requirements for analysis: Instrumentation needed for ELISA methods includes a plate washer, plate reader and various pipettes (approximately US$ 30 000). The cost for materials and supplies is approximately US$ 2–5 per sample, depending on whether the assay used is laboratory-developed or a commercial kit. For a laboratory developed RBP test, a significant amount of time will be necessary to find appropriate antibodies and validate the assay.

Interpretation of results: There are at present no WHO guidelines on the interpretation of vitamin A deficiency prevalence based solely on RBP. When RBP is measured in surveys as a proxy for retinol, it is important to include a caveat if the public health significance of vitamin A deficiency is based on a prevalence of RBP <0.7 µmol/L. Another important consideration to keep in mind is that the prevalence numbers in Table 3.1 are based on serum retinol values that have not been adjusted for inflammation. It is recommended to present both inflammation-adjusted and unadjusted estimates for vitamin A deficiency until guidance from WHO becomes available. Suggested methods for adjusting serum retinol concentrations based on CRP and AGP include the use of regression or arithmetic correction factors, such as those developed by BRINDA (28) and Thurnham (29), respectively. Also, since enzyme immunoassays do not distinguish between holo- and apo-RBP, an additional adjustment (that requires determination of serum retinol) is needed to reflect the RBP:retinol ratio in the population of interest (30, 31). Box 3.2 summarizes the use of RBP for assessing vitamin A status.

Box 3.2. Summary of the use of RBP
At the time of this writing, the preferred method for assessing vitamin A status is serum retinol. When RBP is selected as the main vitamin A indicator for a survey, it is also necessary to measure retinol in a subsample of the population. This will permit the determination of a survey specific RBP cutoff value to define vitamin A deficiency. It is also useful to measure MRDR in a subsample because it provides an assessment of vitamin A liver reserves and the WHO recommendation of two biomarkers to assess deficiency.

It may be complicated to analyse trends if there are different survey-specific population-level cutoff values within the same country from various years. For example, the RBP survey-specific cutoff value to define vitamin A deficiency may be 0.78 µmol/L in one survey cycle and 0.58 µmol/L in the next survey cycle. As such, the prevalence of RBP below those two separate cutoff values may be hard to compare and raises the question of why the RBP-retinol relationship changed in a population between survey cycles. This is important to note, and may be a factor to consider when choosing vitamin A indicators. WHO has defined the retinol cutoff value for deficiency, making it easier to assess trends over time when retinol is collected and analysed using similar methods across surveys. However, many older surveys did not assess indicators of inflammation, which can influence the interpretation of retinol and trends over time as the prevalence of inflammation can vary from survey to survey.
Module 3. Biomarker selection and specimen handling

**Modified relative dose response (MRDR):** RBP is synthesized in the liver as apo-RBP (unbound RBP). When liver reserves of vitamin A are low, apo-RBP accumulates in the liver. When vitamin A becomes available from newly ingested sources the accumulated apo-RBP binds to the retinol and is released into circulation as the holo-RBP complex (retinol bound to RBP). MRDR is a functional test that takes advantage of this process by providing individuals with a measurable “challenge” dose of 3,4-didehydroretinol (also known as DR or vitamin A2) in the acetate form. DR binds to apo-RBP in the liver forming the holo-RBP complex, which is quickly released into the plasma during deficiency. After this challenge dose, DR should appear in serum in significant amounts over baseline (prior to the challenge dose) only when liver reserves of vitamin A are low. Therefore, the amount of DR released is an indication of vitamin A status. MRDR is calculated from the molar ratio of DR to retinol (DR:R). In comparison with other vitamin A indicators, MRDR is less influenced by inflammation and it is not homeostatically controlled in the timeframe of the test (3). An important consideration of MRDR is the time required between administering the challenge dose and collecting the specimen (4-6 hours after the challenge).

The MRDR is useful to assess changes in liver stores of vitamin A, for example, changes in response to an intervention to improve vitamin A status. The MRDR test provides useful semi-quantitative information to evaluate deficiency through low liver reserves of vitamin A. On the contrary, it is not useful in defining excessive vitamin A reserves. MRDR is recommended for inclusion among a randomly selected subsample of individuals, to assess the underlying vitamin A status of the population studied (3). Serum retinol is collected and analysed from the same blood draw as that used for MRDR. When assessing vitamin A deficiency at the population level, it is useful to assess the mean and standard deviation of the MRDR value (namely, DR:R). When comparing results from MRDR with results from RBP and retinol, there may be inconsistencies at the individual level for categorizing deficiency. Thus, comparing vitamin A deficiency prevalence estimates from MRDR, RBP, and retinol may cause confusion. It is most useful to look at two biologic parameters (MRDR plus either retinol (preferred) or RBP) to determine the population status of vitamin A deficiency as a public health problem (3, 18).

**Specimen collection and management:** In preparation for specimens for an MRDR assessment, an individual must consume a small challenge dose of a retinol analog (DR or vitamin A2) along with a fatty snack (lacking in vitamin A) to ensure absorption. This should be done about 4 to 6 hours before collecting 1-3 mL of venous blood. The dose of vitamin A2 can be mixed with 1 mL of olive oil or another edible oil containing no vitamin A. Administering it using a disposable syringe helps ensure that it is completely swallowed, especially with small children. Survey participants must also be questioned about recent consumption of foods rich in vitamin A prior to administering the vitamin A challenge dose. If there has been recent consumption of vitamin A rich foods, it will be necessary to wait two hours before proceeding with the test. Vitamin A rich foods should not be consumed again until after the blood draw for the MRDR test.

The same procedures for transporting, processing, and storing of specimens for other vitamin A indicators apply to the MRDR venous blood specimen.

**Biomarker analysis:** Analysis of 3,4-di-dehydroretinol requires HPLC and can be assessed in the same analytical run as serum retinol. The required sample volume for analysing serum retinol and 3,4-di-dehydroretinol is 250 µL of serum or plasma, and the minimum specimen volume is 500 µL to provide enough sample for a repeat analysis if needed. Retinol acetate is used as an internal standard calibrator for retinol (commercially available at a satisfactory purity >95%) in each analytical run.
Module 3. Biomarker selection and specimen handling

Quality control (QC) samples are recommended, which have a known concentration of retinol that covers the range of retinol concentrations expected in the human population (low, medium and high) and are used to validate each analytical assessment, aiding in correcting analytical bias.

Approximate budget requirements for analysis: Instrumentation needed for this method includes an HPLC, centrifuge, vortex, and various pipettes (costing approximately US$ 50 000 for the complete set of equipment). The cost for materials and supplies is approximately US$ 5 per sample.

Interpretation of results: MRDR is a semi-quantitative indicator of vitamin A status. The MRDR value, which is the ratio of DR to retinol in serum, indicates adequacy of liver reserves. For individuals, the 2016 BOND review (3) recommends a MRDR cutoff value of ≥0.060 to indicate insufficient liver reserves (≤0.1 µmol retinol/g liver vitamin A), and of <0.060 to indicate enough liver reserves (≥0.1 µmol retinol/g liver vitamin A). For groups, a mean MRDR value <0.030 is recommended for indicating adequate vitamin A status (3).

Breast milk retinol: WHO recommends exclusive breastfeeding for infants in the first 6 months of life, followed by continued breastfeeding with appropriate complementary foods for up to 2 years or beyond (32). Breast milk retinol concentrations provide information about both the mother and breastfed infant. They are considered to reflect the recent dietary intake of mothers and can be used to estimate vitamin A intake of infants receiving the breast milk (3, 12). Breast milk retinol concentrations have been used to assess the risk of vitamin A deficiency in populations, determine the efficacy of maternal vitamin A interventions, and for the monitoring and evaluation of programmes providing maternal vitamin A interventions (3). Average breast milk retinol concentrations from well-nourished women are about 485 µg/L (33); however, average concentrations can fall below 400 µg/L in areas where vitamin A deficiency is of public health significance (18). When selecting the population for evaluation, age, stage of lactation, geographic location, season and pregnancy status should be considered (3). Vitamin A content is very high in colostrum (milk secreted in the first 4-6 days postpartum), and remains high in transitional milk (days 7-21 postpartum), after stabilizing in mature milk (after about day 21 postpartum). Therefore, breast milk samples collected after one month postpartum, avoiding colostrum and transitional milk samples, are most useful for assessment of vitamin A status.

Specimen collection and management: Breast milk can be collected as a full milk sample or as a casual sample. The specimen collection method would depend on the survey objectives. A casual sample is appropriate for assessment of population-level prevalence of low breast milk vitamin A, expressed in nmol retinol/g fat, and will be described here. A survey objective of estimating the vitamin A intake of infants from breast milk would necessitate that a full milk sample be collected, which is described in detail elsewhere (34).

Milk collected using the casual sample method (~10 mL) can be hand-expressed into specimen cups or tubes made of polypropylene. A benefit of casual milk collection is that there is no need to standardize ‘time since last feed’; however, milk fat will need to be measured. One option for milk fat measurement is a creamatocrit centrifuge, which is field friendly. Casual milk collection is defined as mid-feed collection 1 minute after let-down, by manual expression. Although women can do the manual expression in privacy and without assistance after receiving adequate instructions, it is important to consider the gender of the field staff and the local context as female health workers may be more appropriate for surveys that include breast milk collection. The breast milk needs to be refrigerated at 4°C immediately and protected from direct light because vitamin A in milk is less stable.
than serum retinol, which is protein bound (34). Protection from light and keeping the milk cold will prevent photodegradation of the vitamin A. If refrigerated, the breast milk must be analysed within 24 hours of collection or it may be stored frozen at -20°C (or colder, such as -80°C) and analysed within one year of collection (3). Before freezing, precise aliquots of milk that will be used for measuring vitamin A content should be prepared as thawed samples can be difficult to homogenize. However, procedures such as centrifugation and an adequate cold chain can be difficult to implement in remote field conditions.

**Biomarker analysis:** The most common and accurate method for measuring breast milk retinol is high-performance liquid chromatography (HPLC) with UV detection after saponification (35). Portable fluorometers are also field friendly equipment that enables mothers to get immediate results on their breast milk vitamin A status (36), and has performed well compared to HPLC (37). Because vitamin A is found in the milk fat, fresh milk should be mixed well so that the cream layer is evenly distributed within the sample taken for measurement. A sample volume of 2 mL of breast milk is required for analysis using HPLC. The minimum specimen volume is 100 µL to provide enough sample for a repeat analysis if needed. Commercially available retinol with greater than 95% purity is used as a calibrator (HPLC-grade reagents are preferred). The base solution to be used for saponification should be mixed and stored in plastic containers to remain stable (35). Either 3,4-didehydroretinyl acetate (3), C23-beta-apo-carotenol (38, 39), or tocol (40), may be used as an internal standard to correct for variations during the analytical procedure. To determine the amount of milk fat in a specimen, the creamatocrit methods can be done in a laboratory (41), or a creamatocrit centrifuge can be used in the field.

**Quality control (QC) measures:** Analytical method imprecision is typically around 5%. Moderate assay differences can occur with analyses conducted across laboratories; however, the National Institute of Standards and Technology (NIST) Health Assessment Measurements Quality Assurance Programme (HAMQAP) does not have certified control for human breast milk, making external quality assurance impractical.

**Approximate budget requirements for analysis:** Instrumentation needed for the HPLC method includes an HPLC with a UV detector, centrifuge, vortex, and various pipettes (costing approximately US$50 000). A calibrated spectrophotometer is also needed for external standard quantification. The cost for materials and supplies is approximately US$ 5 per sample.

**Interpretation of results:** Breast milk retinol concentrations ≤1.05 µmol/L are considered inadequate (18), but this cutoff applies only to full milk collection. It is preferable to express breast milk retinol concentrations per gram of fat to account for fat variability. Retinol concentrations ≤28 nmol/g milk fat (or ≤8 µg/g milk fat) are considered inadequate (18). Vitamin A deficiency is considered a public health problem of mild, moderate or severe importance at a prevalence of inadequate concentrations of <10%, 10-24%, and ≥25%, respectively.

**Clinical and functional indicators:** Clinical or functional indicators of vitamin A deficiency usually focus on xerophthalmia, an eye condition that worsens as the depletion of vitamin A stores progresses (42). Most of these indicators are not recommended for routine cross-sectional surveys due to their rare occurrence, even in areas endemic for vitamin A deficiency. The one exception is assessing whether women have experienced night blindness during a pregnancy within the previous three years or five years. To help interpret reported vision problems, women being assessed should report problems seeing at night as well as during the day during the last pregnancy. The WHO/International Vitamin A Consultative Group (IVACG) states that a
prevalence of night blindness that exceeds 5% among pregnant women would indicate vitamin A deficiency of public health significance among the population (43).

**Haemoglobin**

Anaemia is usually defined by a low haemoglobin concentration adjusted for altitude, and (among adults) adjusted for smoking. One of the most common causes of anaemia is iron deficiency. However, anaemia cannot necessarily be used as a proxy for iron deficiency because anaemia can result from many other factors, including:

- Malaria and other infections
- Other causes of blood loss (such as heavy menses, haemorrhage in childbirth, trauma, gastrointestinal bleeding due to ulcers)
- Deficits in other nutrients (for example vitamin A, folic acid, vitamin B12)
- Haemoglobinopathies (such as sickle cell or thalassemia)
- Overweight, obesity and other causes of chronic inflammation (for example chronic kidney disease) (44)
- Blood loss due to infection (from such conditions as hookworm, schistosomiasis or H. pylori) (45, 46).

Haemoglobin level is an indicator of anaemia, a condition in which the number of red blood cells or their oxygen-carrying capacity is insufficient to meet physiologic needs. Iron deficiency is one of the most common causes of anaemia globally, although anaemia can also be caused by other conditions (47).

Additional haematologic indicators of anaemia include haematocrit, mean cell volume and red blood cell distribution width. It is recommended that haemoglobin be used for assessing anaemia in cross-sectional surveys. For assessing the prevalence of iron deficiency anaemia, it is recommended that countries collect data on haemoglobin and at least one biochemical test for iron deficiency, along with measures of inflammation as appropriate (48).

**Specimen collection and management:** Haemoglobin is typically measured in fresh whole blood samples in the field (49). For non-field-based analysis, haemoglobin is most commonly measured in EDTA blood samples. In this case, the samples should be refrigerated as soon as possible and need to be analysed within 1–2 days of collection.

In the case of field analysis, capillary drops, pooled capillary blood into small blood collection tubes, or venous blood can be used. Capillary blood from a finger prick is collected by capillary action in a cuvette, which is then placed in the photometer that displays the haemoglobin concentration within one minute. The accuracy of haemoglobin measurement may be improved by pooled capillary or venous blood. For pooled capillary blood, collect 250–500 μL in a small blood collection tube containing an anticoagulant such as EDTA or heparin, gently mixing the blood by inverting the tube several times to prevent clotting, and then filling the cuvette with blood from the blood collection tube (50, 51). For venous blood, collect 3-5 mL in a vacuum blood collection tube containing an anticoagulant such as EDTA or heparin, gently mixing the blood by inverting the tube several times, and then filling the cuvette with blood from the collection tube for analysis (49, 51). A comparison of nationally representative surveys measuring haemoglobin using HemoCue® with capillary (DHS) or venous (BRINDA) samples, showed substantial differences in anaemia prevalence estimates, which were consistently lower in venous compared to capillary (52).
The procedures for specimen collection and analysis using a haemoglobinometer must be standardized. This requires careful training of survey technicians. It is particularly important not to squeeze the finger too hard when collecting capillary blood because this can cause interstitial fluid to mix with the blood and result in an incorrect haemoglobin concentration. Poor quality collection of capillary blood can in turn lead to low or high haemoglobin concentrations for population-based surveys (49).

**Biomarker analysis:** The most commonly used method for field-based measurement of haemoglobin in population surveys is photometric determination using a portable haemoglobinometer (49, 52). The procedure does not require specialized laboratory personnel and the haemoglobinometer may be operated on four AA batteries, which makes it particularly useful in the field. Manuals and tutorial videos for haemoglobinometers are available online, making it easier to follow proper operation (50, 51): The HemoCue® haemoglobinometer has been validated against traditional haemoglobin laboratory methods and found to have adequate accuracy and precision in controlled settings (50, 51). The accepted reference method for haemoglobin measurement is the cyanmethaemoglobin method (53).

A systematic review commissioned by WHO for reviewing haemoglobin cutoffs as part of the project to review cutoffs to diagnose anaemia concluded that capillary fingerprick blood usually produces higher haemoglobin concentrations compared with venous blood, that individual drops produced lower concentrations than pooled capillary blood and that compared to automated haematology analysers, other methods (cyanmethaemoglobin, WHO Colour Scale, paper-based devices, HemoCue® Hb-201 and Hb-301, and Masimo Pronto®) overestimated haemoglobin concentrations (49).

**Approximate budget requirements for analysis:** Each haemoglobinometer costs approximately US$ 300–500 depending on the model, and the cuvette cost is approximately US$ 0.50 when both items are procured through UNICEF. While most of the field experience in the use of portable haemoglobinometers has been with HemoCue®, other portable haemoglobinometers are available from other manufacturers at a similar cost (US$ 400–600).

**Adjustments and interpretation of results:** WHO has established cutoff values for haemoglobin to define anaemia by population group, including for pregnant women (48, 54). These are shown in **Tables 3.2–3.4. Table 3.2** presents haemoglobin levels used to diagnose anaemia, while **Table 3.3** defines the public health significance of anaemia in a population. **Table 3.4** shows the adjustments of haemoglobin values that are required to correct for changes that occur due to altitude and smoking (based on the average number of cigarettes per day). Populations living at high altitudes where oxygen pressure is low have higher haemoglobin concentrations, reduced oxygen saturation and an increased production of red blood cells to ensure oxygen supply to tissues. These physiological characteristics would result in identifying fewer cases of anaemia using the cutoff values in **Table 3.2.** The approach shown in **Table 3.4** adjusts everyone’s haemoglobin value first, then applies the haemoglobin cutoff value for anaemia from **Table 3.2.**

The distribution of haemoglobin among sub-groups of the population can also provide important information concerning the aetiology of anaemia (vitamin A deficiency, iron deficiency, other nutrient deficiencies, inflammation status or blood disorders) (55). For example, if the iron deficient population has the same distribution of haemoglobin as the iron replete population, then iron deficiency is
unlikely to be the cause of anaemia. On the other hand, if the iron deficient population has a lower haemoglobin distribution, then it is more likely that iron is a cause of anaemia in that population.

Understanding the aetiology of anaemia is important for the design and evaluation of anaemia prevention strategies and programmes, thus a micronutrient survey should assess some of the factors in the above list. For example, in all malaria endemic countries, malaria should be assessed. Rapid diagnostic tests cost very little (around US$ 1) and are easy to administer. Many resources that explain the samples and analyses required for assessing malaria and other infections, such as helminths, are available from WHO (56–58).

Table 3.2. Haemoglobin cutoff values to define anaemia in individuals for people living at sea level (g/L)\textsuperscript{a}

<table>
<thead>
<tr>
<th>Population</th>
<th>Non-anaemia\textsuperscript{b}</th>
<th>Anaemia\textsuperscript{b}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mild</td>
</tr>
<tr>
<td>Children 6-59 months</td>
<td>≥110</td>
<td>100-109</td>
</tr>
<tr>
<td>Children 5-11 years</td>
<td>≥115</td>
<td>110-114</td>
</tr>
<tr>
<td>Children 12-14 years</td>
<td>≥120</td>
<td>110-119</td>
</tr>
<tr>
<td>Non-pregnant women (15 years and older)</td>
<td>≥120</td>
<td>110-119</td>
</tr>
<tr>
<td>Pregnant women</td>
<td>≥110</td>
<td>100-109</td>
</tr>
<tr>
<td>Men (15 years and older)</td>
<td>≥130</td>
<td>110-129</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Source: reference (54).
\textsuperscript{b}Haemoglobin in grams per litre.

Table 3.3. Classification of public health significance of anaemia at the population level based on estimated prevalence of low haemoglobin\textsuperscript{a}

<table>
<thead>
<tr>
<th>Category of public health significance</th>
<th>Prevalence of anaemia (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Severe</td>
<td>≥40</td>
</tr>
<tr>
<td>Moderate</td>
<td>20.0-39.9</td>
</tr>
<tr>
<td>Mild</td>
<td>5.0-19.9</td>
</tr>
<tr>
<td>Normal</td>
<td>≤4.9%</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Source: reference (54).
### Table 3.4. Haemoglobin adjustment for altitude and cigarette smoking

<table>
<thead>
<tr>
<th>Altitude (metres above sea level)</th>
<th>Adjustment to individual haemoglobin value (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1000</td>
<td>No adjustment</td>
</tr>
<tr>
<td>1000-1499</td>
<td>-2</td>
</tr>
<tr>
<td>1500-1999</td>
<td>-5</td>
</tr>
<tr>
<td>2000-2499</td>
<td>-8</td>
</tr>
<tr>
<td>2500-2999</td>
<td>-13</td>
</tr>
<tr>
<td>3000-3499</td>
<td>-19</td>
</tr>
<tr>
<td>3500-3999</td>
<td>-27</td>
</tr>
<tr>
<td>4000-4499</td>
<td>-35</td>
</tr>
<tr>
<td>&gt;4500</td>
<td>-45</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cigarettes smoked per day</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-smoker</td>
<td>0</td>
</tr>
<tr>
<td>Smoker (All)</td>
<td>-3</td>
</tr>
<tr>
<td>½-1 packet per day</td>
<td>-3</td>
</tr>
<tr>
<td>1-2 packets/day</td>
<td>-5</td>
</tr>
<tr>
<td>&gt;2 packets /day</td>
<td>-7</td>
</tr>
</tbody>
</table>

*SSource: reference (54).*

## Iron

Recommended indicators of iron status for determining iron deficiency include serum ferritin, serum soluble transferrin receptor, and total body iron (2, 59).

**Ferritin:** Serum ferritin is the most specific, non-invasive biochemical test to quantify total body iron stores. In the absence of inflammation, the concentration of serum ferritin is positively correlated with the size of the total body iron stores, with a low serum ferritin concentration reflecting depleted iron stores and therefore iron deficiency (60). However, serum ferritin is an APP and is elevated in response to infectious or inflammatory processes. In population-based surveys, this may artificially lower the prevalence of iron deficiency, thus it is recommended that serum ferritin be assessed along with measures of inflammation (e.g. CRP and/or AGP).

There are several methods to account for the increase in ferritin values caused by inflammation. One method is to adjust serum ferritin concentrations based on CRP and AGP using regression or arithmetic correction factors, such as the BRINDA (61) or Thurnham methods (29), respectively. When using these correction approaches to adjust ferritin concentrations, you can apply the cutoff values recommended for apparently healthy populations. Another method is to apply a higher serum ferritin cutoff value that defines deficiency, 30 μg/L or 70 μg/L, depending on the age group, to individuals with infection or inflammation (60).

*Specimen collection and management:* Most commonly, ferritin is measured in serum or plasma samples that are obtained by centrifugation from whole blood collected by venipuncture or finger prick. The whole blood needs to be refrigerated as soon as possible and processed to serum or plasma within 48 hours of blood collection. Ferritin in serum is stable for at least one week at 4°C and for at least one year at -20°C (16).
**Biomarker analysis:** Ferritin is measured using immunoassays, including methods that can be conducted either with a fully-automated clinical analyser or a manually executed ELISA assay. Commercial assay kits are available for both types of assay. There is no significant difference in within-run imprecision, between-run imprecision, limit of detection, recovery rate or linearity between commercial and home-made assays, or between automated multiple-analytes detection equipment and single laboratory apparatus, showing, overall, that the most common methods used for ferritin determinations are comparable (62).

The required analysis volume is typically <25 μL serum, however a minimum specimen volume of >150 μL serum may be needed to fill the sample cup for the clinical analyser. The product sheet for the intended assay will specify the specimen matrix requirements and should be consulted before deciding on the method and ordering survey supplies. Serum is the preferred matrix, since not all assays can use EDTA or heparin plasma. Sandwich ELISAs are also available to measure serum ferritin along with other indicators, including those assessing other iron indicators and vitamin A and inflammation status (63).

Quality control measures are required to ensure high quality results (64). Commercial assay kits include calibration materials and may also include quality control materials. It is nonetheless recommended to establish “in-house” quality control materials that can be tracked over a longer period to verify that the method did not shift over time. The method imprecision is typically 5–10% for clinical analyser assays and around 10% for ELISA assays. Ferritin is also part of the CDC VITAL-EQA program and of CDC’s Performance Verification Program for Serum Micronutrients (22).

A WHO-developed serum-based international standard (recombinant ferritin RM 94/572) (65), used to verify method accuracy, is available through the National Institute of Biological Standards and Control (NIBSC). However, not every assay may be able to use this material because the assay performance may differ between native patient samples and reference materials that have undergone some processing. Quality control materials for serum micronutrients including ferritin are available from CDC to support in-house quality assurance programs for laboratories engaged in public health work (23). Moderate assay differences (e.g. 2.0–5.0% variability depending on the assay) are common in proficiency testing programmes, such as the US College of American Pathologists (CAP) Chemistry Survey (66).

**Approximate budget requirements for analysis:** Instrumentation needed for this method includes either a clinical analyser (approximately US$ 100 000) or a plate-washer, plate-reader, and various pipettes (approximately US$ 30 000). The cost for materials and supplies is around US$ 2–5 per sample for a commercial kit assay. Material costs may be slightly lower for laboratory-developed ELISA assays that measure serum ferritin in addition to other micronutrients.

**Interpretation of results:** The classification of iron stores based on serum ferritin concentrations by the presence or absence of inflammation are presented in Table 3.5. The generally accepted serum ferritin cutoff value for defining depleted iron stores is <15 μg/L for children over 5 years of age, adolescents, adults and women in the first trimester of pregnancy, while a cutoff value of <12 μg/L is used for children under 5 years of age (60). These cutoffs are appropriate when a regression or arithmetic correction approach has been applied to the ferritin concentrations to account for inflammation. If no mathematical correction for inflammation is applied to the ferritin concentrations, then the higher concentrations may be used to define deficiency (e.g., <30 μg/L or <70 μg/L).
Module 3. Biomarker selection and specimen handling

Thresholds for elevated serum ferritin to identify risk of iron overload should be used only in clinical care with additional indicators and evaluation to establish the underlying cause.

Table 3.5. Recommended cutoffs to define iron deficiency in apparently healthy and non-healthy individuals by age group

<table>
<thead>
<tr>
<th>Serum ferritin (μg/L)(^b)</th>
<th>Apparently healthy individuals(^c)</th>
<th>Individuals with infection or inflammation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infants and young children 0-4 years of age</td>
<td>&lt;12</td>
<td>&lt;30</td>
</tr>
<tr>
<td>Children and adolescents 5-19 years of age</td>
<td>&lt;15</td>
<td>&lt;70</td>
</tr>
<tr>
<td>Adults ≥20 years</td>
<td>&lt;15</td>
<td>&lt;70</td>
</tr>
<tr>
<td>Pregnant women first trimester(^d)</td>
<td>&lt;15</td>
<td>&lt;70</td>
</tr>
</tbody>
</table>

\(^a\) Source: reference (60).
\(^b\) Markers of inflammation should be assessed along with the ferritin concentration, and ferritin adjusted as necessary.
\(^c\) For the purposes of this guideline, an apparently healthy individual is defined as an individual with physical well-being for their age and physiological status, without detectable diseases or infirmities.
\(^d\) There are several physiological changes occurring in pregnancy that may contribute to the variation in thresholds of iron deficiency in pregnancy as defined by serum ferritin, including a physiological rise in acute phase proteins secondary to pregnancy; second trimester plasma volume expansion; and changes in inflammatory measures in the final trimester of pregnancy.

Transferrin receptor: Transferrin receptor is found on the cell membrane and allows iron-bound transferrin to enter the cell. When the iron supply is inadequate, the number of transferrin receptors on a cell surface increases. This enables the cell to compete more effectively for iron. The number of membrane receptors is in proportion to the soluble transferrin receptor (sTfR), a truncated form of transferrin receptor found in plasma. An increase in sTfR levels is seen in patients with iron deficient erythropoiesis or iron deficiency anaemia with increased erythropoiesis (67).

Because sTfR is not an acute phase protein, it may be less influenced by inflammation than ferritin. Other advantages of sTfR are that cutoff values do not vary with age or gender, or by physiologic state. However, the BRINDA project found that the relationship between sTfR and AGP was consistently significant and therefore recommended adjusting sTfR for AGP and malaria (68). The BRINDA project reported that the relationship between sTfR and CRP was most often not statistically significant. The decision to include malaria in the BRINDA adjustment was based on the physiologic response of sTfR during infection. Circulating sTfR levels may be elevated when there is increased red blood cell production or turnover or both, such as in the case of haemolytic anaemia (67).

Specimen collection and management: Most commonly, sTfR is measured in serum samples that are obtained by centrifugation from whole blood collected by venipuncture or capillary sample. Whole blood should be refrigerated as soon as possible and processed within a few days of blood collection. The serum is stable for at least one week at 4°C, and for at least one year at -20°C (16).
**Biomarker analysis:** sTfR is measured by immunoassays (clinical analyser or ELISA assay), most often using commercial assay kits. The required analysis volume is typically <25 µL serum, however a minimum specimen volume of >150 µL serum may be needed to fill the sample cup for the clinical analyser. The product sheet for the intended assay will specify the specimen matrix requirements and should be consulted before deciding on the method and ordering survey supplies. Serum is the preferred matrix, since not all assays can utilize EDTA or heparin plasma. Sandwich ELISAs are also available to measure sTfR along with other indicators, including those assessing other iron indicators, vitamin A and inflammation status (63). The method imprecision is usually around 10%.

A WHO-developed serum-based reference reagent (recombinant sTfR RR 07/202) is available through the NIBSC. This reagent has an assigned value based on protein content because assays have not yet been standardized and assay results are not comparable. The US College of American Pathologists proficiency testing offers a performance programme for laboratories performing sTfR measurements. CDC’s Performance Verification Program for Serum Micronutrients (22) covers sTfR and CDC also offers quality control materials for serum sTfR to support in-house quality assurance programmes for laboratories engaged in public health work (23).

**Approximate budget requirements for analysis:** sTfR is measured on the same instrumentation as serum ferritin, however the cost for materials and supplies is higher (approximately US$ 5–10 per sample for a commercial kit assay). Material costs may be slightly lower for in-house developed ELISA assays that measure serum sTfR in addition to other micronutrients.

**Interpretation of results:** Assay-specific normal ranges for sTfR are available (for example, 2.9–8.3 mg/L for one brand of ELISA), however, there is no universally agreed normal range for sTfR. Similarly, there are no WHO definitions of public health problems based on sTfR prevalence. Using data generated with the Roche sTfR assay for the United States population in the National Health and Examination Survey (NHANES), cutoff values indicating iron deficiency were proposed as ≥6.0 mg/L for children 1-5 years and ≥5.3 mg/L for non-pregnant women 15-49 years (69).

**Body iron index:** The body iron index provides a quantitative assessment of body iron stores (index value >0 mg/kg) and indicates the size of the functional iron deficit. The functional deficit can be described as the amount of iron needed before it can be accumulated in the body’s stores, in an individual who is iron deficient (index value ≤0 mg/kg). The index is not a measure of total iron in the body. Previous terms used to describe this measure include “body iron”, “total body iron”, and “total body iron stores”.

In a controlled phlebotomy study, the sTfR only increased once the iron stores (measured by serum ferritin) were completely exhausted (70). When serum ferritin fell below 12 µg/L, the sTfR began to rise, roughly in proportion to the deficit in functional iron. This indicates that sTfR measures the deficit in tissue iron once stores are depleted. The combination of serum ferritin and sTfR levels can portray the spectrum of iron status from normal to severe deficiency. The formula to calculate the body iron index using ferritin and sTfR values (adjusted for measurement using the Ramco ELISA assay) in µg/L is as follows (71):

\[
\text{Body iron index (mg/kg)} = -\left[\log(\text{Ramco sTfR/ferritin}) - 2.8229\right]/0.1207
\]

If the sTfR has been measured by the Roche assay instead of the Ramco ELISA assay, the relationship of that assay to the Ramco ELISA assay needs to be taken into consideration. The adjustment equation is as follows (72):

\[
\text{Adjusted Body iron index (mg/kg)} = -\left[\log(\text{Roche sTfR/ferritin}) - 2.8229\right]/0.1207
\]
Iron deficiency anaemia

Individuals with iron deficiency anaemia are anaemic (based on low haemoglobin) and iron deficient (based on one of the recommended indicators of iron status). Fig. 3.1 shows two examples of the relationship between iron deficiency, iron deficiency anaemia and anaemia in populations with the same prevalence of anaemia. The level of overlap between these three conditions will vary between populations, and within a given population. In setting A, the prevalence of iron deficiency in the population is high, as is the prevalence of iron deficiency anaemia, while non-iron deficiency causes of anaemia are infrequent. In setting B, the prevalence of iron deficiency and iron deficiency anaemia are lower than in setting A, indicating that other factors, such as malaria, hookworm, and/or genetic blood disorders (for example, haemoglobinopathies) may be the main causes of anaemia.

Fig. 3.1. Visual representation of the overlap between iron deficiency, iron deficiency anaemia and anaemia

Adapted from: reference (73).

Iodine

Iodine is necessary for proper foetal brain development, and there is mixed evidence that high levels of iodine may affect thyroid function (74). The most usual indicator of population iodine status is the median urinary iodine concentration (UIC) of the population.

There are clinical and other biological indicators of iodine status, including goitre and thyroid volume by ultrasonography, however these are not currently recommended for large national cross-sectional surveys. Assessment of goitre is not recommended either, because it is a more subjective method, especially if conducted by palpation. More importantly, thyroid size responds very slowly after the introduction of an intervention (such as iodized salt) to improve iodine intake, and does not provide a reliable indication of recent individual or population iodine intake (74).
Module 3. Biomarker selection and specimen handling

More recently, thyroglobulin has been proposed as a sensitive indicator of both low and excess iodine intake for use at the population level, following a U-shaped association with the urinary iodine concentration (UIC) in school-age children, pregnant women and infants. Due to high day-to-day variability, however, the utility of thyroglobulin as an individual biomarker of iodine status is uncertain (75).

**Urinary iodine concentration (UIC):** The recent iodine intake of an individual can be assessed by measuring urinary iodine excretion (UIE), the iodine level in a 24-hour collection of urine that mitigates diurnal variations in iodine excretion. However, it is not feasible to include 24-hour urine collections as part of a large cross-sectional survey, and thus it is not possible to estimate the iodine status of individual survey participants. With a sufficiently large number of single urine samples, the median UIC will represent the status of the entire sample population.

*Specimen collection and management:* Urine samples are normally collected at the household but depending on the survey they can be collected from a clinic or laboratory. Samples do not require refrigeration but are usually kept in cool boxes or refrigerated with other specimens until they are processed.

Urine samples are relatively easy to collect from older children and adults. Only a small amount of urine is required (approximately 1 mL) for duplicate laboratory testing of iodine content. Infants and very young children may have difficulty urinating on demand or into the collection cup.

*Biomarker analysis:* The urinary iodine ammonium persulfate method (76) is considered the “gold standard” assay for analysis of UIC. Reagents and calibration materials, including ammonium persulfate, arsenious acid, ceric ammonium sulphate solutions and sulfuric acid are necessary for the sample analysis. A sample of 250 µL is needed, but a minimum of 1 mL of urine should be collected for potential repeat analysis. It is recommended to use internal quality controls during each analytical run in order to assess the accuracy and precision of the results. CDC also offers the Ensuring the Quality of Urinary Iodine Procedures (EQUIP) programme for urinary iodine, available globally to all laboratories so that bias and imprecision of their method can be tested against the CDC method three times a year (77). The urinary iodine ammonium persulfate method is relatively simple to perform but requires special attention to prevent iodine contamination of the laboratory area and equipment. Although urinary iodine excretion can be expressed in relation to creatinine excretion (µg iodine/g creatinine), the ratio of urinary iodine to creatinine can be misleading (78). Therefore, WHO recommends reporting urinary iodine as µg/L (79).

*Approximate budget requirements for analysis:* Instrumentation needed for this method includes a spectrophotometer, laboratory glassware, regents, a vortex, a heating block with a timer, and various pipettes (costing approximately US$ 10 000). The cost for materials and supplies is approximately US$ 2 - 5 per sample.

*Interpretation of results:* Table 2.1.6 presents the median UIC that indicates iodine status among different population groups (79). It is important to note that only population-level assessments of iodine status are possible from the survey methodology of casual, spot urine sample collection. Iodine status estimates based on the methodology of casual spot urine sample collection cannot be used to classify individual status and should not be presented as a prevalence of deficiency or adequacy (74). The information provided in Table 3.6 is frequently misinterpreted to reflect the situation of individuals. The correct interpretation is that populations with a median urinary iodine <20 µg/L have
“severe” iodine deficiency, populations with a median urinary iodine 20-49 μg/L have “moderate” iodine deficiency, and populations with a median urinary iodine 20-49 μg/L have “mild” iodine deficiency.

Table 3.6. Epidemiologic criteria for assessing population-level iodine nutrition based on median urinary iodine concentrations in different population groups

<table>
<thead>
<tr>
<th>Median urinary iodine concentration (µg/L)</th>
<th>Indication of population iodine intake</th>
<th>Indication of population iodine status</th>
</tr>
</thead>
<tbody>
<tr>
<td>School-age children (6-12 years of age)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;20</td>
<td>Insufficient</td>
<td>Severe iodine deficiency</td>
</tr>
<tr>
<td>20-49</td>
<td>Insufficient</td>
<td>Moderate iodine deficiency</td>
</tr>
<tr>
<td>50-99</td>
<td>Insufficient</td>
<td>Mild iodine deficiency</td>
</tr>
<tr>
<td>100-199a</td>
<td>Adequate</td>
<td>Adequate iodine nutrition</td>
</tr>
<tr>
<td>200-299</td>
<td>Above requirements</td>
<td>May pose a slight risk of more than adequate iodine intake in these populations</td>
</tr>
<tr>
<td>≥300</td>
<td>Excessivec</td>
<td>Risk of adverse health consequences (iodine-induced hyperthyroidism, autoimmune thyroid disease)</td>
</tr>
<tr>
<td>Pregnant women</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;150</td>
<td>Insufficient</td>
<td></td>
</tr>
<tr>
<td>150-249</td>
<td>Adequate</td>
<td></td>
</tr>
<tr>
<td>250-499</td>
<td>Above requirements</td>
<td></td>
</tr>
<tr>
<td>≥500</td>
<td>Excessivec</td>
<td></td>
</tr>
<tr>
<td>Lactating women and children under 2 years of age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;100</td>
<td>Insufficient</td>
<td></td>
</tr>
<tr>
<td>≥100</td>
<td>Adequate</td>
<td></td>
</tr>
</tbody>
</table>

a Source: reference (79).
b A UIC range of 100-299 µg/L has been proposed to indicate optimal iodine status among school age children (74, 80).
c The term “excessive” means in excess of the amount required to prevent and control iodine deficiency.

Vitamin B12

Vitamin B12 is found in animal source foods. A chronic dietary deficiency of vitamin B12 contributes to failure to thrive in infants and to neurologic disorders among all age groups. It is one nutrient deficiency that causes macrocytic anaemia. Although strict dietary deficiencies are rare among populations consuming a Western diet, some population groups consume minimal or no animal source foods due to abject poverty, to religion or to other customs (81, 82). More common amongst the elderly, vitamin B12 deficiency may also result from inability to absorb vitamin B12 based on an underlying disorder of the stomach or intestine, such as hypertrophy of the intestines, reduced gastric acidity, lack of intrinsic factor, or an interference with medications. The autoimmune condition known as pernicious anaemia, most commonly experienced by elderly populations, is a rare but important disease that inhibits absorption of vitamin B12 and will result in deficiency if untreated (1).

The most commonly used biomarker to assess vitamin B12 status in population-based surveys is serum vitamin B12 (total cobalamin) (1).
Specimen collection and management: Serum samples are obtained by centrifugation from whole blood collected by venipuncture. The whole blood needs to be protected from light and processed to serum within a few days of blood collection. Serum vitamin B12 is stable for at least one week at 4°C, and for at least one year at -20°C (16).

Biomarker analysis: Serum vitamin B12 is commonly measured via a competitive protein-binding assay on a fully-automated clinical analyser using commercial assay kits. The required analysis volume is typically around 25 µL, however a minimum specimen volume of 150 µL may be needed to fill the sample cup for the clinical analyser. The product sheet for the intended assay will specify the specimen matrix requirements and should be consulted before deciding on the method and ordering survey supplies. Serum is the preferred matrix, since not all assays can utilize EDTA or heparin plasma. Appropriate quality control measures must be followed to ensure high quality results. The assay kits include calibration materials, and in many cases they also include quality control materials. It is nonetheless recommended to establish “in-house” quality control materials that can be tracked over a longer period to verify that the method did not shift over time. The method imprecision is typically 5–10%. A WHO developed serum-based international standard (IS 03/178) is available through the NIBSC to verify method accuracy. However, not every assay may be able to use this material because the assay performance may differ between native patient samples and reference materials that have undergone some processing. Moderate assay differences can be observed in proficiency testing programmes, such as the US College of American Pathologists (CAP) Ligand Survey (66). CDC's Performance Verification Program for Serum Micronutrients (22) covers serum vitamin B12 and CDC offers quality control materials for this analyte to support in-house quality assurance programmes for laboratories engaged in public health work (23).

Approximate budget requirements for analysis: The cost for a clinical analyser can vary widely but is usually around US$ 100,000. The cost for materials and supplies is approximately US$ 3–5 per sample.

Interpretation of results: To estimate vitamin B12 deficiency at the individual level, the WHO recommended cutoff value is 203 pg/mL (150 pmol/L) (83). This is the point where the slope of the relationship between serum vitamin B12 and methylmalonic acid changes and where serum methylmalonic acid concentrations rise steeply in response to decreasing serum vitamin B12 concentrations. This is nearly identical to the clinically derived cutoff value of 200 pg/mL (148 pmol/L), below which there are often metabolic abnormalities present. Serum vitamin B12 concentrations between 200 and 300 pg/mL are frequently characterized as “subclinical” deficiency, or at risk of deficiency (depletion), but it is less clear whether these concentrations have a negative health impact (81, 83).

Folate

Folate is a generic term for several forms of folate with vitamin activity. Serum and red blood cell folate are key indicators used to assess folate status. Folate deficiency causes macrocytic anaemia, and low folate status among women increases the risk of bearing a child with neural tube defects. It is useful to consult the 2016 Biomarkers of Nutrition for Development (BOND) review for folate (5) when considering indicators for folate assessment and analytic methods.

Because folates are sensitive to temperature, oxygen, and light, special consideration must be given to sample collection, processing, storage, and shipment.
Red blood cell (RBC) folate: RBC folate indicates long-term folate status and is well correlated with liver folate stores. It is not affected by fasting and has traditionally been used to assess folate status in a population, both in terms of folate deficiency (risk of megaloblastic anaemia) and folate insufficiency (among women of reproductive age, risk for neural tube defects in the baby) (84).

Specimen collection and management: Whole blood specimens need to be protected from light and from elevated temperatures to avoid folate losses (16) and must be stored in a refrigerator or a cold box with ice packs. It is best to process them on the day of collection, and they should be processed within 48 hours after collection.

A whole blood haemolysate needs to be prepared. For this, exactly 100 µL of carefully mixed EDTA whole blood (blood collection tube inverted 8–10 times) is added to a vial containing 1 mL of ascorbic acid solution (1% weight/volume). The vial with the haemolysate is mixed well and stored immediately in a -20°C freezer, for a maximum of one month, and shipped on dry ice to a laboratory for analysis. For storage beyond one month, the haemolysate must be stored at -70°C.

If dried blood spots are generated in the field, the cards need to be completely dry prior to storing them in re-sealable plastic bags with desiccant sachets. Cards can be kept refrigerated for a maximum of one week; for a longer period, they need to be frozen at -20°C or colder to avoid folate losses (85).

Biomarker analysis: RBC folate concentration can be measured using a variety of assays, including microbiologic assay, protein-binding assay, and high-performance liquid chromatography coupled to tandem mass spectrometry. There are also various methods within these assay types. WHO recommends the microbiologic assay because it requires the fewest resources to generate accurate results (84). WHO thresholds for RBC folate are specific to the microbiologic assay and may not apply to other methods (86–88). The microbiologic assay measures “total” folate and does not distinguish among various folate forms. Protein-binding assays also measure “total” folate, although several manufacturers have stopped marketing clinical RBC folate assays due to questions of assay validity and comparability, combined with decreasing demand. Chromatography-based assays differentiate individual folate forms, but it is a highly resource intensive approach that is not suited for laboratories with limited capacity and infrastructure.

The volume required for analysis is largely dependent on the assay used. While the microbiologic assay only needs about 25 µL per test, the other techniques mentioned generally need >100 µL per test. To allow for potential repeat analysis, the microbiologic assay also requires a minimum specimen of ≥100 µL.

Haematocrit or haemoglobin values and serum folate values (if available) can be used to calculate the RBC folate value using the following formula, where for example, a haematocrit of 40% would be entered into the equation as 0.4:

\[
\text{RBC folate} = \frac{\text{[whole blood folate} - \text{serum folate (1} - \text{haematocrit)]}}{\text{haematocrit}}
\]

However, RBC folate values can be calculated if only the haematocrit or haemoglobin values are available by ignoring the serum folate contribution. This approach leads to slightly overestimated RBC folate values (89).
Dried blood spots can be used instead of whole blood haemolysates for the microbiologic assay, but generally not for the other mentioned techniques. Using the microbiologic assay, folate and haemoglobin are measured in the extract of the dried blood spot, and a haemoglobin-folate value is calculated (nmol folate per g haemoglobin). This value can be converted to RBC folate by multiplying haemoglobin-folate by the mean corpuscular haemoglobin concentration (MCHC, or g haemoglobin per L whole blood). An average MCHC value of 345 g/L can be used instead of individual MCHC values (85).

It is recommended to establish “in-house” whole blood haemolysate quality control materials that can be tracked over a longer period to verify that the method did not shift over time. The method imprecision for the microbiologic assay is around 10%, while for clinical analyser assays it is 5–10%. A lyophilized whole blood reference material is available from NIBSC (IS 95/528) with an assigned consensus value from multiple assays (65). Large assay differences that can exceed 100% can be observed in proficiency testing programmes, such as the CAP Ligand Survey (66) and the UK NEQAS programme (90). CDC’s Performance Verification Program for Serum Micronutrients (22) covers folate microbiologic assays and CDC also offers quality control materials to support in-house quality assurance programmes for laboratories engaged in public health work (23).

**Approximate budget requirements for analysis:** Instrumentation needed for the microbiologic assay includes a plate reader, an incubator, and various pipettes and small equipment (approximately US$ 40 000). Sample dilution and pipetting can be automated to increase sample throughput and reduce laboratory errors, however this requires additional resources (around US$ 50 000). The cost for materials and supplies is approximately US$ 2 per sample. The instrumentation cost for a clinical analyser can vary widely but is typically around US$ 100 000. The cost for commercial kits is approximately US$ 2–5 per sample.

**Interpretation of results** for RBC folate is described below in the section on serum folate.

Serum folate: Serum folate represents recent folate intake. The serum folate status of an individual can only be interpreted when the specimen has been collected in a fasted state. However, population status can be interpreted from non-fasted samples because on average serum folate concentrations are only about 10% higher in non-fasted compared to fasted individuals (91).

**Specimen collection and management:** For serum folate, blood is collected in a blood collection tube without an anticoagulant. To avoid folate losses, the whole blood needs to be protected from light and from elevated temperature, thus stored in a refrigerator or in a cold box with ice packs. Storage of unprocessed whole blood at room temperature is unacceptable (16, 92). It is best to process them on the day of collection, and they should be processed within 48 hours after collection.

Samples are centrifuged, and the serum is stored frozen until shipped on dry ice to a laboratory for analysis. Serum folate is stable for maximum one week at 4°C and maximum one month at -20°C, but for long-term storage the sample needs to be at -70°C (22). While serum is the preferred matrix, EDTA or heparin plasma can usually be used, however folate in EDTA plasma is particularly sensitive to oxidative losses at ambient or elevated temperatures. After the whole blood haemolysate is generated, the EDTA whole blood collection tube can be centrifuged to collect the plasma, if needed, which should be stored under the same conditions as specified for serum.
Biomarker analysis: The same assays used for RBC folate are employed to measure serum folate concentrations. Similar to RBC folate, WHO recommends the microbiologic assay for serum folate. Because serum folate concentrations are about 20 times lower than RBC folate concentrations, the sample dilution factor for serum is lower than for whole blood haemolysates. Serum and whole blood haemolysate specimens must be carefully diluted using calibrated pipettes to ensure accurate results (93).

A sufficient quantity of “In-house” serum quality control materials is needed to track assay performance over a longer time period than would be done with commercial materials. Serum-based reference materials are available from NIST (SRM 1955 and SRM 1950) and from NIBSC (IS 03/178) (65). However, none of these materials has certified values for total folate, which is what the microbiologic assay measures and is considered the gold standard method. Assay differences for serum folate are somewhat smaller than for RBC folate, but they can still be in the range of 30–50%, as can be observed in proficiency testing programmes such as the CAP Ligand Survey (66). CDC’s Performance Verification Program for Serum Micronutrients (22) and quality control materials for folate support in-house quality assurance programmes for laboratories engaged in public health work (23).

Approximate budget requirements for analysis: The same resources and instrumentation described in the section on RBC folate are needed for the measurement of serum folate, and costs are similar.

Interpretation of results: The cutoff values for folate status in all age groups, using macrocytic anaemia as a haematological indicator, are presented in Table 3.7. Additional cutoff values for folate deficiency, based on rising homocysteine concentration as a metabolic indicator, of <14 nmol/L for serum folate and <624 nmol/L for RBC folate can be used with data produced from the microbiologic assay calibrated with 5-methyltetrahydrofolate, the main form of folate found in serum and RBCs. This data would serve to determine metabolic risk (elevated homocysteine) (83). Note that these values are not listed in the table. Table 3.8 shows the red blood cell folate cutoff values defined for the prevention of neural tube defect-affected pregnancies in women of reproductive age (88). These values are at the population level. WHO recommends the relevant mean population cutoff value as RBC folate <400 ng/mL or <906 nmol/L (88). The recommended value was derived from epidemiologic data produced using a microbiologic assay calibrated with folic acid. If instead data are produced with a microbiologic assay calibrated with 5-methyltetrahydrofolate, a cutoff value of <748 nmol/L should be used (86). These thresholds should not be used at an individual level for determining risk of a neural tube defect-affected pregnancy, and there is no individual threshold to recommend (88).

Table 3.7. Folate concentrations in serum and red blood cells for determining individual-level folate status in all age groups, using macrocytic anaemia as the haematological indicatora

<table>
<thead>
<tr>
<th>Serum/plasma folate level</th>
<th>Red blood cell folate level</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>ng/mL (nmol/L)b,c</td>
<td>ng/mL (nmol/L)b,c</td>
<td></td>
</tr>
<tr>
<td>&gt;20 (&gt;45.3)</td>
<td></td>
<td>Elevated</td>
</tr>
<tr>
<td>6-20 (13.5-45.3)</td>
<td></td>
<td>Normal range</td>
</tr>
<tr>
<td>3-5.9 (6.8-13.4)</td>
<td></td>
<td>Possible deficiency</td>
</tr>
<tr>
<td>&lt;3 (&lt;6.8)</td>
<td>&lt;100 (&lt;226.5)</td>
<td>Deficiency</td>
</tr>
</tbody>
</table>

a Source: reference (84).
b Folic acid conversion factor: 1 ng/mL = 2.265 nmol/L.
c Assayed by Lactobacillus casei via microbiologic assay.
Table 3.8. Mean RBC folate concentrations in red blood cells for preventing neural tube defect-affected pregnancies in women of reproductive age at the population levela

<table>
<thead>
<tr>
<th>Red blood cell (RBC) folate level, ng/mL (nmol/L)b,c</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt; 400 (&gt;906)</td>
<td>Folate sufficiency</td>
</tr>
<tr>
<td>≤ 400 (≤906)</td>
<td>Folate insufficiency</td>
</tr>
</tbody>
</table>

a These thresholds should not be used at an individual level for determining the risk of a neural tube defect-affected pregnancy. These cutoff values are only appropriate when the microbiologic assay calibrated with folic acid is used to determine red blood cell folate concentration. Source: reference (84).

b Folic acid conversion factor: 1 ng/mL = 2.265 nmol/L.

c No individual level threshold is recommended for the prevention of neural tube defects in women of reproductive age.

Vitamin D

Vitamin D is produced endogenously when ultraviolet-B (UVB) rays from sunlight strike the skin and trigger vitamin D synthesis. It can also be obtained from the diet in the form of vitamin D3, cholecalciferol, from animal sources (e.g., fatty fishes such as salmon, tuna and mackerel, fish liver oils, beef liver, cheese and egg yolks) or vitamin D2, ergocalciferol, in mushrooms irradiated with UVB light, vitamin D-fortified foods (dairy products, oils, margarine and spreads, and some breakfast cereals) and vitamin D-containing supplements (94, 95). Vitamins D2 and D3 are similar compounds except for the structure of their side chains. The conversion of vitamins D2 and D3 into active compounds requires a two-step enzymatic hydroxylation process, although they have different conversion efficacy (96). A meta-analysis indicates supplementation with vitamin D3 had a significant and positive effect in raising serum 1,25-dihydroxyvitamin D (1,25(OH)2D) concentrations, the physiologically active form also known as calcitriol, compared to supplementation with vitamin D2 (P = 0.001) (97). However, vitamin D2 is considered an active substance and is not excluded as a source of dietary vitamin D.

The most widely accepted and used indicator of vitamin D status is plasma or serum 25-hydroxyvitamin D (25(OH)D), which is reflective of exposure to vitamin D from both cutaneous synthesis and dietary intake from food and supplements (98). However, there is no international consensus about the blood concentration associated with optimal status in different population groups (99) and WHO has not yet issued guidance. The United States’ Institute of Medicine (IOM) of the National Academies (now referred to as the Health and Medicine Division of the National Academies of Sciences, Engineering, and Medicine [the National Academies] established vitamin D recommended nutrient requirements for populations based on preventing serum 25(OH)D concentrations below 30 nmol/L for musculoskeletal outcomes (100). The Endocrine Society, a global organization representing professionals from the field of endocrinology, defines vitamin D deficiency as 25(OH)D concentrations below 50 nmol/L (20 ng/mL) and vitamin D insufficiency as 52.0–72.5 nmol/L (21–29 ng/mL), based on multiple health outcomes, including but not limited to musculoskeletal outcomes (101). The European Food Safety Authority (EFSA) has suggested similar cutoff values (102).

*Specimen collection and management:* 25-hydroxyvitamin D is usually measured in plasma or serum specimens obtained by centrifugation of whole blood collected by venipuncture. 25-hydroxyvitamin D is very stable, so whole blood processing can be delayed for up to two days. Serum is stable for at least two weeks at 4°C and for at least one year at -20°C (16).
Biomarker analysis: Serum 25-hydroxyvitamin D is commonly measured by a competitive protein-binding assay on a fully automated clinical analyser using commercial assay kits, or by using high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS). The latter approach is resource intensive and not suited for laboratories with limited capacity and infrastructure. The required analysis volume is typically >100 µL, although a minimum specimen volume of >300 µL is needed for repeat analysis. The product sheet for the intended assay will specify the specimen matrix requirements and should be consulted before deciding on the method and ordering survey supplies. Serum is the preferred matrix, since not all assays can utilize EDTA or heparin plasma. Appropriate quality control measures must be followed to ensure high quality results. The assay kits include calibration materials and often also include quality control materials. It is nonetheless recommended to establish “in-house” quality control materials that can be tracked over a longer period to verify that the method did not shift over time. The method imprecision is typically 5–10%.

Serum-based certified reference materials are available from NIST (SRM 972a) to verify method accuracy. However, not every assay may be able to use this material because the assay performance may differ between native patient samples and reference materials that have undergone some processing. Because of the variations in results between assays and between laboratories, efforts have been made improve assay standardization. The United States’ Office of Dietary Supplements of the National Institutes of Health established the Vitamin D Standardization Program to improve the standardization of 25(OH)D assays. Additionally, the United States Centers for Disease Control Vitamin D Standardization Certification Program (VDSCP) provides participating laboratories with one-time sets of 40 different reference materials for bias assessment and calibration, as well as 40 blinded samples per year with assigned values measured by a reference LC-MS/MS method for both 25(OH)D2 and 25(OH)D3, to certify analytical performance such as bias and imprecision (103). Over 20 laboratories and assay manufacturers are currently participating in the CDC programme (104). Additionally, the Vitamin D External Quality Assessment Scheme, from the Charing Cross Hospital, UK, provides participating laboratories with 20 samples per year that have reference values for 25(OH)D2 and 25(OH)D3, for assessment of bias and to allow for inter-assay and between-laboratory comparisons (105).

Approximate budget requirements for analysis: The cost for a clinical analyser can vary widely but is typically around US$ 100 000. The cost for materials and supplies is approximately US$ 10–20 per sample.

Interpretation of results: There are no WHO recommended cutoff values for defining risk of deficiency at the individual or the population level. The United States IOM of the National Academies identified serum 25(OH)D concentrations for determining vitamin D status at the individual level in all age groups (100) (see Table 3.9). These values are similar to those proposed by a group of experts for the context of skeletal mineralization and mineral ion metabolism for the prevention of nutritional rickets (106).
Table 3.9. 25-Hydroxyvitamin D concentrations in serum for determining individual level vitamin D status in all age groups

<table>
<thead>
<tr>
<th>Serum levels (nmol/L)</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;30</td>
<td>Risk of deficiency</td>
</tr>
<tr>
<td>30-&lt;50</td>
<td>Risk of insufficiency</td>
</tr>
<tr>
<td>50-75</td>
<td>Likelihood of sufficiency</td>
</tr>
<tr>
<td>&gt;75-125</td>
<td>No increased benefit</td>
</tr>
<tr>
<td>&gt;125</td>
<td>Risk of excess</td>
</tr>
</tbody>
</table>

* Source: reference (100).

**Zinc**

Serum zinc is the key indicator for assessing zinc deficiency among populations. Serum zinc reflects dietary zinc intake and responds consistently to zinc supplementation. Reference data are available for most age and sex groups. Assessing serum zinc allows the identification of specific populations and subgroups who have an elevated risk of zinc deficiency (107). Serum zinc cannot be used in the diagnosis and treatment of individuals who are zinc deficient because serum zinc concentrations are not a direct reflection of the individual’s zinc status (108). However, serum zinc can provide an assessment of the magnitude of the risk of zinc deficiency within a specific population or subgroup (107).

There are special considerations when measuring serum zinc concentration. Recent meals, the time of day, the age and sex of the participant, the use of hormonal contraception, inflammation and systemic infections all can have a direct impact on serum zinc concentration (108). The International Zinc Nutrition Consultative Group (IZiNCG) suggests to collect information on inflammatory proteins when assessing zinc, and if there is a significant negative correlation between either inflammatory protein and serum zinc to adjust serum zinc concentration statistically (109).

Zinc concentrations in hair, nail and urine have been used in some studies to assess a population’s exposure to zinc; however they are currently not recommended as a single indicator in the assessment of zinc status (4, 110).

**Specimen collection and management:** There are special considerations when collecting serum specimens to assess zinc deficiency. Specimens can be easily contaminated by ambient sources of zinc during collection, processing and analysis. To reduce the risk of ambient zinc contamination, the blood specimen must be taken directly from the vein using zinc-free needles and trace-metal-free vacuum blood collection tubes. When processing the samples, zinc-free sterile storage vials and transfer pipettes or pipette tips must be used to reduce the risk of contamination. If possible, specimens should be collected from survey participants during a pre-agreed time of day, and the participants should have been fasting for at least eight hours prior to collection. Fasting conditions are not always possible, so if non-fasting specimens are collected, an effort must be made to reduce variation by collecting samples from the entire survey population during the same time of day. It is important to record the time of the previous meal, especially for calculating non-fasting cutoff values and interpreting data. Once a blood specimen has been collected, it should be stored in a cold box or a refrigerator until centrifuged to collect the serum.
Ideally, all specimens should be centrifuged within 20 to 30 minutes after collection. If this is not possible, it is recommended that processing be done within 24 hours, provided that the cold chain is reliable and blood specimens can be kept refrigerated (2–10°C). Specimens should also be centrifuged in a controlled laboratory environment and serum should be separated into vials under a laboratory hood. In situations where this is not feasible, it is recommended that a tent be used so that a sealed laboratory can be configured. Plastic boxes can be used along with plexiglass or plastic wrap to develop a temporary field hood. All necessary information specific to zinc-free laboratory supplies and the construction of a hood from a plastic box is available from IZiNCG.

**Biomarker analysis:** Zinc concentration can be measured using several different analytical methodologies. The gold standard is inductively coupled plasma mass spectrometry (ICP-MS), however, this method is very expensive. Other acceptable analytical methods to measure zinc concentration include inductively coupled plasma atomic emission spectrometry, flame atomic absorption spectrometry (AAS), graphite furnace atomic absorption spectrometry, and neutron activation analysis.

**Approximate budget requirements for analysis:** The cost for instrumentation can vary widely depending on use of AAS or ICP-MS, but is typically around US$ 50 000–US$ 400 000. The cost for materials and supplies is approximately US$ 10–20 per sample.

**Interpretation of results:** Data on low serum zinc concentration should be compared with the appropriate reference data for age, sex, time of day and time since last meal. Reference data have been derived from NHANES II and represent individuals older than 2 years of age. The IZiNCG-recommended cutoff values for low serum zinc concentration are presented in Table 3.10. It is important to note that if 20% of the population has low serum zinc concentration there is a risk for zinc deficiency and a public health concern (108).

<table>
<thead>
<tr>
<th>Time of day and fasting status</th>
<th>Suggested lower cutoff values for low serum zinc concentration in µg/dL (µmol/L)(^b)</th>
<th>(\leq 10) years</th>
<th>(\geq 10) years</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males and females</td>
<td>Non-pregnant females</td>
<td>Males</td>
</tr>
<tr>
<td>Morning, fasting(^c)</td>
<td>not available</td>
<td>70 (10.7)</td>
<td>74 (11.3)</td>
</tr>
<tr>
<td>Morning, non-fasting</td>
<td>65 (9.9)</td>
<td>66 (10.1)</td>
<td>70 (10.7)</td>
</tr>
<tr>
<td>Afternoon, non-fasting</td>
<td>57 (8.7)</td>
<td>59 (8.6)</td>
<td>61 (9.3)</td>
</tr>
</tbody>
</table>

\(^a\) Source: reference (108).

\(^b\) Values converted to µmol/L by dividing µg/dL by 6.54.

\(^c\) Fasting is defined as no food or beverage consumption for at least 8 hours.
Overview of specimen collection containers, storage and transport conditions

*Table 3.11* presents a summary of the collection device requirements for different specimen types and main factors related to specimen storage and transport.
### Table 3.11. Overview of specimen collection containers, storage and transport conditions

<table>
<thead>
<tr>
<th>Biological matrix and collection method</th>
<th>Collection, testing and storage device/container</th>
<th>Specimen type</th>
<th>Biomarker(s)</th>
<th>Storage and transport considerations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capillary blood from a finger prick</td>
<td>Microcuvette for portable photometer (tested in field) Small blood collection tube with EDTA or heparin</td>
<td>Whole blood</td>
<td>Haemoglobin&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Test immediately, ensure that cuvettes are kept in dry and air tight container. Do not expose un-used cuvettes to the air. Test immediately or refrigerate specimen and test (Hb) or process specimen (RBC folate) within 1-2 days of blood collection. Refrigerate whole blood and generate plasma within 2-3 days of blood collection. Plasma stable for 1 week refrigerated and 1 year at -20°C. Ship frozen samples on dry ice.</td>
</tr>
<tr>
<td></td>
<td>Small blood collection tube without anticoagulant</td>
<td>Serum&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Ferritin, sTfR, RBP, AGP, CRP</td>
<td>Refrigerate whole blood and generate serum within 1 day of blood collection. Serum stable for 1 week refrigerated and 1 year at -20°C; ship frozen samples on dry ice.</td>
</tr>
<tr>
<td>Venous blood from venipuncture</td>
<td>Vacuum tube with EDTA</td>
<td>Whole blood</td>
<td>Haemoglobin&lt;sup&gt;a&lt;/sup&gt;, folate (RBC), B12</td>
<td>Refrigerate and protect whole blood from light and generate haemolysate within 1-2 days of blood collection. Haemolysate is stable for 1 month at -20°C; long-term storage at -70°C; ship frozen samples on dry ice.</td>
</tr>
<tr>
<td></td>
<td>Vacuum tube without anticoagulant and with a serum separator – trace metal free&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Serum (after centrifugation)</td>
<td>Ferritin, sTfR, RBP, AGP, CRP, retinol, MRDR</td>
<td>Refrigerate whole blood and generate plasma within 2-3 days of blood collection. Plasma stable for 1 week refrigerated and 1 year at -20°C. Ship frozen samples on dry ice.</td>
</tr>
<tr>
<td></td>
<td>Dried blood spot (RBC folate)</td>
<td>Dried blood spot (RBC folate)</td>
<td>Folate (RBC)</td>
<td>Refrigerate and protect whole blood from light and generate DBS within 1 day of blood collection. Dry DBS at ambient temperature; DBS stable for 2 days refrigerated and 1 year at -20°C. Long-term storage at -70°C. Ship frozen samples on dry ice.</td>
</tr>
<tr>
<td></td>
<td>Urine sample</td>
<td>Serum (after centrifugation)</td>
<td>Zinc, ferritin, sTfR, RBP, AGP, CRP, retinol, MRDR, folate (serum), vitamin B&lt;sub&gt;12&lt;/sub&gt; &amp; vitamin D (25OHD)</td>
<td>Refrigerate whole blood and generate serum within 1-2 days of blood collection. Serum stable for 1 week refrigerated and 1 year at -20°C. Ship frozen samples on dry ice.</td>
</tr>
</tbody>
</table>

**EDTA:** ethylenediaminetetraacetic acid; **sTfR:** soluble transferrin receptor; **RBC:** red blood cell; **RBP:** retinol binding protein; **AGP:** alpha-1-acid glycoprotein; **CRP:** C-reactive protein; **MRDR:** modified relative dose response.

<sup>a</sup> Whole blood can be collected into microcuvette directly from finger or heel prick or from whole blood collected in a small blood collection tube or vacuum tube.

<sup>b</sup> Trace metal free tubes needed for zinc assessment.
References


Module 3. Biomarker selection and specimen handling


Module 3. Biomarker selection and specimen handling

Module 4. Survey design

In this module, we will discuss:

- Types of sampling design
- National and subnational estimates, principles and purpose of stratification
- The survey approach: independent or combined with another
- Method of collecting data: paper-based or electronic
There are many types of survey design that can be used in different contexts. The design determines whether the outcomes of the survey will meet the agreed objectives. It also affects most aspects of a survey, including sample size calculations and data analysis. This module describes the more common types of household-based surveys used to assess indicators for the coverage of interventions, micronutrient status, and other relevant factors (for example, knowledge of a specific micronutrient deficiency and practices in relation to an intervention).

Certain aspects of the survey design will depend on whether the survey is intended as a single assessment of micronutrient status or intervention coverage, or whether it is part of a series of periodic assessments over time. A single survey may be conducted to assess the prevalence of micronutrient deficiencies and anaemia in one or more population groups to determine the need for an intervening or for policy and programme changes. The data may also be used for global reporting. A series of periodic assessments or an endline survey may be conducted to assess the status or effect of an intervention or programme compared to a baseline survey.

This module focuses on the most typical aspects of large-scale micronutrient survey design. For updates on basic sampling theory and terminology, it may be useful to review the document “Definitions of survey sampling terminology” prior to reading this module. Words in italics below are defined in Box 4.1.

### Types of sampling design

The most common sampling designs are:

1. **Simple random sampling (SRS):** The sample is randomly selected from a comprehensive listing of elements.
2. **Stratified survey with SRS:** Elements are divided into two or more mutually exclusive geographic areas (called strata, singular is stratum) and, within each stratum, a sample of elements is randomly selected.
3. **One-stage cluster survey:** Primary sampling units (PSUs) are selected from a sampling frame, and all elements within each PSU are sampled.
4. **Multi-stage cluster survey:** Primary sampling units (PSUs) are selected from a sampling frame, and a sample of elements is selected from within each PSU. Where the selected elements are households or schools, there may be a third level of selection, namely, of individuals (as elements) from within the households or schools.
5. **Stratified multi-stage cluster survey:** Elements are divided into two or more mutually exclusive geographic areas (strata) and, within each stratum, a two- or three-stage cluster survey selection of elements is implemented.

Population-based micronutrient surveys are often based on a multi-stage cluster design, usually stratified. In large-scale surveys, a multi-stage cluster design is a much more cost-effective method of data collection than simple random sampling (SRS). It also works well in situations where reliable nationwide household listings may not be available. The first stage of sampling, the selection of geographical units (primary sampling units, or PSUs), allows the development of lists of households, sometimes including a listing of individual household members, within the selected PSUs. In the second and third stages, samples of households are then randomly selected from those lists and, depending on the survey design, individuals may then be selected from within the identified households.
The survey design will affect how data analysis is done. More information on this can be found in Module 15: Data processing and analysis.

This manual focuses on multi-stage cluster and stratified multi-stage cluster designs. An SRS design may be relevant for assessing micronutrient status in relatively small, defined areas, for example, a refugee camp, therefore some information on sample size calculation using SRS is briefly included in Module 5: Sample size.

**Box 4.1. Definitions related to sampling design**

- **Sample**: the subset of elements drawn from the frame (the survey sample).
- **Element**: the basic unit that represents whatever is being sampled and from which survey data are to be gathered. For health-related surveys, this is frequently the individual or the household.
- **Primary sampling units (PSU)**: the sampling units that are selected in the first stage of a multi-stage sample. The PSUs may be an area with clearly defined, non-overlapping geographic boundaries, for example villages in rural areas and wards or blocks in urban areas.
- **Frame (sampling frame)**: a listing of PSUs, or of elements within a PSU, from which the sample is drawn.
- **Strata**: Groups into which elements are divided for stratified sampling (for example regions: North, South, Central).

**National and subnational estimates, principles and purpose of stratification**

Surveys designed to produce single representative national estimates of, for example, micronutrient status, anaemia, or intervention coverage provide useful information for reporting the national status and possibly for comparing that status to the results of a previous survey. However, national estimates are generally not useful in determining where a deficiency is most prevalent or where an intervention is performing poorly. A more common option is to design a survey that will produce representative estimates for two or more subnational areas, referred to as “strata.” A primary purpose of stratification is to improve the precision (reliability) of the survey estimates for areas of programmatic interest.

Stratification partitions the national listing of PSUs into mutually exclusive and collectively exhaustive subgroups. This means that there is no overlap between the populations of different strata, and the population of all strata combined represents the national population. Separate samples are then selected from each stratum to produce representative data for each selected subpopulation. This would show for example, differences in the prevalence of a specific micronutrient deficiency by province or by urban/rural location.

National estimates can be obtained by combining strata results after adjustment as appropriate. This is described in Module 15: Data processing and analysis.

Two important considerations in defining strata are:

- **Familiarity with where and how policy and programme decisions are made.** In countries with a decentralized administration, stratification should make it easier to provide data for decision-making at the appropriate subnational level. This may be less important for large scale food fortification which tends to be mandated at the national level.
• The expected status of the primary variable of interest for the survey. This should be as different as possible (heterogeneous) across the various strata. For example, if it is known that the status of the primary variable and any intervention is similar (relatively homogeneous) across two subregions, then these two subregions could be combined as one stratum. Note that this also depends on available information about the homogeneity of other variables of interest.

When a survey is being designed without recent data on the status of micronutrients or related interventions of interest, decisions about stratification could be based on geography, on other factors known to be associated with the primary variable, or on administrative areas where programme decisions are made.

Box 4.2 summarizes these points and additional factors to consider in the selection of strata.

Specifically defining the strata usually takes place during the survey design stage and is known as “explicit stratification.” Another type of stratification is “implicit stratification”. This relates to the order in which PSUs are listed prior to selection and is referred to briefly in Module 6: Selecting clusters.

The present module is based on considerations for the design of a national survey. However, the same principles apply where a survey aims to obtain information from a subnational area only. If micronutrient

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**Box 4.2. Main factors to consider when using stratified sampling design**

1. What is the basis of the stratification, and what characteristics should be used to subdivide the population into strata? In the case of micronutrient surveys, stratification could be based on:
   - Known or suspected differences in the etiology and magnitude of micronutrient deficiencies according to:
     - Residence type, for example urban and rural
     - Geographical features, such as inland and coastal, mountains and lowlands
     - Administrative factors, such as districts with smoothly functioning health care systems compared to districts subject to conflict or other disruptive factors.
   - Intervention-related issues, for example, if implementation of an intervention to improve micronutrient status is known to face specific challenges in geographically defined areas, then these areas should be considered as separate strata so that representative, programmatically useful data are obtained for each stratum.
   - Programme/policy decision-making administrative units.
   - Stratification used in previous surveys. If the planned survey is a follow-up, using the same stratification will allow a comparison of stratified data between surveys.

2. The number of strata to be constructed and the stratum boundaries to be used relate to the basis for stratification.

3. The number of observations to be collected in each stratum relates to the desired precision within each stratum and nationally. National estimates (made up of all the strata) will have the largest sample size and tightest precision compared to stratum-specific estimates.

4. The available budget: a separate survey sample needs to be drawn for each stratum. Therefore, total resource requirements are dictated by the number of strata and the selected precision for the main indicator estimates in the survey. Some strata may require a larger budget if there are challenges related to logistics, terrain access issues or insecurity.
status or other indicators of interest vary considerably within that area, then stratification along lines that divide the population of the area into groups with more similar status should be considered.

During the survey planning stage, a range of sample sizes with associated precision for the indicator estimates and expected budget are calculated. When combined with information on survey objectives and available resources, these can be used to guide decisions about design. You can find an abbreviated example of this process in Box 4.2, and a detailed description of the process in Module 5: Sample size.

The survey approach: independent or combined with another

Information on micronutrient status and on the implementation of relevant interventions may be collected either through an independent survey or as a component of another population-based survey. There are many other surveys to which a micronutrient module could be added. It is important to assess in the earliest stages of planning whether other surveys that cover the same population group or geographic area are being planned. If so, stakeholders should discuss whether it would be possible to expand the scope of the other survey(s) to include a micronutrient module and share resources. Some advantages and disadvantages of incorporating a micronutrient component into or linking with another planned survey are presented in Box 4.3.

To determine whether or not to integrate a micronutrient module with another survey, three important questions should be considered:

- Will the government and the organizers of the other survey agree to incorporate the micronutrient module?
- Will it be possible to collect data from a representative and large enough sample in the population groups of interest in the desired geographic areas to meet the objectives of the micronutrient survey? (Sample size is discussed in Module 5: Sample size).
- Will the integration save costs and reduce the burden on participants?

Gaining agreement and commitment from the team implementing another survey to incorporate a micronutrient module may be a significant challenge. There may be many concerns from the organizers, including that the timelines and survey design requirements might not align or that the added complication of collecting biological specimens might compromise the main survey.

If integrating into another planned survey is not an option, it may still be possible to use the sampling frame from a previously conducted survey. The advantage to this is that the first-stage sampling may have been performed and the maps and information to identify households and subjects may already be developed and available. One example of an integrated survey is the 2015-16 Malawi Micronutrient Survey, which was carried out jointly with the 2015-16 Malawi Demographic and Health Survey.¹

Method of collecting data: paper-based or electronic

The method of data collection affects many subsequent decisions and processes, including the budget, the timeline, hiring, training, logistics, equipment and supply needs, questionnaire development and the roles and responsibilities of data entry and management personnel. A decision on whether to collect data on paper or electronically needs to be made early on in the survey planning process.

Both systems of data collection have their security risks. These should be detailed and addressed in the protocol and the application for ethical approval. Risks include:

- Loss of information: Paper-based forms may be lost or damaged (during transport, due to theft or from fire/flood during transport or storage), while electronically completed forms could be lost due
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to a device malfunction or if the device is stolen prior to sharing with the supervisor and/or uploading to the selected storage-based system. In addition, electronically-collected data needs to be protected from possible accidental or deliberate deletion.

- Unauthorized access to information: Both paper-based and electronic forms require secure storage with authorized access only, whether this be a physical location for paper forms or a password-protected storage-based system (with a backup) for electronic data.

Electronic data collection: Electronic data collection is increasingly the default option. However, it is not always feasible or may not be the preferred option.

Electronic data collection has numerous advantages. A well-designed electronic data entry system greatly improves data quality, minimizes missing data, is more efficient to implement in the field, and saves on data entry and management time preparing for analyses. Inconsistencies in the interview can be prevented even before the data are entered into the device because the data entry programme can be designed to allow only the entry of unique values (for identification codes, for example), values within predetermined ranges, and responses that match with previously entered values. It can also automatically skip questions in accordance with previous responses. These features permit supervisors and team leaders in the field to focus on community relations and on supporting optimal enumerator and field technician performance, instead of investing time checking the quality of questionnaires as they are completed.

Uploading electronic data allows for checks on data frequency and quality to be shared with the Survey manager who will assess progress against data targets. The survey management team will be alerted to any unexpected results so that the associated cause can be explored, and ideally resolved. If needed, refresher training materials can be developed and delivered by survey coordinators or monitoring teams.

The availability and use of global positioning system (GPS) technology on mobile devices helps the survey management team ensure that the PSUs and households were visited in accordance to plan. GPS can also generate a map of all selected and visited PSUs for the survey report and archives. In addition, a GPS can provide data on altitude, which may be required for interpreting certain biomarkers, such as the haemoglobin levels for defining anaemia.

Some software can incorporate methods to assist with the sampling and selection of participants. System links are also usually set up to take photographs, for example, of a food brand or a salt iodine field test, and to scan household, individual, and sample identification barcodes into the questionnaire. Data will not be accepted if, for example, the individual part of the identifier does not match the household identification.

Budget and personnel resources required to design and set up a high-quality electronic data collection system can be substantial. Typically, electronic data collection requires technical expertise for programming and a longer lead time to develop and extensively test the electronic data entry platform. This expertise is also required during training, piloting and field work for troubleshooting.

It is also important to consider the capacity needed and available to handle hierarchical data (such as linking individuals with household data). This has previously been a weakness in some survey settings, however, working with a sampling statistician or data manager is advisable to appropriately manage hierarchical data.
Assessing national capacity for and feasibility of electronic data collection: The Technical committee should assess the preparedness and capacity of the survey team to undertake data collection using mobile devices, and verify the availability of equipment and network access. Factors to consider in the assessment are described in Box 4.4.

**Box 4.4. Factors to consider in assessing the feasibility of electronic data collection**

- **Budget availability for purchasing electronic devices and accessories:**
  - This should be weighed against the savings that will be made on paper, printing, computer access, and personnel time required for paper-based data collection and entry. Purchase of the devices could also be considered an investment because the equipment could be used again in subsequent surveys or for other purposes.
- **Availability of personnel with the specialized programming and data management skills necessary to set up and manage the system, or the ability of the Technical committee to work with an external individual or team who can provide these skills.**
- **Ease of locally obtaining spare devices, batteries, and SIM and memory cards.**
- **Any anticipated periods of time away from power supply. Enumerators would need to be able to recharge batteries regularly and the team should have spare batteries and/or devices for backup purposes.** Take into account:
  - The proportion of selected PSUs that are in remote areas and how long a team may spend there; and
  - Whether in such areas, teams will have access to a car where devices could be charged.
- **Infrastructure of the region, especially related to internet connections:**
  - There must be an expectation of reasonably frequent access to internet connections to upload data. Another option is the ability to purchase mobile wireless internet devices.
  - Cost of internet connectivity if wireless internet devices for the survey must be purchased.
- **Security of the team:**
  - How safe do enumerators feel carrying the tablets/phones in all survey locations?
  - Where security is a concern in only a small number of PSUs, paper-based forms could be used instead of electronic-based forms for these locations.
- **Any government data security concerns and approvals required.**

Even when electronic data collection is the selected method, all teams should carry paper-based forms in case of 1) security issues, 2) community discomfort with the use of mobile devices and 3) the risk of device failure. Where, despite extensive pilot testing, an error in the electronic data entry form is discovered that would lead to loss of data, for example from an incorrect skip, paper-based forms can be used until the issue has been resolved and new data entry forms are downloaded into survey devices.

Any data from paper-based forms that are sent to a central location should be entered using the same data entry system as on the survey devices, so that data are in the same format and can be easily merged.

To fully understand the supply requirements and implementation process for electronic data collection, refer to the relevant sections in **Module 8: Survey supervision and personnel, Module 9: Supplies and equipment,**
Module 4. Survey design

Module 10: Budget and timelines, Module 11: Data collection tools, field manual and database, Module 12: Training and pilot testing, Module 13: Field logistics, and Module 14: Data entry and cleaning.

Paper-based data collection: Most national survey management teams are familiar with the use of paper-based data collection. Paper-based data collection is usually quicker to develop for field use and generally requires fewer resources in terms of budget and technological expertise. However, significantly more time is required at the “back end” to double-enter data and then compare, reconcile, and clean these data. Supervision and review of questionnaires in the field and a highly skilled data entry and cleaning manager are essential to efficiently produce the highest-quality information from the data generated.

Development of data entry forms, with checks and skips, is still required. It should be completed prior to the pilot test so that the data entry process can also be piloted and adjusted as needed. Data entry should begin as soon as paper questionnaires are transferred from the field, reducing the time required for post-completion of the survey. Data comparison and data cleaning can be done while waiting for laboratory results of sample and specimen analyses.

Paper-based collection does not enable all questionnaire-related errors and issues to be systematically identified and addressed in the same close-to-real-time manner as electronic data collection. However, if the survey management team decides to use paper-based data collection, it can be accompanied by the use of an electronic survey monitoring tool that can be run on any phone with SMS capability. This tool can provide information to track the PSU visited, the number of households with complete questionnaires and the number of different types of samples collected, so that fieldwork progress can be tracked and actual implementation numbers can be compared with survey target numbers.
Module 5. Sample size

In this module, we will discuss:

- General sampling considerations
- Considerations for household-based surveys
- Calculation of sample size for a single cross-sectional cluster survey
- Calculation of sample size for baseline and follow-up cluster surveys
- Subgroup comparisons and their effect on sample size

This module provides examples and information on sample size determination. It is essential that an experienced sampling statistician be included in the team to develop and implement the sampling plan, and to undertake quality control measures as the survey progresses.
Module 5. Sample size

General sampling considerations

The right sample size is crucial for meeting survey objectives and for calculating costs. Sample size calculations depend on a number of factors, and it is worth investing time to consider the advantages and disadvantages of different scenarios. Individuals with sampling expertise should either be part of the Technical committee or consulted to check that the proposed sample size will meet the survey’s objectives.

If the survey is intended to measure many indicators among different population groups, a choice must be made as to which indicator will drive the sample size. The major factors that influence sample size decisions are related to the survey purpose and design (see Module 4: Survey design), and include:

- stratification and proposed number of survey strata;
- key indicators, population groups of interest, and whether estimates are required at the stratum or national level;
- precision and level of statistical confidence required for the indicator of interest in the specific population group at the stratum and national levels; and
- available budget.

Each of these four factors will influence decisions about sample size in different ways.

1. Stratification and proposed number of survey strata.
   - The sample size calculation should initially be based on desired strata-level precision for the indicator and population group. The national sample size is the sum of the strata specific sample sizes.
   - In some cases, stratified data are collected for certain indicators, while feasibility issues may mean that only national estimates, or estimates for more than one stratum combined, are collected for other indicators. Feasibility may be limited by the magnitude of the calculated sample size or by factors such as laboratory costs for analysing sufficient samples to obtain reliable estimates at the stratum level.

2. Key indicators, population groups of interest, and whether estimates are required at the stratum or national level.
   - In cases where the principal aim of the survey is to obtain stratified data for one key indicator, the survey sample size may be based on the calculated sample size for this indicator in the main population group of interest. For example, it may be decided that due to the implementation of an oil fortification programme, vitamin A deficiency is the driving micronutrient of interest. Sample size for this key indicator is then determined largely by:
     - the level of stratification
     - the desired precision for the estimate
     - the expected survey design effect on the indicator.
   - If the survey objectives include more than one key indicator, or measurements of a key indicator in different population groups, the stratum-level sample size requirements should be calculated for each indicator for each population group separately. The resulting sample sizes should then be considered against the feasibility of obtaining these sample sizes at the stratum or national level. Box 5.1 presents an example of sample size calculations for several key indicators and for more than one population group of interest. It also includes discussion about the feasibility of obtaining estimates at the stratum and national levels.

Box 5.1 presents an example of sample size calculations for several key indicators and for more than one population group of interest. It also includes discussion about the feasibility of obtaining estimates at the stratum and national levels.
Module 5. Sample size

3. Precision and level of statistical confidence around point estimates required for the indicator of interest in the specific population group at the stratum and national levels.
   - The reason for including a specific indicator will help determine how precise or reliable the indicator measurement needs to be. For example, if a micronutrient indicator is being measured to obtain an initial estimate of the prevalence of deficiency, lower precision may be acceptable. If it is being measured to assess the impact of a targeted intervention, higher precision may be needed to assess the change in prevalence.
   - A balance needs to be found between acceptable precision, logistics and cost. Box 5.1 includes discussion of this for the example presented. In general, micronutrient surveys are conducted to provide estimates with programmatically applicable precision, as opposed to research-level precision. For a stratified survey, having good precision at a sub-national level will lead to a more precise national estimate.
   - The confidence level describes the confidence interval (CI) around the measurement derived from the survey. The CI is presented as a range of values within which the true value is likely to fall. A 95% CI is used as the standard in most surveys, and is used in the sample size calculations in this module. The width of the CI around the estimate, for example ± 0.05 (± 5%) or ± 0.10 (± 10%) is a measure of the level of precision. For example, if the prevalence of iron deficiency was estimated to be 40% among women of reproductive age, a precision of ±10% would provide 95% confidence limits that range between 30% and 50%. This means that one can state with 95% confidence that the prevalence in the population lies somewhere between 30% and 50%. Whether this is an acceptable level of precision depends on the expected use of the survey results.
   - Precision is affected by a number of features, including the design effect for each indicator. Further information about precision and the design effect, as well as about how these are affected by the balance between number and size of clusters, is provided in later sections of this module.

4. Available budget
   - Sample size is the principal factor for the total cost of both fieldwork and sample analysis. Therefore, the sample design, sample size, and available budget need to be considered together.

The interplay between these factors and their effect on sample size is illustrated in Fig. 5.1.

Other factors to consider in calculating sample size include:
   - Finite population correction (FPC) factor:
     - Most cluster survey sample size formulae assume an infinite or very large number of PSUs in the geographic area of interest. If the total number of PSUs is “small,” as may be the case in some Pacific Island nations, for example, a smaller sample size can be used by taking into consideration the FPC factor. There is no exact value for what makes a “small” target population, but in general the FPC factor is considered when the total number of PSUs from which the sample is selected is less than 1000. For geographic areas with at least 1000 PSUs, the FPC factor will not substantially change the sample size and is very rarely used.
   - Response rate:
     - In calculating the sample size, an estimate of the response rate is needed, and the sample size needs to be increased to account for non-response. For household surveys that include individual-level indicators, there are two levels of response: the household-level response and, for participating households, the individual response.
Fig. 5.1. Factors affecting sample size

*The clustering effect can be lowered by increasing the number of clusters and decreasing the number of samples per cluster. The concept of clustering effect is described in later sections of this module.

Considerations for household-based surveys

For individual-level indicators in a household-based survey where households are randomly selected from a complete listing of households in the cluster, the number of households to visit depends on:

- the number of individuals needed to obtain estimates with sufficient precision for the indicator within that population group;
- the average size of a household;
- the number of individuals from the population group of interest expected within each household; and
- the expected response rate.

More detail on how these factors are accounted for is described in Box 5.2.

The decision about selecting the survey sample from all households or from households that meet a specified criterion requires expert consultation to clearly understand the advantages and disadvantages of each approach and their effect on interpreting the resulting data.

If the number of survey households to include in the sample is based on the numbers required for preschool-age children, then it may not be necessary to collect data from all eligible WRA in the households. In such a case, it may be best to do a random selection of WRA. Possible methods include the random selection of one WRA per household, or selecting all WRA from every third household. The approach needs to be decided at
### Box 5.1. Sample size estimates for multiple indicators across a single stratum (based on household survey design)

<table>
<thead>
<tr>
<th>Nutrition-related measure of interest</th>
<th>Indicator</th>
<th>Population group</th>
<th>Precision at 95% confidence</th>
<th>Estimated prevalence (%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Estimated design effect&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Required sample size&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Number of individuals needed to achieve target sample size&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Number of households needed to obtain target sample size&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stunting</td>
<td>Height/length</td>
<td>Children under 5 years</td>
<td>± 7</td>
<td>40</td>
<td>1.5</td>
<td>280</td>
<td>370</td>
<td>530</td>
</tr>
<tr>
<td>Anaemia</td>
<td>Haemoglobin</td>
<td>Children 6-59 months</td>
<td>± 10</td>
<td>45</td>
<td>2</td>
<td>190</td>
<td>235</td>
<td>430</td>
</tr>
<tr>
<td>Anaemia</td>
<td>Haemoglobin</td>
<td>Women 15-49 years</td>
<td>± 10</td>
<td>30</td>
<td>2</td>
<td>160</td>
<td>190</td>
<td>165</td>
</tr>
<tr>
<td>Iron Deficiency</td>
<td>Ferritin</td>
<td>Children 6-59 months</td>
<td>± 10</td>
<td>50</td>
<td>2</td>
<td>192</td>
<td>240</td>
<td>430</td>
</tr>
<tr>
<td>Iron Deficiency</td>
<td>Ferritin</td>
<td>Women 15-49 years</td>
<td>± 10</td>
<td>50</td>
<td>2</td>
<td>192</td>
<td>225</td>
<td>195</td>
</tr>
<tr>
<td>Household coverage of adequately iodized salt</td>
<td>Household salt iodine level</td>
<td>Household</td>
<td>± 10</td>
<td>60</td>
<td>3</td>
<td>280</td>
<td>300</td>
<td>300</td>
</tr>
</tbody>
</table>

<sup>a</sup> An estimated prevalence of 50% will give the largest required sample size if all other factors remain the same. Therefore, for an indicator with no information on estimated prevalence, 50% is generally used to ensure an adequate sample size.

<sup>b</sup> Design effect estimated based on previous surveys, estimates from other countries, and knowledge from the national (iodized) salt supply system.

<sup>c</sup> The formulae used to calculate the (rounded) target sample size and number of individuals needed to achieve this sample size (accounting for expected response rates) are described in later sections of this module.

<sup>d</sup> This column applies where the survey is designed based on a random selection of households from a complete household listing in each cluster, and takes into consideration the expected proportion of household members within each population group. However, not all surveys are designed in this way. In some cases, a census of households is conducted in advance, and then only households with, for example, children under 5 years of age are visited to obtain the required sample size for this population.

After making the initial calculations of sample sizes for the desired precision at the stratum level, decisions need to be made about feasibility. Where one population group (in this case children under 5 years of age) requires visiting significantly more households, then the following can be considered:

1) Identifying in advance households with this population group and randomly selecting as many of these as are required to find 370 children (this would bias other indicators to be representative of households with children under 5).

2) Accepting a reduced stratum level precision for estimates of 10% (stunting among children under 5), 11% (household coverage of adequately iodized salt), and 13% (anaemia and iron deficiency among children 6-59 months). (This would reduce the required number of households to approximately 250 per stratum).

3) It may be determined that reliable estimates for indicators among this group are only possible at the national level rather than at sub-national (e.g., stratum) levels.
Box 5.2. Household- versus individual-level indicators

For household-level indicators, the number of households to visit will be determined by the number of completed household interviews (and, where included, food tests or samples) required to obtain estimates with the desired precision, accounting for the expected number of occupied households and the response rate for interview and sample collection.

For example, 95% of selected households may be occupied and have an adult household member willing to answer questions about the household, while food sample collection may only be feasible in a lower proportion due to non-availability of the food item and/or non-response for collection.

For individual-level indicators in a household-based survey with a random selection of households from a complete household listing, the number of households to visit depends on four factors:

1. the required sample size for the number of individuals within a specific population group
2. the average household size
3. the proportion of the population comprising the population group of interest
4. the expected household and individual response rates for population-group specific interviews and for sample collection.

Multiplying the average household size by the proportion of the population group of interest in the national population provides the average number of eligible individuals expected per household. The number of households that need to be visited to achieve the required sample size can then be calculated from this, taking into consideration the expected response rate.

The final number of households to visit to obtain data for the required number of subjects from a specific population group may be calculated by dividing the sample size (in this case 766) by the product of: [the average household size (3.9) multiplied by the proportion of the specific population group in the population (0.31) multiplied by the expected response rate for households (90%) and individuals (85%)]. This must also take into account the design effect, DEFF, which is a measure of the homogeneity within a cluster and the variability between clusters.

As an example, where a survey sample size for non-pregnant women of reproductive age (WRA) in a geographic area has been determined to be 766 (already accounting for DEFF), the average household size is 3.9, the proportion of non-pregnant WRA in the population is 0.31, the household response rate is expected to be 0.9 (90%), and the individual response rate for consenting to biological sample collection is expected to be 0.85.

The number of this population group (WRA) per household would be expected to be $3.9 \times 0.31 = 1.2$. Therefore, the team can expect to obtain data from more than one eligible woman per household on average. However, the response rate also needs to be considered. The final number of households to visit to obtain information or samples from 766 WRA, based on the information above, could be calculated as:

$$\frac{766}{3.9 \times 0.31 \times 0.9 \times 0.85} = 828$$
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the survey design stage and cannot be changed during the fieldwork. In all cases, it is important to document the total number of eligible individuals in each household, because this information will be needed to determine the sampling weight at the data management stage.

It is also important to keep in mind that not all information needs to be collected on every survey subject or household. For example, it may be reasonable to perform more expensive tests on a subsample of biological samples, such as every second survey participant within one population group, so long as minimum sample size requirements for that indicator are satisfied.

**Calculation of sample size for a single cross-sectional cluster survey**

Because sample size calculations are based on a number of different decisions and estimates, a range of sample sizes may be produced for a single indicator and population group. The assumptions behind the differences between sample sizes should be discussed with the Steering committee and Technical committee. A rationale for acceptable precision and the final proposed size should be agreed for each indicator and population group. Whatever decisions are made regarding sample size calculations, they should all be documented in the methodology sections of the survey protocol, human subject documents and final survey report.

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**Box 5.3. Special considerations for calculation of sample size in surveys assessing iodine status**

Current recommendations for survey sample collection and data analysis are based on the determination of population - not individual - iodine status. For example, a median urinary iodine concentration (UIC) of 100-199 µg/L indicates adequate iodine intake among a population of school-age children.¹

Iodine status is normally assessed in cluster surveys using casual (single sample) urine samples. Because there is high variability in individual iodine excretion throughout the day, a single urine sample and resulting UIC cannot be considered to reflect an individual’s iodine status. Therefore, it is not valid to calculate or present prevalence of iodine deficiency (which implies a count and comparison of people with adequate and inadequate iodine status).

According to the UNICEF 2018 guidance,² there is uncertainty on the best methods for power calculations to determine population iodine status using spot UIC measurements. Therefore, programme managers may act conservatively and have sample sizes higher than required to determine population iodine status. For example, a common starting point is a 30-cluster survey with 30 urine specimens per cluster for nationally representative estimate. Any subnational stratification requires additional consideration, such as residence, region, socio-economic status, or level of salt iodization. Further, sample sizes need to be adjusted for expected DEFF and non-response. The UNICEF guidance points readers to technical documents for sample size calculations specific to urinary iodine.


Cluster survey sample size calculations start with the same calculation as would be used for a survey using the single random sampling (SRS) method. However, the calculation then takes into consideration the survey design, the expected proportion of the population group of interest within a household, and the expected response rates.

**Step 1: Calculate the sample size using the SRS method**

This calculation is based on the formula:

\[ n = \frac{z^2 \times p(1 - p)}{d^2} \]

Where:

- \( n \) is the calculated sample size
- \( z \) is the statistic that defines the level of confidence required
- \( p \) is an estimate of the key indicator to be measured by the survey in the population group of interest, for example, the prevalence of iron deficiency among WRA, expressed as a proportion of that population
- \( d \) is the desired level of precision, or the margin of relative error to be obtained.

Generally, \( z = 1.96 \), which is the \( z \)-statistic for the 95% confidence level. If the expected estimate of the key indicator (\( p \)) is not known, the value of 0.5 (or 50%) is used because it produces the largest sample size (for a given value of \( d \)). If the proportion is expected to be between two values, select the value closest to 0.5. For example, if the proportion is thought to be between 0.65 and 0.75, use 0.65 for the sample size calculation.

Common values for \( d \) for national level estimates are usually around ±5% for indicators with estimated prevalence in the range of 20%–80% (for example, anaemia), and around ±3% for less common or very common events (for example, wasting, or household coverage of iodized salt). It is often acceptable to use a value of \( d \) higher than ±5% at a stratum level, with the knowledge that the precision around the national estimate will be narrower.

**Step 2: Account for the design effect in calculating sample sizes for cluster surveys**

Cluster sampling is more feasible than SRS for large-scale surveys because it reduces the number of locations to visit and to map, and in which to set up field laboratories. However, cluster sampling introduces the “clustering effect”, which describes the fact that households in the same cluster tend to be more alike in terms of certain characteristics (for example, income, education and access to a fortified food product) than households across the general population. This clustering effect increases the variance between clusters so that it lowers the precision around an estimate that would have been found based on the same sample size calculated for SRS sampling. This change in precision due to clustering is described as the design effect (DEFF). The DEFF is a measure of the homogeneity within a cluster and the variability between clusters. It indicates how much larger the sampling variance (square of the standard error) is for the cluster sample compared to a simple random sample of the same size.

Unless the DEFF was calculated from previous survey data, it will need to be estimated at the stage of sample size calculation, based on prior field experience (unpublished data or subject matter expertise) or from published literature. Some indicators such as wasting (low weight-for-height) may have a small DEFF, while other indicators such as vitamin A capsule distribution may have a large DEFF. In rare cases, the DEFF may be
less than 1, in which case a value of 1 should be used for sample size calculations. The DEFF is calculated using the intra-class correlation ($ICC$), which is a metric of the similarity of clusters in the outcome of interest. Box 5.4 provides more details on the ICC and how this affects the DEFF.

Calculation of sample size for a cluster survey incorporating the DEFF requires a modified version of the previous equation:

$$n = \frac{z^2 x p(1 - p)(DFE)}{d^2}$$

There are four important principles that can be applied to keep the DEFF (and sample size) as low as possible:

1. Use as many clusters as is feasible. For a non-stratified survey, the recommended minimum number is 30 clusters in the first stage of sampling. For a stratified survey, the recommended minimum is 25 per stratum. Up to a certain limit, the DEFF will continue to decrease as the number of clusters increases. Sampling more than 40 clusters per stratum does not provide much additional benefit to survey precision, in general.

2. Use the smallest cluster size (number of households per cluster) as is feasible, generally aiming for a minimum of 10 observations for each population group per cluster.

3. Use a constant cluster size.

4. Select a random sample of households at the final stage to increase geographic dispersion (see Module 7: Selecting households and participants).

Note: The DEFF (and hence the precision) for an indicator is affected by the number of observations per cluster, not the number of households visited. As an example, for a sample of 1200 households, a lower DEFF and higher precision would be achieved by selecting 40 clusters of 30 households each instead of 20 clusters of 60 households each.

**Step 3: Account for response rates**

Determining the sample size for household- and individual-level indicators must take into consideration the expected response rates of households and of individuals, and the availability of fortifiable foods. Box 5.2 describes factors to consider. Household and individual response rates can be estimated based on experience from previous similar surveys. Typically, response rates are higher for questionnaire-related indicators, such as an assessment of knowledge or behaviour, and they are lower for food sample collection (usually considered a household-level indicator) and for biological specimens, especially from young children or pregnant women. Ensuring community sensitization to the survey can greatly improve response rates, but when calculating the sample size, it is better to use a relatively conservative estimate.

Non-response can occur at different levels, for example the household interview, individual interview, and food sample and biological specimen collection. Here are potential reasons for non-response:

- None of the household members may be available during the time of the survey (the household is away on a temporary basis).
- The entire household may choose not to participate.
- Some individuals within a household may refuse to participate, may be sick, or may not be available during the survey.
- Some individuals may partially participate, such as agreeing to answer questions but refusing to provide a food sample or biological specimen collection.
• The fortifiable food of interest may not be available for collection within the household.
• The volume of blood or food sample collected may be insufficient for laboratory analysis.

**Box 5.4. Relationship between the design effect and the intra-cluster correlation (ICC)**

The ICC is a measure of the degree of homogeneity (similarity with respect to the variable of interest) of the units (households or individuals) within a cluster. It is sometimes referred to as the “rate of homogeneity”, or ROH. Because units in the same cluster tend to be similar to one another in terms of income, climate/environmental conditions and attitudes, the ICC is almost always positive. The DEFF can be described in terms of the ICC and vice versa.

For a given DEFF and average number of units sampled per cluster (\(\bar{n}\)), the ICC can be estimated as:

\[
ICC = \frac{DEFF - 1}{\bar{n} - 1}
\]

If estimates of the ICC and average number of units per cluster are available, the DEFF can be calculated as:

\[
DEFF \equiv 1 + ((\bar{n} - 1) \times ICC)
\]

The DEFF is significantly affected by the number of observations within one cluster. However, the relationship between the DEFF and the ICC means that the DEFF can be estimated for a subsequent survey that may have a different number of observations per cluster, using the equations above.

For example, analysis of data from the 2015-16 Malawi Micronutrient Status Survey found a DEFF of 1.9 for anaemia among children 6–59 months of age.\(^1\) This estimate was based on 105 clusters with an average of 13.8 children per cluster (\(n=1452\)). If another survey was being planned with a design to select an average of 16 children per cluster, the ICC-DEFF relationship could be used to estimate the expected DEFF for this second survey.

\[
ICC = \frac{1.90 - 1}{13.8 - 1} = 0.07
\]

The DEFF for the second survey with an average of 16 children per cluster would be estimated as:

\[
DEFF \equiv 1 + ((16 - 1) \times 0.07) = 2.05
\]

Therefore, the estimated DEFF for anaemia for a survey with 16 children per cluster is 2.05, and this would be used in the sample size calculation.

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Where:

- \( j \) is the expected response rate as a proportion (household response multiplied by individual response for interview/biological specimen collection)
- \( k \) is the average household size
- \( l \) is the proportion of the total population accounted for by the population group of interest
- \( d \) is the desired level of precision, or the margin of relative error to be obtained
- DEFF is the design effect
- \( z \) is the statistical value derived from the normal distribution table for a given level of confidence (for example, \( z=2.58 \) when the type I error, or level of significance: alpha \((\alpha)\)=0.01).

An example calculation for one indicator is shown in Box 5.5.

The “Survey sample size calculator” online tool can facilitate the process of sample size calculation and makes it easy to see, in the above equation, the effect of changing precision or other variables on final sample size. Additional computer-based programs to perform sample size calculations can be found at [www.OpenEpi.com](http://www.OpenEpi.com).

There may be differences between hand-calculated sample size estimates and computer-based calculations due to rounding and slight variations in sample size formulae, but these differences are usually minor.
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Box 5.5. Example sample size calculation
The required (unit) sample size to assess vitamin A deficiency among children 6–59 months of age was calculated assuming \( p = 0.35 \) (prevalence of deficiency 35%), level of acceptable precision \( d = 0.05 \) (or \( \pm 5\% \)), and \( \text{DEFF} = 2.5 \):

\[
n = \frac{1.96^2 \times 0.35(1 - 0.35)(2.5)}{(0.05^2)} = 873.96
\]

Sample sizes are always rounded up, so this would be a desired sample size of 874 children.

The average household size is 3.9 and the proportion of this age group in the population is 0.09, while the household response rate is 90% and the individual response rate is 85%. In order to achieve the target sample size of 874 children, 3255 households should be visited.

\[
n = \frac{1.96^2 \times 0.35(1 - 0.35)(2.5)}{(0.90 \times 0.85)(3.9)(0.09)(0.05^2)} = 3254.81
\]

Decreasing the acceptable level of precision by 2% reduces the number of households to visit by more than 1500. Using an acceptable precision of 0.07, or \( \pm 7\% \), results in a household sample size of 1661 households.

\[
n = \frac{1.96^2 \times 0.35(1 - 0.35)(2.5)}{(0.90 \times 0.85)(3.9)(0.09)(0.07^2)} = 1660.62
\]

Depending on the setting, different precision values or different expected \( \text{DEFF} \) values may be appropriate. For example, an estimated household coverage of adequately iodized salt of 20% would indicate significant problems with implementing universal salt iodization. It could be decided that a precision of \( \pm 10\% \) would be sufficient because the programme response would be the same whether the true prevalence was anywhere between 10% and 30%. On the other hand, if the prevalence of the indicator of interest is very low or very high, or expected to be close to the cutoff value for public health significance, it may be desirable to have a precision of \( \pm 5\% \) or, in rare cases, an even higher precision of \( \pm 2.5\% \). However, the impact of such increased precision on sample size must be weighed against the programmatic application of the outcome.

Calculation of sample sizes for baseline and follow-up cluster surveys
Household surveys are frequently designed to estimate the prevalence of certain indicators and to assess changes in these indicators over time. Often, an initial survey serves as a baseline to identify the need for an intervention or to assess status before its implementation. A follow-up survey is then conducted to assess changes in selected indicators, and potentially to introduce additional indicators. The sample size for each survey should be estimated using survey design parameters that account for:

- assumptions about expected changes in the indicator estimates over a proposed time period and the reliability of the data to capture this change; and
- whether the same or different clusters and households will be included in the initial and follow-up surveys.
Module 5. Sample size

There are different methods to calculate the required sample size. One example is provided in the *Feed the Future population-based survey sampling guide*¹ and calculator.² You can also find details of the OpenEpi method at [www.OpenEpi.com](http://www.OpenEpi.com). To calculate the required sample size, the following estimates and assumptions are needed:

- $n$ is the calculated sample size
- $DEFF$ is the estimated design effect (while the formula allows for one $DEFF$ across the two surveys, it is recommended to use the larger $DEFF$ for the sample size calculation)
- $Z$ is the statistic that defines the level of confidence required
- $\alpha$ (“alpha”) is the desired level of two-sided significance of the difference in estimated proportions between surveys, usually 0.05 or 5% (corresponding to a 95% CI)
- $p$ is an estimate of the key indicator to be measured by the survey in the population group of interest, for example, the prevalence of iron deficiency among WRA, expressed as a proportion of that population
- $q_i = 1 - p_i$
- $1 - \beta$ (the type II error) is the expected chance of detecting a difference between the two surveys, usually 0.8 (80%) or 0.9 (90%), also known as power
- $p_i$ is the estimate of the key indicator to be measured in the population group of interest, for example, prevalence of anaemia or proportion of households using adequately iodized salt at the time of the baseline survey
- $q_i = 1 - p_i$
- $p_1$ is the estimate of the key indicator to be measured in the population group of interest, expressed as a proportion, at the time of the follow-up survey
- $q_2 = 1 - p_2$

In the formula below, it is assumed that the sample size in each of the two surveys will be the same. The formula is:

$$n = DEFF \times \frac{Z_{\alpha/2} \sqrt{2pq} - Z_{1-\beta} \sqrt{p_1 q_1 + p_2 q_2}}{(p_1 - p_2)^2}$$

Where:

- $\bar{p} = \frac{p_1 + p_2}{2}$ and $\bar{q} = 1 - \bar{p}$ when sample sizes for each survey are equal
- $Z_{\alpha/2}$ is the Z value for the level of significance; $\alpha$ generally $= 0.05$, therefore $Z_{0.025} = 1.96$
- $Z_{1-\beta}$ is the Z value for power; $\beta$ generally $= 0.20$, therefore $Z_{1-0.20} = -0.842$

---


Tables 5.1 and 5.2 display two-sided Z values ($Z_2\alpha$) that can be used for different significance levels, and one-sided Z values ($Z_{1-\beta}$) that can be used for various power ($1 - \beta$) levels.

**Table 5.1. Two-sided Z values for different significance levels**

<table>
<thead>
<tr>
<th>Significance level ($\alpha$)</th>
<th>Two-sided Z value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01</td>
<td>2.576</td>
</tr>
<tr>
<td>0.05$^a$</td>
<td>1.960</td>
</tr>
<tr>
<td>0.10</td>
<td>1.645</td>
</tr>
</tbody>
</table>

$^a$ Value used in example.

**Table 5.2. One-sided Z values for various power levels**

<table>
<thead>
<tr>
<th>$\beta$ value</th>
<th>Power ($1 - \beta$)</th>
<th>One-sided Z value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01</td>
<td>.99</td>
<td>-2.326</td>
</tr>
<tr>
<td>0.05</td>
<td>.95</td>
<td>-1.645</td>
</tr>
<tr>
<td>0.10</td>
<td>.90</td>
<td>-1.282</td>
</tr>
<tr>
<td>0.20$^a$</td>
<td>.80</td>
<td>-0.842</td>
</tr>
</tbody>
</table>

$^a$ Value used in example.

An example of a calculation is shown in Box 5.6. It is important to remember that a higher power and lower significance level will increase the needed sample size.

Once the baseline survey has been completed, the components assumed for the sample size calculation for the baseline (namely prevalence at baseline, DEFF, response rates and accuracy of projected estimates) for the follow-up survey should be revised based on the known information from the baseline survey.

You can find additional help in comparing the sample sizes of a baseline and a follow-up survey using the “Survey sample size calculator” online tool.

**Subgroup comparisons and their effect on sample size**

The discussion of factors affecting sample size is based on surveys designed to obtain national and subnational estimates for the prevalence of certain nutrient-related indicators. However, sometimes an objective of the survey will be to compare subgroups, such as comparing males to females, or comparing the prevalence of a specific deficiency among a defined population group in households that do not use a fortified food to the equivalent estimate in households that do. If these types of comparison are important, then the sample size will usually need to be increased to ensure that precision for each subgroup is adequate to make a reliable comparison.

If it is expected that the two subgroups are fairly equally distributed in the population (such as females and males in most populations), then the sample size presented in the previous section could be used with
Box 5.6. Example sample size calculation for baseline and comparative follow-up surveys

A country is going to begin fortifying flour with iron. The survey team estimates that the baseline prevalence of anaemia is 50% among WRA, and expects that iron fortification of flour will lower the anaemia prevalence in this group to 40% over 12 months.

Example of sample size calculation when calculating this by hand:
- \( p_1 \) (proportion of anaemia in the selected population group at baseline) = 0.50, \( q_1 = 0.50 \)
- \( p_2 \) (proportion of anaemia in the selected population group at follow-up after intervention) = 0.40, \( q_2 = 0.60 \)
- \( \alpha = 0.05 \), therefore \( Z_{\alpha} = 1.96 \)
- \( \beta = 0.20 \), therefore \( Z_{1-\beta} = -0.842 \)
- DEFF = 2

Assuming equal sample sizes, \( p \) is calculated as:
\[
\hat{p} = \frac{0.50 + 0.40}{2} = 0.45
\]
\[
\hat{q} = 1 - 0.45 = 0.55
\]
\[
n = 2 \times \left[ \frac{1.96 \sqrt{2(0.45)(0.55)}}{(-0.842) \sqrt{(0.5)(0.5) + (0.4)(0.6)}} \right]^2 = 2 \times \frac{3.876}{0.01} \approx 776
\]

In this example, the sample size would be 776 individuals in each cross-sectional survey, that is, 776 for the baseline survey and 776 in the follow-up survey. The number of households to visit to obtain information from 776 individuals would depend on the expected response rate and the proportion of the population group in each household.

Suppose the prevalence of anaemia among WRA in households using iron-fortified flour is to be compared to the prevalence of anaemia among WRA in households not using iron-fortified flour, and it is estimated that 80% of households in the population use iron-fortified flour. The sample size formula is:
\[
n_1 = \text{DEFF} \times \frac{Z_{\alpha/2} \sqrt{(r + 1)pq} - Z_{1-\beta} \sqrt{rp_1 q_1 + p_2 q_2}}{r(p_1 - p_2)^2}
\]
\[
n_2 = r n_1
\]

Where:
\[
\hat{p} = \frac{p_1 + rp_2}{r+1} \quad \text{and} \quad \hat{q} = 1 - \hat{p} \quad \text{when sample sizes are to be unequal.}
The term \( r \) is an element added to the previous formula for calculating sample size. In this example, \( r \) would be the proportion of households \textit{not} using iron-fortified flour divided by the proportion of households using iron-fortified flour. In the above example, \( r = 0.2 \div 0.8 = 0.25 \).

Two sample sizes are calculated:
- \( n_1 = \) the number of households using the fortified product
- \( n_2 = \) the number of households \textit{not} using the fortified product

For example, assume the following:
- \( p_1 = 0.40 \), the prevalence of anaemia in households using iron-fortified flour
- \( p_2 = 0.50 \), the prevalence of anaemia in households \textit{not} using iron-fortified flour
- \( r = 0.2 \div 0.8 = 0.25 \)
- \( \alpha = 0.05 \), therefore \( Z_{\frac{\alpha}{2}} = 1.96 \)
- \( \beta = 0.20 \), therefore \( Z_{1-\beta} = -0.842 \)
- \( \text{DEFF} = 2 \)

\[
\bar{p} = \frac{0.4 + (0.25 \times 0.5)}{0.25 + 1} = 0.42 \quad \text{and} \quad \bar{q} = 1 - 0.42 = 0.58
\]

\[
n_1 = 2 \times \left[ 1.96 \sqrt{(0.25 + 1)(0.42)(0.58) - (-0.842)\sqrt{0.25(0.40)(0.60) + (0.5)(0.5)}} \right]^{-2} \times \frac{(1.0816 + 0.4688)^2}{0.0025} = 1923
\]

\[
n_2 = 0.25(1923) = 481
\]

Therefore, the survey would need to include 2404 individuals, of whom 1923 would be expected to be from households that use iron-fortified flour and 481 would be expected to be from households that do not use iron-fortified flour.
Module 6. Selecting clusters

In this module, we will discuss:

- Determining the appropriate number of clusters and the number of units per cluster
- Methods for selecting clusters
- Examples of cluster selection using probability proportional to size, simple random sampling and systematic sampling

This module provides examples and information on sampling. It is essential that an experienced sampling statistician be included on the team to develop and implement the sampling plan, and to undertake quality control measures as the survey progresses.
Determining the appropriate number of clusters and the number of units per cluster

There are two main issues to consider when applying sample size calculations to fieldwork plans for cluster surveys: how many clusters are needed, and how many units (individuals or households) are needed per cluster. The two values are interrelated, meaning that decisions about one affect the value of the other.

As noted in Module 5: Sample size, the number of units per cluster affects the DEFF and therefore the required overall sample size. The more clusters it is possible to include, the fewer units are needed per cluster and the more diverse the sample will be. As a general rule, up to around 40 clusters per stratum is a good estimate, with the aim of at least 10 observations per cluster. A greater number of clusters and fewer units per cluster will decrease the DEFF and either improve precision for the same sample size, or maintain precision with a smaller sample size. In the example given in Module 5: Sample size, for a sample of 1200 households, higher precision can be achieved by selecting 60 clusters of 20 households each as opposed to 40 clusters of 30 households each. On the other hand, visiting 60 clusters rather than 40 increases the cost of the survey. This underscores the need to weigh the cost of collecting data and specimens against programmatic needs for a specific level of precision.

General guidelines for deciding on the number of clusters and number of units per cluster are provided in the section on DEFF in Module 5: Sample size. Other factors that influence this decision are geography and time per cluster:

- **Geography:** In larger countries, the cost and time required for teams to move from one cluster to another can be substantial. In this case it might be better to select a smaller number of clusters, but never less than 25. If the country is very small, or if the survey is being conducted in a region or province only, having a larger number of clusters is a reasonable way to improve precision.

- **Time per cluster:** The number of household visits and data collection that can be completed in a single day can vary. In some surveys, the questionnaire and specimen collection might be brief, whereas in others it may be much more time-consuming. In some surveys, the specimen collection and interviews are conducted in the household, while in others, survey participants may be asked to go to a central laboratory set up in the cluster. Depending on the survey design, the size of the team, the traveling distance between households, and the complexity of the survey, a single team can typically complete visits to five to ten households in one day.

The logistics required for cold chain management are also crucial to consider. In some harder-to-reach clusters with limited access to electricity, it may be necessary to minimize the number of days spent in the cluster if there are specimens that require processing and freezing in the field. Portable freezers and centrifuges that can be plugged into a car or portable generator are available, but the less time spent in areas without a direct power source, the more likely it is that the cold chain can be maintained. There are several ways to minimize the time spent in one cluster: increasing the number of enumerators per team, increasing the number of teams per cluster and increasing the total number of clusters so that there are fewer households per cluster.

In some circumstances, the collection of specimens for a specific biomarker may be complicated and time-consuming. If this is the case, it may be possible to collect data only from a random subsample of the population group of interest within each stratum, and focus on generating a reliable national estimate. The modified relative dose response (MRDR) test for assessing vitamin A deficiency is an example of a biomarker that requires a random subsample. Specimen collection for the MRDR test requires the survey participant to
avoid vitamin A rich foods for at least two hours before the initial blood draw, consume a dose of vitamin A2 mixed with oil, continue to avoid vitamin A rich foods, and then have a second blood specimen drawn four to six hours later. In addition, sample analysis for the MRDR test is costly. For reasons of feasibility and cost, it may be sufficient to collect specimens for the MRDR test from one single household per cluster.

**Methods for selecting clusters**

Cluster selection must be random. The first stage in selecting clusters is generally based on a comprehensive listing of all primary sampling units (PSUs). For household surveys, PSUs often have the same boundaries as census enumeration areas (EAs). The PSUs are often referred to as “clusters” because the survey elements, namely households, are clustered within the PSU.

For surveys that concern attendees of government facilities, PSUs can be defined as all government-run health facilities. For primary school-based surveys, PSUs could be all primary schools, including private and religious schools. The estimates obtained would represent only children who attend school.

In household-based surveys, the comprehensive listing of PSUs would require the population size or the number of households within each cluster. In clinic-based surveys, the comprehensive listing of PSUs would require the number of clinic enrollees, while in school-based surveys, the number of students enrolled in and regularly attending each school is necessary for the PSU listing.

If relatively accurate data on population size are available, then the preferred method for selecting clusters is the probability proportional to size (PPS) method. If reasonably reliable population data area not available, then either a random or systematic sampling (SS) of clusters could be used. Each of these methods is described in more detail in the following sections.

**PPS method:** Using the PPS method, the likelihood of a PSU being selected is proportional to the size of its population (the number of individuals or households). Thus, larger PSUs are more likely to be selected than smaller ones.

The PPS method starts by obtaining the “best available” census data for all the PSUs in the geographic area to be surveyed. This information is usually available from the government agency responsible for the census, such as a national bureau of statistics. The list from which survey PSUs are selected must cover the whole area intended to be represented by the survey estimates. If it is a nationally representative survey, all national households must be represented in the list.

Depending on the country, PSUs may cover relatively small geographic areas, with a population size between 100 and 1000 individuals or between 20 and 200 households. It is important to confirm the sizes of PSUs, as there may be circumstances in which there are not enough potential survey units in one PSU to meet the required sample size. In those cases, two nearby PSUs should be combined to form a single one, prior to the selection process.

To use the PPS method to select PSUs, first create a table with four columns, as is shown in Box 6.1:

- The first column lists the name or code of each PSU. As a general rule, it is best for the list to be in geographic order and organized by urban, rural, district, and province (implicit stratification).
- The second column contains the population size of each PSU.
Module 6. Selecting clusters

- The third column contains the cumulative population that is obtained by adding the population of each PSU to the cumulative population of PSUs preceding it on the list.
- The fourth column indicates selected clusters within PSUs.

A sampling interval (labeled as “k”) is obtained by dividing the total population size by the number of PSUs to be selected for the survey. A random number between 1 and the sampling interval (k) is chosen to identify the initial PSU. The value of the sampling interval (k) is added to this to select the second PSU. This continues, adding the value of k to each selected PSU cumulatively until the desired number of clusters is chosen. Note that the last selected PSU should be less than the value of k away from the end of the PSU listing.

Where there is a large number of PSUs, the selection process is usually performed using a computer. For SAS users, the PROC SURVEYSELECT command has an option to select data using PPS. With SPSS, the optional Complex Samples module has a “Select Sample…” option. Use of spreadsheets and appropriate formulae is another method for performing the selection.

The “How to select PSUs” online tool contains an extract from a spreadsheet with instructions on how to select PSUs using the PPS method. Box 6.1 also shows an example of how this is done.

Random and systematic selection of clusters (where population size is inaccurate or unknown): When a list of PSUs is available but the population size for each PSU is not known or could be very inaccurate, simple random sampling (SRS) may be used. SRS means that the predetermined number of PSUs is randomly selected from a total list of PSUs. In this case, sampling is based on the sequential numbering of PSUs rather than on population size. Selection proceeds according to a random starting point and a fixed sampling interval (k). In this method of sampling, k is calculated by dividing the total number of PSUs by the desired number of PSUs. This value of k should be used to select the PSUs by rounding up the value of k. Many software packages are available that can easily select the number of PSUs desired.

As is done in the PPS method, a random integer between 1 and the sampling interval (k) is chosen as the initial PSU, and the value of the sampling interval (k) is added to this PSU number to select the second PSU number. Once the list of selected PSU numbers is completed, they should be rounded down as needed to identify the actual PSU to select. See Box 6.2 for an example.

To be able to analyse the data collected with some adjustment for population size, an estimate of the population size in each selected PSU should be collected when the survey team arrives on site. Typically, a mini census is conducted to determine this number. Definitions of households and an explanation of how to select households and individuals from within selected clusters are provided in more detail in Module 7: Selecting households and participants.

If equal numbers of households (or a different survey unit) are randomly selected using the same method within a cluster, then they can have equal weight. Using the PPS sampling method at the first stage above would result in a self-weighted (equal weighted) sample of units within the stratum. All households in a stratum have the same probability of selection regardless of which PSU they are located in.

Implicit stratification spreads the sample evenly among geographically important subgroups of the population, such as urban or rural areas, or administrative regions. The process involves arranging the PSUs in geographic order, such as urban by province, within each province by district, followed by rural by province, then within each province by district before systematically applying the PPS method.
Examples of cluster selection using probability proportional to size (PPS) and systematic sampling (SS)

Box 6.1. Small-scale example of selecting clusters from a listing of PSUs using the PPS method

**Step 1:** Calculate the sampling interval \((k)\) by dividing the total population by the number of clusters to be surveyed. In this example, the total population is 24,940, and the number of clusters to be surveyed is 30, thus the sampling interval is \(24,940 ÷ 30 = 831\) people. Always round down to the nearest whole integer.

**Step 2:** Use a random number table or generator to determine a random starting point between 1 and the sampling interval \((k)\). In this example where the sampling interval is 831, the number 710 was randomly selected as the starting participant.

**Step 3:** Based on the cumulative population column, individual n° 710 is found in the first cluster. In this example, the first cluster is in the PSU of Mina because it includes the population from individual 601 to individual 1300.

**Step 4:** Continue to assign clusters by adding 831 \((k)\) cumulatively. For example, the second cluster will be in the PSU where the value 1541 is located (710 + 831 = 1541), which is Bolama. The third cluster is where the value 2372 is located (1541 + 831 = 2372), and so on. In PSUs with large populations, more than one cluster could be selected. Note that if two clusters are selected in the same PSU (in this case Hilandia), the survey team will divide the PSU area into two sections of approximately equal population size and treat each area as an independent cluster. Similarly, if three or more clusters were in a PSU (for example, Cococopa), the PSU would be divided into three or more sections (clusters) of approximately equal population size.

<table>
<thead>
<tr>
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<th></th>
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<td>320</td>
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<td>4,520</td>
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<td>Noszip</td>
<td>1,780</td>
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<td></td>
<td>42</td>
<td>Plitok</td>
<td>420</td>
<td>18,500</td>
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<td>18</td>
<td>Aisha</td>
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<td>8,250</td>
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<td>Dopoltan</td>
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<td>300</td>
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<td>8,830</td>
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<td>Mewoah</td>
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<td>24,980</td>
<td>30</td>
</tr>
</tbody>
</table>

In this example, there are only 50 PSUs in the listing. In practice, the number of PSUs will be much larger. The spreadsheet and formula used to generate the table above are shown in the “How to select PSUs” online tool.
Box 6.2. Small-scale example of systematic sampling of clusters from a listing of PSUs

**Step 1:** Obtain the list of the PSUs and number them from 1 to the total number of PSUs. In this example there are 50 PSUs.

**Step 2:** The number of PSUs to sample should have already been determined. In this example it is 20.

**Step 3:** Calculate the sampling interval \( k \) by dividing the total number of PSUs by the number to be sampled. In this example, there are 50 PSUs, of which 20 should be sampled, thus the sampling interval is \( k \) is 50 ÷ 20 = 2.5.

**Step 4:** Using a random number table or generator, select an integer between 1 and \( k \). Whichever number is randomly selected, go to the PSU list and include that PSU as the first selected PSU. In this example, the first selected PSU is number 2.

**Step 5:** Select the subsequent PSUs by adding \( k \) to the selected PSU number, then round down to the nearest whole integer. In this example the second PSU would be 2 + 2.5 = 4.5, rounding down makes it PSU number 4, and the third selected PSU is 4.5 + 2.5 = 7. The fourth selected PSU is 7 + 2.5 =9.5, rounded down to 9. This table shows the 20 PSUs selected from PSUs numbered 1 to 50.

<table>
<thead>
<tr>
<th>Number</th>
<th>PSU name</th>
<th>Selected</th>
<th>Number</th>
<th>PSU name</th>
<th>Selected</th>
</tr>
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<tr>
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<td>Kegalni</td>
<td></td>
</tr>
<tr>
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<td>Taluma</td>
<td>x - 2</td>
<td>29</td>
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<td>Mapazoko</td>
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<td>Sanbita</td>
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<td>25</td>
<td>Mezan</td>
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<td>50</td>
<td>Andidwa</td>
<td>x</td>
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</table>

In this example, there are only 50 PSUs in the listing. In practice, the number of PSUs will be much larger. You can find the spreadsheet and formula used to generate the table above in the “How to select PSUs” online tool.
Module 7. Selecting households and participants

In this module, we will discuss:

- General principles of mapping and listing
- Definitions
- How to segment primary sampling units (PSUs)
- How to select households in a cluster
- Identifying and selecting eligible individuals in the household

This module provides examples and information on sampling. It is essential that an experienced sampling statistician be included on the team to develop and implement the sampling plan, and to undertake quality control measures as the survey progresses.
General principles of mapping and listing

After having selected the clusters, the next steps are to map them and to list, select and identify households to visit for data collection. This process is essential to ensure the random selection of the survey sample, and must be included in the survey budget and timeline despite its significant field expense.

Mapping and listing can be done slightly in advance of the survey, by specialist teams that do not include survey fieldwork team members. Alternatively, they can be done immediately before data collection by the survey teams themselves. Advance mapping and household listing is the recommended option, as it has been found to be more reliable. The national statistics office usually has staff with training and experience in mapping and household listing.

Some countries have electronically available maps, in other countries the maps are hand-drawn. Maps may or may not be up to date. The household listing team needs to establish the level of accuracy before beginning segmentation or household listing from any pre-existing maps. If the available information is more than one year old, a new household listing should be conducted.

The household listing team usually consists of two to three listers. Supervisors generally oversee several teams.

The listing team should identify the physical boundaries of the PSU, commonly with the help of a local guide. If the PSU is large it may require segmentation (see below) before the team can begin to map and list. Any problems encountered during the mapping and listing process should be communicated to the supervisor.

The materials needed for the household listing activity include:

- A manual describing all procedures for mapping and household listing
- Felt-tipped pens (alternatively, marker or chalk) to be used in numbering structures
- A notebook
- Pencils and erasers
- Maps of the selected clusters
- A cluster information form
- A household listing guide and form
- A segmentation form.

Definitions

Clear definitions of survey-related terms are essential because they may vary from country to country. In particular, the terms “household” and “dwelling” must be well defined to ensure that survey teams operate consistently when identifying and selecting households in the field. The Demographic and Health Survey (DHS) and the Multiple Indicator Cluster Survey (MICS) are good resources to check for meanings that are culturally appropriate, especially in societies where the family structure and marital arrangements (for example, polygamy) may add complexity to definitions.

This manual uses the following definitions: 1

A **household** consists of a person or a group of persons who live together in the same dwelling unit, who share common living arrangements, who eat together, who acknowledge the same person as the head of household, and who are considered as one unit.

The **head of household** is a resident member of the household who is acknowledged by the other members as the primary decision-maker.

A **dwelling unit** is a room, or a group of rooms, normally intended as a place of residence for one household. This could be a single house, an apartment, or a group of rooms in a house. However, a dwelling unit can also be shared by more than one household.

A **structure** is a free-standing building that can have one or more dwellings for residential or commercial use. A residential structure can have one or more dwelling units, for example, it may be a single house or an apartment building.

### How to segment primary sampling units (PSUs)

Selected PSUs can be made up of one single or multiple clusters. PSUs may vary widely in the number of households or population size. It would not be cost- or time-efficient to conduct a full listing of all households in large PSUs. Instead, it would be more useful to subdivide them into smaller geographic areas, called segments (sometimes referred to as quadrants). Only one of these segments will be selected as the cluster eligible for sampling, then it will be mapped and listed. **Box 7.1** presents the main rationale for PSU segmentation.

### Box 7.1. Rationale for PSU segmentation

Large PSUs may need to be segmented to accommodate numerous clusters within a single PSU (an example is provided in Box 5.1). When PSUs are split into discrete segments, it is important to adopt segment boundaries that are easily identifiable. If clearly identifiable boundaries are not present, it may not be appropriate to subdivide the area.

Segmentation of large PSUs into multiple clusters is also advantageous for survey logistics. A micronutrient survey generally requires the transport of a large amount of equipment, for conducting anthropometric measurements and for collecting and storing samples and specimens. In some surveys, a central laboratory is set up in each cluster, and eligible participants are asked to go to this location for measurement and for collection of samples or specimens. It therefore makes sense to have smaller cluster sizes. Ideally the cluster should not contain more than three to five times the number of households that need to be selected. Thus, if 30 households are needed, a range of 90–150 households in the cluster is ideal. Larger clusters will have more variation in outcomes, increasing the intra-cluster correlation and the design effect.

[http://mics.unicef.org/files?job=W1siZisljslwMTkvMDivMjJvMTkvMzEvMzAvOTU5L01JQ1NfTWFiudWFiX2ZvbGRfTGlzdGlzZ18yMDE5MDiyN3s6aXAIxV0&sha=1822015d5f32e1e5; accessed 17 June 2020](http://mics.unicef.org/files?job=W1siZisljslwMTkvMDivMjJvMTkvMzEvMzAvOTU5L01JQ1NfTWFiudWFiX2ZvbGRfTGlzdGlzZ18yMDE5MDiyN3s6aXAIxV0&sha=1822015d5f32e1e5; accessed 17 June 2020).
Module 7. Selecting households and participants

Upon arrival in a large PSU that may need segmentation, the listing team should first tour the PSU and do a quick count to estimate the number of households. In general, any PSU with well over 100 households should be subdivided into segments of approximately equal size, ideally around 90-150 households each.

Each listing team should have segmentation forms available to them in the field. These may be paper or electronic forms. Instructions and examples of forms are provided in the “Mapping household listing and segmentation” online form. Segmentation and selection of a sample segment will be carried out as described in Box 7.2.

Box 7.2. How to segment and select a sample segment

1. Draw a location map of the entire PSU (see Fig. 7.1).
2. Conduct a quick approximate count of the number of dwellings in the whole area. Where there are large multi-dwelling structures that are likely to include many households, such as an apartment block, information should be obtained about the likely number of households in the structure.
3. Using clear boundaries, such as roads, paths, or streams, divide the PSU into segments that contain roughly equal numbers of dwellings. There may be considerable differences in geographic size between segments, depending on population density.
4. Indicate the boundaries of the newly created segments on the location map.
5. Number the segments sequentially.
6. For each segment, do a quick approximate count of the number of dwellings and likely number of households.
7. If the segment is still too large (well over 100 households), then divide it further into smaller areas, called sub-segments.
8. Using the “Mapping household listing and segmentation” online form, record the PSU number and locality, and indicate the number of dwellings, percentage and cumulative percentage per segment in the appropriate columns.
9. Using a random number table or generator, select a random number between 1 and the total number of segments. A random number table is available in the online tools.
10. Select the segment with this number as the cluster to be surveyed.
11. Draw a full sketch map of the selected segment and list all the households found (see Fig. 6.2); a sample listing form is available in the “Household listing” online tool.
12. Select households using a systematic random process (SRS) described in Module 4: Survey design.

For the purpose of segmenting a cluster, the initial count of households does not have to be precise. A close approximate count of dwellings and likely number of households is sufficient. It is acceptable to have a slightly unequal number of households per segment in order to create segments with clearly identifiable boundaries.

Fig. 7.1 shows an example of a cluster location map, while Fig. 7.2 shows a detailed sketch map of the cluster.
Fig. 7.1. Example map showing the location of the selected cluster Ngaku, Code EA01009\(^a\)

Fig. 7.2. Sketch map showing structures within cluster Ngaku, Code EA01009

Module 7. Selecting households and participants

Segmenting urban areas may be easier than segmenting rural areas. Cities and towns are usually organized into blocks or other similar units. When using census enumeration areas (EAs) in cities and larger towns, maps are often available that show streets and blocks. If they are not available, such maps can be easily drawn. A quick drive through the area will provide a sense of whether there are an approximately equal number of dwellings of similar size per block. If so, the cluster could be segmented by block or parts of blocks.

For example, in an urban PSU that includes 18 very similar blocks, where the number of dwellings per block would also be expected to be similar, estimate the number of dwellings per block. If each block contains approximately 50 dwellings, the total number would be 900. This could be divided to give seven segments of approximately 125 dwellings (or 2.5 blocks) per segment. If the number of dwellings per block or the expected number of households per dwelling varies considerably, the number in each would need to be estimated before dividing into approximately equal segments.

In rural areas, it is likely that several households may exist in the same compound and that the number in each compound may need to be estimated. Large clusters, such as cities, may already have political subdivisions with an estimated number of households or population size. These can be used to define segments.

**Fig. 7.3** illustrates the standard decisions and steps to take depending on the original PSU size. In this example, it is assumed that the average household size is 7.4 individuals, the number of households to be assessed is 30, and the goal is to identify a segment with approximately 100 households (in other words, a segment with around three times the number of households that will be included in the sample).

There are several advantages and disadvantages to be weighed in determining the segment size. For example, if households are selected systematically over a large area, the amount of time required for the teams to get from one household to the next may make supervision more difficult. However, if a cluster size is very small, the diversity of the selected sample is reduced and the design effect (DEFF) is increased.

If the number of households in the PSU is not known, but an estimate of the population size is available, then this information can be used to determine the expected number of segments required, based on the average household size in the country and the desired number of households per segment. For example, if there is an average of 7.4 persons per household, and the aim is to identify segments of around 100 households, then the expected total population of these households would be 740. If it is known that the PSU has a population of approximately 1350, the number of segments would be:

\[
1350 \div 740 = 1.8 \text{ (this will be rounded up to 2)}
\]

In this example, the PSU should be divided into two segments of approximately equal size, one of which should be randomly selected and its households listed.

If the total estimated population of the PSU is less than 450, segmentation is not usually necessary unless the average household size is below 2.5, equivalent to approximately 180 households. It should be possible to count the total number of households in a PSU that has fewer than 150 households and select the required number of households to be surveyed.
How to select households in a cluster

All households in the selected cluster must be identified, and each should have an equal probability of being selected. To ensure this, the listing teams should follow the instructions and complete the household listing form (electronic or paper-based) provided in the “Generic household listing form” online tool. In some situations, only households with a specific population group (for example children 6–23 months of age) will be selected for the survey, in which case information to identify these households needs to be included in the household listing. Only those households will be included on the list used for the selection procedures described below.
Module 7. Selecting households and participants

As mentioned in Module 6: Selecting clusters, most cluster surveys sample the same number of households in each cluster. This is generally conducted using systematic random sampling of the predetermined number of households from an ordered household list to obtain wide geographic distribution.

If the cluster mapping and household listing have been conducted in advance, then household selection needs to be done centrally once the listing forms are completed. If it has not been done in advance, the same process will be conducted in the field using the listing developed immediately prior to the survey sample collection.

This is an example from a MICS survey:

**Step 1:** In the first column of the “Household listing” online form, “Survey HH number,” start with the number 1 and assign a sequential number to each household listed that meets one of the three following criteria:
- Occupied residential dwelling;
- Household that refused to cooperate during household listing; or
- Household whose occupants were temporarily absent during household listing.

Leave the cell blank if the dwelling unit is not occupied or the structure is not a residential structure. For each cluster, the number assigned to the last household on the list corresponds to the total number of households for that cluster.

**Step 2:** Record this total number of households in the MICS template for systematic random selection of HHs1 (“MICS household selection template”). If the cluster was selected after segmentation, record the proportion that the selected segment represents in the PSU/EA in the “Proportion of the selected segment” column. See the “Mapping HH listing and segmentation” online tool for this information. If no segmentation was carried out, leave the value of 1 in the column “Proportion of the selected segment”.

**Step 3:** The MICS template for systematic random selection of HHs will automatically generate the numbers for those households to be interviewed in the survey for each cluster. The selected households should be indicated on the “Household listing” online form by circling the corresponding number in the “Survey HH number” column. If household selection is done in the field and if it is culturally acceptable, where possible, mark the number on the door frame of the structures selected, using a marker or chalk.

Other approaches are sometimes used to identify survey households, such as having a random starting household and then selecting households in a specified direction, or using the “next nearest household,” as is frequently done in Expanded Program on Immunization (EPI) surveys. This is not recommended for micronutrient surveys because it results in a less-dispersed random selection and increases the design effect (specifically, it decreases the likely diversity of selected households).

**Identifying and selecting eligible individuals in the household**

Rules for selecting individuals who meet different eligibility criteria need to be defined in the survey protocol. Methods include selecting all eligible individuals in each selected household, or randomly selecting one or more of the eligible individuals in a selected household, or selecting all or a random selection of individuals who meet certain eligibility requirements in every nth household, for example every 2nd or 3rd household. The agreed approach must be systematic and applied in the same way in all clusters. For example, if non-pregnant

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1 The [http://mics.unicef.org/tools#survey-design](http://mics.unicef.org/tools#survey-design) site has a useful Excel sheet for the random selection of households.
women of reproductive age is a population group of interest and there are three women meeting this criterion in the household, all three could be requested to participate. Alternatively, one of the three women may be randomly selected and requested to participate. (Note that in this case there would not be substitution by another woman if the first one refuses consent.) This methodology may be applied in all selected households or in a pre-determined number of households that are systematically selected from the household listing.

The decision on selection methodology is usually based on the target sample size for a specific population group, the proportion of this group in the population and the total number of selected households per cluster, as described in Module 5: Sample size. For example, in the 2015–2016 Malawi Micronutrient Survey where 22 households were selected per cluster, survey participants from different eligibility groups were enrolled according to the following schematic (see Fig. 7.4):

- All preschool-age children from all households
- All non-pregnant WRA from each of nine households
- All school-age children from each of six households
- All men from each of four households.

The required number of households for each eligible group was selected by systematic sampling from the 22 selected households. This schematic enabled the survey to meet the sample sizes required to achieve the survey objectives for each population group of interest, while ensuring that there was no unnecessary burden of purchasing and transporting survey supplies.

Fig. 7.4. Malawi 2015–16 data collection schematic indicating demographic group eligibility per cluster

<table>
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<th>Household</th>
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<td>Women of reproductive age</td>
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Module 8. Survey supervision and personnel

In this module, we will discuss:

- Survey oversight: Steering committee, technical expertise and subcommittees
- Personnel needs, organizational structure, and main responsibilities
- Field team numbers, composition and roles
- Survey field team recruitment
Module 8. Survey supervision and personnel

A high-quality, timely micronutrient survey requires strong supervision and appropriately skilled personnel to plan, implement, analyse, report on and manage the entire process.

Large-scale population-based surveys are generally initiated by the primary users of the information that will be collected. This is frequently the ministry of health, the national nutrition division/department, some other government body, an academic institution, or a nongovernmental organization (NGO). The initiating organization takes primary responsibility for implementation and assigns a senior-level person to chair the Steering committee and provide overall policy guidance and leadership.

Operational management and responsibility for survey coordination, implementation, reporting, and quality assurance are usually assigned to an experienced, full-time survey coordinator. The coordinator works closely with the Management team, and reports to the Steering committee at its meetings and at additional times as needed.

**Survey oversight: Steering committee, technical expertise and subcommittees**

**Steering committee:** The Steering committee provides high-level leadership, with representation from each of the key stakeholders who have identified a need for or will use the data from the survey. This representation helps to ensure that survey results are accepted, endorsed and used for programme improvements. These decision makers provide guidance throughout the survey process, and may be from:

- Government ministries, agencies, and departments (ministry of health, office of statistics, and others as appropriate)
- United Nations agencies (World Health Organization (WHO), UNICEF, World Food Programme (WFP), and others as appropriate)
- Academic institutions (national and international)
- Nutrition partners and stakeholders (national and international)
- Other donors
- Agency or organization implementing the survey

The Steering committee should meet at critical stages of the survey and as needed when challenges arise. Terms of reference for the committee should be agreed upon by all members. The usual primary functions of the committee are listed in **Box 8.1.**

**Technical expertise and subcommittees:** The Steering committee must ensure that the survey has the technical support necessary to advise and guide the Management team on survey planning, implementation, data analysis, reporting and dissemination decisions.

Technical expertise is often available from the senior personnel from the ministry of health, the national office of statistics (or equivalent), other ministries, United Nations agencies, NGOs (national and international),
academic institutions working in nutrition and other nutrition partners with technical expertise as appropriate. The survey coordinator, the laboratory coordinator, and the database manager can advise the Steering committee on what sort of technical expertise may be needed for the survey.

The Technical experts may be needed for subcommittees, such as protocol and questionnaire development, budget and finance, logistics and supplies, laboratory, and data and analysis. Roles of these subcommittees are described in Box 8.2. The number of individual experts to involve or subcommittees to establish, and their scope of work, will vary according to the national context and complexity of the survey. The survey coordinator should work closely with and participate in all subcommittees.

External survey support: In some contexts, the Steering committee may recommend securing contracts with outside experts (national or international) who have specialized skills for a specific aspect of survey planning or implementation. Such skills could include developing the survey design, calculating appropriate sample sizes, developing data entry forms for electronic data collection, and data management and analysis, including Computer Assisted Personal Interviewing (CAPI) programming and testing. In some cases, an external group may be contracted to advise and closely oversee the entire micronutrient survey, in partnership with the national team.

Personnel needs, organizational structure, and main responsibilities

In addition to the committees and subcommittees, there is a range of personnel needed to successfully implement a micronutrient survey. Complex surveys, especially in large countries, are likely to require multiple teams and a significant amount of logistical and supervisory support. Fig. 8.1 shows an example of an organizational structure for a national micronutrient survey. The organizational levels and main tasks for each role are explained further below. The table in the “Tasks and roles for survey personnel” online tool provides
Module 8. Survey supervision and personnel

an overview of typical roles and responsibilities for different survey personnel, teams and committees at the central, regional, and field levels.

Box 8.2. Survey subcommittees and their tasks

Protocol and questionnaire development
- draft, review and finalize the survey protocol, including workplan and timeline;
- define procedures for referring survey participants identified as having a health condition (for example, anaemia) in conjunction with the ministry of health;
- develop the survey questionnaires, the training, field, and laboratory manuals, the training plan and the outline result tables;
- develop listing and control forms for the fieldwork;
- develop, pretest and pilot test the survey questionnaire and all related tools.

Budget and finance
- develop the survey budget;
- secure mechanisms for disbursement of funds to relevant institutions;
- track the budget and expenditures throughout the survey process.

Logistics and supplies
- develop a detailed survey procurement plan in coordination with the Laboratory coordinator and the budget and finance subcommittee;
- prepare the necessary documentation for customs clearance for laboratory and field supplies;
- identify secure storage facilities for supplies;
- ensure that procurements are within budget and timeline constraints;
- prepare logistical and supply requirements for the training and fieldwork and submit to the Technical committee;
- ensure that all field logistical and supply needs are in place and timely, especially transportation and fuel;
- monitor the distribution and stocks of all supplies.

Laboratory
- prepare a costed list of laboratory supply and equipment needs;
- develop a detailed laboratory work plan for cold chain, transport, and analysis of all survey samples and specimens;
- prepare standard operating procedures for all methods;
- conduct assessment and selection of laboratories as needed;
- ensure laboratories are participating successfully in active external quality control systems,
- obtain necessary approvals to ship biological specimens (if needed).

Data and analysis
- prepare the sampling framework and select PSUs;
- determine whether to use paper-based or electronic data entry and develop a resource list for supplies and personnel;
- develop and test a protocol for data entry;
- develop a database and data management system and ensure that it functions,
- develop syntax to generate results according to the result table outlines.

Many of these tasks (for example, preparing a costed list of laboratory supplies) may be the responsibility of a single individual rather than an entire subcommittee, but the subcommittee should provide oversight and guidance on the developed tools.

All subcommittees should report to the Technical committee on all tasks, in line with the implementation plan, budget and timeline.
Module 8. Survey supervision and personnel

Survey management team: The management team provides overall management and coordination of all processes at the central and regional levels. Members usually include the survey coordinator, the deputy coordinator, administrative staff, the Data coordinator and the Fieldwork support team (see the subsection, Fieldwork support team below). Depending on the size of the area to be surveyed, the complexity of the survey, and the geographic distribution of clusters, the management team structure may be replicated in full or in part at a regional level. The management team also has overall responsibility for supervising fieldwork progress and monitoring indicators of quality assurance in close collaboration with the Technical committee. Good supervision is dependent on strong familiarity with all aspects of the survey methodology, therefore all personnel involved with supervision should participate in the entire survey training and piloting process.

Survey coordinator and Deputy coordinator: The Survey coordinator should have prior experience in managing large population-based surveys, should be assigned on a full-time basis, and must be able to participate effectively in all aspects of the survey. It is critical that he or she works on the survey full time to ensure the quality and timeliness of implementation. More information can be found in the “Job description for Survey coordinator”.

A Deputy coordinator should be included on the team from the initial design and planning stage to support the coordination, management and supervision of the entire survey team as needed. The Deputy coordinator backstops the Survey coordinator and shares duties when the workload is very heavy. The Deputy coordinator often plays a particularly crucial role during the training, pilot testing, and early days of survey fieldwork.

Administrative staff: Capable administrative staff support the Survey coordinator to manage survey processes and logistics, including human resources, procurement, photocopying and printing, financial management, transportation and accommodation.

Data coordinator and the central data team: The Data coordinator leads and has overall responsibility for the performance of the central data team, as well as for the collection, storage, transport, and security of data at all levels. The Data coordinator should have experience in coordinating data collection in large population-based surveys, in database management, and in supervising interviewers in the field.

The central data team is often located in the place where data are ultimately entered (and stored if paper-based). The central data team is typically composed of:

- A specialist programmer, if using electronic data entry, to develop the data collection form and manage the use of appropriate field devices, software and accessories
- A number of data entry personnel (see the “Job description for data enterer”)
- A data entry manager and data editor (if using paper-based data collection) (see the “Job description for data entry manager”)
- A database manager to assure the quality of data format, the checking, cleaning, and security of final data sets
- A statistician to ensure that analyses are conducted accounting for the survey design and that the output fits with the result tables agreed with the Survey steering committee and the Technical committee.

Fieldwork support team: The Fieldwork support team supports the team leaders, manages the efficient implementation and monitoring of fieldwork and troubleshoots any problems that arise. The Fieldwork support team includes the Field coordinator, the Laboratory coordinator, and Regional supervisors (for
fieldwork and, in some cases a separate regional supervisor for laboratory work). It may be part of the national Management team.

**Laboratory coordinator:** The Laboratory coordinator leads the laboratory team, which may be located in the national laboratory of the ministry of health or in an academic institution that has expertise in micronutrient analyses.

The Laboratory coordinator, in coordination with the Regional laboratory supervisor (where this role is included), oversees all stages of collecting, storing, and transporting biological specimens and food samples from the field to the laboratory. In addition, if sample or specimen analysis takes place outside the country, the Laboratory coordinator is responsible for the appropriate packaging, labelling, and shipping of samples.

The Laboratory coordinator needs to work closely with:

- The Field coordinator and the Survey coordinator, to ensure that supplies and equipment with the correct specifications are ordered, that they function well throughout the training, pilot, and fieldwork, and that they are provided in appropriate quantities to the appropriate Regional supervisor(s).
- The budget and finance subcommittee and the Survey coordinator to plan for and ensure that the budget is adequate to purchase all the supplies and equipment needed.
- The administrative staff to verify that the correct supply and equipment orders are made, to check on progress with shipping, expected arrival date, customs clearances and to arrange for transfer to an appropriate and secure storage area at the laboratory.
- Regional laboratory supervisors (where these are included), to supervise their role.

You can find additional information on the Laboratory coordinator’s role online in the “Job description for Laboratory coordinator”.

**Field coordinator:** The Field coordinator is responsible for survey logistics and fieldwork implementation according to the implementation plan. This person manages:

- Communication between the Survey coordinator and Regional fieldwork supervisors;
- Overall logistics for the survey fieldwork, including transport and accommodation;
- Survey implementation and data collection in the field.

**Regional supervisors:** Regional field and laboratory supervisors must have a detailed understanding of all survey aspects and related protocols to ensure the appropriate support, coordination, and monitoring of fieldwork and laboratory specimen collection and transfer in their region. The roles include the following functions:

- Communication between the Field coordinator and field teams to facilitate team logistics and travel (Field supervisor);
- Communication between the Laboratory coordinator and field teams to facilitate sample collection, storage and transport (Laboratory supervisor);
- Management of supplies and equipment for all field teams under their supervision; and
- Data storage and transfer (where paper-based collection is used) for teams under their supervision.
Fig. 8.1. Organizational chart showing personnel needs for a typical national micronutrient survey
Field team numbers, composition and roles

The size and composition of each field team, as well as the type and quantity of field equipment and supplies required, depend on the number of households to be visited within each cluster, the number of people to be interviewed, the expected length of individual interviews, the number and type of data and biological specimens to collect, and whether anthropometry is included. Additional determining factors include the expected travel time between clusters and the number and size of vehicles available.

Fieldwork is usually organized with an aim to minimize the time in any one cluster while also limiting inter-individual error, which can occur with large numbers of interviewers.

Micronutrient surveys generally require substantial amounts of equipment and supplies as well as multiple team members. This may make it necessary to have a bus-type vehicle or two cars for each team. Having two vehicles per team can be helpful in case of cold chain failures, vehicle malfunction, and for transporting additional passengers such as community advocates, local guides, and village leaders. It also allows one sub-team to move ahead to mobilize the next cluster, while the other remains behind to complete interviews or specimen collection. Depending on the mix of indicators and the relative time required for interviews versus sample collection, it may be reasonable to have two interviewer sub-teams covering one cluster each, while a single team of phlebotomists and anthropometrists covers both clusters. It is worth the time for the Management team to work through various potential team and fieldwork configurations to create the most efficient model for the survey design and context.

Fieldwork support team: At the start of survey fieldwork, it is important that the Fieldwork support team (Field coordinators, Laboratory coordinators, and Regional supervisors) visit as many teams as quickly as possible to verify the quality of field implementation. It is recommended that survey fieldwork should begin with all teams working in relatively accessible central locations. Initial field assessments will review the quality of data collection, help make any adjustments where needed, and identify any retraining that might be required. This is logistically easier to do if teams are near a central site. The Fieldwork support team should also provide strong support, oversight and encouragement to field teams during the later stages of a large survey when data quality may otherwise suffer as a result of field team fatigue and low morale.

Field team members: A typical micronutrient survey field team consists of

- 1 Team leader
- 1 Interviewer supervisor (if divided into sub-teams)
- 1 Laboratory supervisor (if divided into sub-teams)
- 2–4 Interviewers (possibly more if divided into sub-teams)
- 1–2 Field laboratory technician/phlebotomist
- 1–2 Drivers
- Additional personnel depending on survey objectives

The number of teams needed to perform the survey depends on multiple factors, including the budget, the sample size, the number of clusters selected, the amount of time needed to complete the survey in each cluster and the number and type of vehicles, personnel and equipment for each team. The smaller the number of teams, the greater the consistency of data collection will be. However, this must be balanced with the burden of longer field time for each team. In contrast, a large number of teams results in quicker overall
implementation, but with potentially less standardized data collection and possible inefficient investment in training additional staff.

To optimize standardized survey implementation, it is recommended to train the smallest number of teams that can feasibly collect the required data in a quality-assured manner over a reasonable period of time. Backup field personnel will also be needed, although this must remain within the budgeted cost for survey training and implementation. If resources permit, it is a good idea to train more personnel than are needed in order to select the best and have some people in reserve if needed.

Where the survey includes anthropometry, multiple members of the team can be trained to conduct the measurements. There may be a main measurer and an assistant measurer. Specification of whom to train for these roles will depend on the expected site of specimen collection. If collection takes place in a mobile field laboratory, then the phlebotomist and laboratory technician may take on the roles of the anthropometrists. If collection takes place at the household, the interviewers may be trained to carry out this task. It is most important that any person collecting length/height and weight measurements is adequately trained in this process before going to the field and that the tasks are not performed by any other person.

Roles of the various members of the field team are described briefly below. Sample job descriptions with detailed tasks and responsibilities for each of the positions are available in the “Tasks and roles for survey personnel” online tool.

Team leader: Each field team should be supervised by a Team leader. As the senior supervisory member of the field team, this person must understand all aspects of data collection and field logistics in order to manage and supervise the implementation of all appropriate procedures specified in the protocol. In surveys with large teams and/or sub-teams the Team leader role may be divided between an Interview supervisor and a Laboratory supervisor. Whether the role is individual or shared, the overall responsibilities include:

- coordinating and communicating with community leaders at all levels to organize data collection;
- monitoring, supervising, and supporting team members to ensure quality performance;
- quality control checking of approximately 10% of interviews conducted in each cluster;
- regular observations of each interviewer and discussion of any concerns;
- daily checking and editing all completed questionnaires (paper-based or electronic);
- conducting interviews on an as-needed basis, for example, if an interviewer is absent;
- managing survey equipment and supplies, checking availability and functionality;
- supervising phlebotomists and field laboratory technicians to collect and process specimens and to maintain the cold chain.

At the end of each day, the Team leader should make time to discuss with the team members, assess any issues that arose and plan how to address them. Examples of individual mistakes should be handled sensitively and as privately as possible, although some discussion of examples in a group can create awareness and help the team improve together. Encourage team members to support each other within their respective roles.

If the Team leader finds that tasks such as interviewing, anthropometry, or biological data collection are being conducted with consistent errors, it is important to stop the fieldwork and conduct refresher training on an individual or team level, as needed. Any such incidences should be reported to the appropriate Regional supervisor, who may check whether the same concern is being experienced by other teams. Trained backup people should be available to replace any team member who has consistent problems with collecting data of
sufficient quality and whose performance does not improve with additional supervision. This replacement may also be needed in cases of persistent illness or absence of a team member (see online “Job description for the Team leader”).

Interviewers: Interviewers carry out all interviews and food sample collection. They may also be trained to perform anthropometry tasks (see online “Job description for the interviewer”). The responsibilities of interviewers include:

- collecting and checking information from survey participants (including informed consent);
- collecting household food samples where required;
- transferring all information and any food samples to the Team leader or other designated person, according to the survey protocol; and
- providing guidance to participants on any additional components of the survey, for example what to expect and where to go for specimen collection.

Phlebotomists and laboratory technicians: Phlebotomists and laboratory technicians are responsible for

- collecting any blood and urine specimens;
- field processing of blood, urine, and stool specimens, as needed; and
- maintaining the cold chain until collected specimens are transferred to the district or regional laboratory.

See online “Job description for the Phlebotomist” and “Job description for laboratory technician”. These team members may also be trained to perform anthropometry tasks.

Drivers: Drivers are responsible for transporting team members safely to each destination. Ideally, they should have the mechanical skills to maintain the vehicle and fix vehicle problems on the road. Drivers should also be able to assist the survey staff when needed, for example, in carrying equipment. It is the driver’s responsibility to keep the vehicle and equipment inside the vehicle secure and, where needed, to help maintain the cold chain by keeping portable freezers plugged in to the vehicle or assisting with the setup of portable generators. Drivers should be under the supervision of, and report directly to, the Team leader and must not leave the survey team without permission (see online “Job description for driver”).

Community advocates and focal points: Appropriate community mobilization should have taken place prior to the survey. On arrival in each cluster, the Team leader will contact and establish a good working relationship with the village/community leader. The community leader may be requested to recommend one or more people from the cluster to serve as community advocates for the survey team. Sometimes called local focal point or local guide, this may be the community health worker or another recognized position in the community. Where household listings are conducted in advance of the survey team’s arrival, the listing team can introduce the concept of the survey to the community leader and work with the local focal points to identify cluster boundaries and landmarks. The focal points can provide information about and assistance with locating survey households, health facilities, stores, and lodging, among other things. The local focal point may also help carry supplies, find an appropriate site for the mobile laboratory (if required), and generally facilitate survey implementation. Importantly, the involvement of a local focal point can generate trust by and access to community members and households to be surveyed. Local focal points need to be available during the entire time that a team is in the cluster/community and should be properly compensated for their time spent with the team.
Module 8. Survey supervision and personnel

See the “Terms of reference for local focal points” in the online tools.

Survey field team recruitment

The composition and skills of the survey field team are essential to ensure that the work is performed systematically and is of high quality. Personnel must be qualified, highly motivated, and available to work full time for the duration of the survey training and field work.

Key skills for interviewers include the ability to:

- efficiently ask questions, record and manage information in both paper-based and electronic-based formats according to the expectations of the role and the training guidelines;
- enlist household cooperation, put respondents at ease and ask questions in a calm, natural way;
- demonstrate an understanding and consideration of any local cultural practices or beliefs that might affect a respondent’s willingness to respond to certain questions;
- troubleshoot problems that may arise; and
- communicate and work as part of a team.

Specific skills required for phlebotomists and field laboratory technicians include the ability to:

- collect and process biological specimens according to the survey protocol;
- conduct phlebotomy correctly and quickly (whether venous puncture or capillary (finger stick or heel stick collection), in particular from young children, where this is a requirement for the survey;
- collect samples in a calm, confident manner, putting the respondent at ease; and
- demonstrate an understanding and consideration of any local cultural practices or beliefs that might affect a respondent’s willingness to provide biological specimens.

Some national contexts require that government nurses, phlebotomists and laboratory technicians are included on each survey team. Where these staff are not able to be away from their usual job for the duration of the training and field work period, it is recommended to assign them to work only in their home region. This strategy has the advantage of potentially improved trust and survey response rates among the community due to the government personnel’s understanding of the local culture, traditions, and language, and because they are a recognized part of the government health system. In these situations, it will be necessary to recruit a larger number of personnel to participate as part of the survey team, each for a shorter period of time. This will increase the demand for training and supervisory support.
In this module, we will discuss:

- Anthropometry
- Collecting, storing, transporting and analysing biological specimens and food samples
- Communication and data collection
- Labelling procedures
- Supply lists and ordering
- Storing, tracking, inventory, and transport of supplies
Having the correct equipment and supplies, and having enough of them, are essential to the success of any survey. The Management team, in consultation with the Technical committee and subcommittees, will need to invest time to review each aspect of the survey and the related needs for supplies and equipment.

A number of components of the survey design must be confirmed in order to develop an adequate procurement list:

- whether or not to include anthropometry in the survey, and for which population groups (see Module 1: Planning and designing a micronutrient survey);
- which specimens and food samples to collect, and how they will be collected, transported, stored, and analysed (see Module 2: Indicators of programme coverage, specimen selection, management and analysis);
- which laboratories will be used to analyse samples and specimens, and whether or not they are in the country. If analyses will be done in another country, there will be shipping requirements to consider (see Module 2: Indicators of programme coverage, specimen selection, management and analysis);
- whether data collection will be paper-based or electronic (see Module 4: Survey design);
- whether reimbursements will be provided to participants (for example, replacement food samples); and
- how labelling will be done, for example by barcode or other methods.

This module first discusses considerations for the points above that are not included in other modules, then it provides guidance on initial and final listings, procurement, storage, calibration, transport and tracking of equipment and supplies.

**Anthropometry**

The main equipment required for anthropometric measurements are:

- scales for measuring weight
- boards for measuring height and length
- mid-upper arm circumference (MUAC) tapes
- standard weights and length measures for calibrating the equipment.

Issues to consider when selecting anthropometric equipment include:

- The population group(s) being assessed.
- The availability of equipment used in a previous survey. If it is not possible to purchase new scales, those available from a previous survey should be recalibrated by the manufacturer before reuse. The manufacturer should provide a warranty that the scales have been properly recalibrated to their original standard. 28
- The budget available for purchasing equipment that has the required accuracy, reliability, portability and ease of use. If purchased internationally and shipped, allow time for shipping and release from customs, as well funds for potential shipping fees.

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Module 9. Survey equipment and supplies

The “Equipment and supplies list (complex worksheet)” online tool provides details of anthropometry equipment recommended for field surveys.

Collecting, storing, transporting and analysing biological specimens and food samples

The equipment and supplies required for biological specimen and food sample collection, transport, storage and analysis depend on the selection of biomarkers and related methods of analysis. Module 2: Indicators of programme coverage, specimen selection, management and analysis describes possible options and factors related to these decisions.

The list of equipment and supplies varies according to the following main factors:

• the method of blood collection (venous or capillary) and type of blood fraction required for specific biomarker analysis;
• specific nutrient-related equipment requirements (for example, zinc requires the use of zinc-free tubes);
• the method of assessing haemoglobin for anaemia (field based or central laboratory);
• inclusion of urine specimen collection (for example, for assessing iodine);
• inclusion of stool specimen collection (for example, to assess parasitic infection);
• types of household food sample(s) to be collected for analysis, for example salt, oil, sugar and flour; and
• inclusion of the test for presence of food nutrients during fieldwork, for example iodine in salt, using a rapid test kit.

For each biological specimen or food sample, the equipment, supply and budget-related needs should be listed for the following categories, as appropriate:

• biological specimen or food sample collection and handling (including disposal of collection devices and other supplies);
• biological specimen or food sample labels (resistant to rubbing off);
• bar code reader;
• field testing of biological specimens (such as point of use haemoglobin testing) or food samples (such as rapid qualitative iodine testing for salt samples);
• field processing of biological specimens (including adding ascorbic acid to whole blood for preserving or centrifuging whole blood and aliquoting serum prior to freezing specimens);
• storage and transit to the central laboratory, including any power adapters required to use the power supply of the survey vehicle;
• cold chain requirements;
• storage at the laboratory; and
• laboratory assessment and shipping costs if being sent to a laboratory out of the country.

The “Equipment and supplies list (complex worksheet)” online tool provides a detailed list of supplies for collecting different biological specimens and food samples. This should be used together with the information in Module 2: Indicators of programme coverage, specimen selection, management, and analysis and in Module 3: Biomarker selection and specimen handling, as well as the “Standard operating procedures for ordering supplies and equipment” online tool, in consultation with experts in planning this type of fieldwork.
Communication and data collection

Electronic data collection and internet connectivity are fast-evolving fields of technology. The expected connectivity in different areas of the country needs must be considered and appropriate equipment (e.g. phones and SIM cards) for communication within the team and with regional and central supervisors are required.

Electronic data collection

Devices: There is a wide range of mobile data collection devices to choose from. The choice of which ones to use will depend on a number of factors, primarily functionality, comfort of use for long periods of time, availability and cost. The “Electronic data collection hardware” online tool provides a list of factors to consider when selecting the devices as well as typical additional hardware needed per enumerator, team, and Regional supervisor.

The section below on labelling describes the need for easy-to-use scanners and camera settings. The ability to install and use these also needs to be taken into consideration when choosing devices.

Accessories: The mobile devices must function reliably without loss of data throughout the fieldwork period. Interviewers need to be able to recharge device batteries regularly, and data should be uploaded consistently to back up the information and monitor fieldwork progress.

These actions will require power sources, data storage capacity, and connectivity. Mobile wireless internet devices (similar to hotspots for wireless internet devices) might be needed in areas of low connectivity. The number and power of portable battery packs needed will depend on the anticipated number of days away from a standard power source. It may not be necessary to upload data every day, however this option should be considered in addition to storing a copy of the data on the device and options for data backup. The Survey coordinator will also need access to a computer to review uploaded data and generate monitoring and progress summaries.

Where data are collected electronically, some paper-based versions of the questionnaire should be available to each team in case of hardware malfunction. There may also be areas of a country where carrying a mobile device could be a security risk, in which case paper forms may be preferred.

Paper-based data collection

The main equipment and supply requirements for paper-based data collection are paper, printers and printer ink, a photocopier, clipboards, pens, pencils, erasers, staples and staplers and other basic stationery. Storage containers for blank and completed questionnaires will also be needed, along with filing space with secure storage facilities, and access to computers for data entry. These are listed in the “Equipment and supplies list (complex worksheet)” online tool.

Food sample replacement

If food samples (such as salt, sugar or flour) are collected as part of the survey, the household may be offered a replacement of the same type of food. In cases where a quality-assured fortified version of the food is available, for example adequately iodized salt, then the fortified food should be provided as the replacement.
Module 9. Survey equipment and supplies

In some surveys, participants may be offered a beverage, depending on the tests being undertaken and the likely duration of their participation. In cases where participants must walk some distance to a mobile or central field laboratory for specimen collection and anthropometry, they may be offered a small snack or beverage at the end of the process.

The costs associated with replacement food or reimbursement items need to be included in the budget. Additionally, all field team members should be aware of the logistics of purchasing, securely storing, transporting, and documenting the distribution of these items to the participants.

**Labelling requirements to consider when purchasing or generating them**

Proper and accurate labelling is one of the most important aspects of a survey. Each selected survey household and participant must be assigned his or her own unique identification number (ID). The household ID should reflect the cluster ID and household number. Any individual ID should include reference to the household ID. In this way, results from a participant can be linked with information from his or her household, and data from each household can be linked with the cluster from which it was selected. Labelling also needs to take into consideration hierarchical data, for example, how a selected child is linked to a selected woman and her selected household.

Because labels play such an important role in linking data from an individual and his or her household to the corresponding samples and specimens, the Laboratory and Field coordinators should work with the Survey coordinator to agree on the final label template early on in the survey planning process. Forms, database entries, and training guidelines can then be designed accordingly and related supplies (such as labels and scanners) ordered. Labels need to be received (if ordered) or printed before the training and fieldwork. Labels can be printed in many formats and can include a variety of information, that sometimes depends on the size of the label.

Special labels are required for training and field piloting. These labels should have distinct codes, for example, easily apparent different numbering systems with a unique prefix or by adding a suffix “T” to the same numbering that will be used in the fieldwork. In this way, forms and samples from these processes will not get confused with those from the final survey fieldwork. The best labels and printer ink to use are those that can adhere to any surface (paper, storage vials, plastic), will not peel off when the vials are stored in freezing temperatures, and will not smear if they come into contact with water. You can find additional useful information on labelling in the “Tips for labelling specimens” and “Overview of labelling procedures” online tools.

**Supply lists and ordering**

The Management team should develop an initial list of equipment and supply requirements to meet the survey objectives and agreed data collection method. This will be used when developing the preliminary survey budget estimate, and is discussed in detail in Module 10: Budget and timeline.

After approval of the initial supply list and associated budget estimates, the Management team should develop a fully comprehensive list, usually in a spreadsheet. The “Equipment and supplies list [complex worksheet]” online tool, modified according to the specific survey design and factors listed earlier can be useful for this process. Bear in mind that purchasing supplies requires a significant amount of time, which affects the project
Module 9. Survey equipment and supplies

timeline. Training should begin only when all necessary equipment, including laboratory equipment, is on hand.

The Laboratory coordinator and the Survey coordinator should take the following steps in the procurement process, with assistance from administrative staff:

- Develop a comprehensive list of equipment and supplies for each sample and specimen collection and planned analysis. The list must include specific item reference numbers and required quantities.
  - Final quantities will depend on the number of clusters and the final composition of field teams.
  - The list should also include supplies that are needed for training and piloting all field procedures.
  - It is standard practice to increase the quantities by 15% to 20% above the calculated needs, to ensure that adequate supplies are purchased.
- Identify equipment that may already be available or can be borrowed, for example, anthropometry equipment or data collection devices.
  - Available and borrowed items should be of high quality and need to be thoroughly checked at the time of finalizing the list, in case the items do not function well and need to be procured elsewhere. Budgeting also needs to cover replacement of any borrowed equipment that is damaged or lost during the survey.
- For items that will be purchased or rented, determine whether to procure them locally or from international suppliers. Although procuring supplies locally can significantly reduce costs, the majority of supplies for a micronutrient survey usually need to be procured from overseas to meet the necessary standards for quality.
- Begin the requisition for supplies and equipment through the appropriate approval channels. This will vary depending on the agency responsible for procurement and release of funds.
  - This must be done by someone with a working knowledge of the national system for generating a purchase order (PO) and requesting estimates from vendors.
  - This step requires significant attention to detail to ensure that the POs list the correct items and quantities. It is recommended that more than one person conduct an extensive review of the POs prior to requesting estimates.

Storing, tracking, inventory, and transport of supplies

Storing: Before any supplies are purchased, secure storage should be identified by the Management team. Ideally, the storage facility should be near the office of the Laboratory coordinator and/or the training site. It should be of adequate size, clean, well-ventilated and cool, as some supplies might deteriorate if exposed to excessive heat or moisture. Supplies should be well organized in the storage facility, and only the required people should have access. If supplies must be stored in multiple locations, the same conditions should exist at all sites.

Tracking: The Laboratory coordinator is usually responsible for the receipt, organization, distribution and tracking of supplies and equipment from the laboratory to the field and national laboratories. The Laboratory coordinator should develop a tracking sheet that will be used in coordination with the Survey coordinator. A tracking sheet should include the following information for each item:

- item description
- quantity ordered
- quantity received
Module 9. Survey equipment and supplies

- date of receipt in the country
- date of clearance from customs
- storage location
- condition of item
- any additional useful information.

A similar sheet should be developed for distributing equipment and supplies to field teams at the start of and throughout the survey fieldwork. This sheet should include the item, quantity, date of distribution and intended recipient, with a duplicate copy for the Regional supervisor or Team leader to sign when they receive the material. More information is available in the “Supply tracking and distribution” online tool.

Management of equipment and supplies is a crucial activity during survey planning, training, pilot and fieldwork. Good management will limit any delays of the training and fieldwork, and ensure that all teams have sufficient quantities of correct supplies to carry out their work.

Inventory and equipment checks: Before going to the field, each team should complete an inventory to ensure that there are adequate supplies for the assigned fieldwork over a defined period. The team should also check that all equipment is in good working condition, with all necessary components. If portable freezers and centrifuges are included in the survey, a servicing electrician should test them before teams disperse to the field.

Some equipment needs to be tested on a routine basis in the field. Haemoglobin photometers, for instance, should undergo daily cleaning, maintenance and testing during data collection. All electronic and mechanical equipment should be routinely tested according to manufacturer’s recommendations to ensure that they are in good working condition. All portable electronic devices should be fully charged and backed up each night so that they are ready for the next day. Any equipment malfunction should be immediately reported to the Team leader, who should report it to the appropriate Regional supervisor. Backup devices should be stored at a site that is accessible to teams during fieldwork.

In addition, the vehicles used for the survey need to be serviced and in good working condition prior to the start of data collection, and they must be well maintained throughout the fieldwork period. Vehicle breakdown can cause major delays during data collection and make it difficult for the field teams to complete their work.

Transport of supplies: Secure storage and transport of survey supplies can be complex, depending on the number of teams, the composition of the teams, and the types and quantity of equipment and supplies needed daily. Certain equipment, such as portable freezers, centrifuges, and tents require quite a bit of space. There should be a clear chain of responsibility for equipment and supplies. For laboratory work this chain goes most frequently from the Laboratory coordinator to the Regional supervisor and the Team leaders.
Module 10. Budget and timeline

In this module, we will discuss:

- Factors to consider when developing a budget
- How to develop a survey timeline
Micronutrient surveys generally require large budgets and an extensive planning period. The survey preparation phase must be given sufficient time and attention, with national ownership and commitment from the highest possible level. Budgeting and planning need to allow for the ethical review process, the procurement of specialized laboratory supplies from international companies, conducting cold chain assessments, recruiting specialized staff and analysing specimens and samples for a range of micronutrients.

The Management team, the Steering committee and the Technical committee should be kept informed of any major changes that might alter the overall budget or timeline for the survey. Challenges that may arise in securing funding following changes to an agreed-upon budget will require input from high-level oversight, and advanced planning to ensure survey success.

**Factors to consider when developing a budget**

The principal costs for equipment and supplies for a micronutrient survey concern collecting and analysing biological specimens and food samples, alongside the cost of field work. During the initial design phase, a preliminary budget is developed to ensure that the design of the survey, the selection of the target groups and the planned analyses of biological specimens can be covered with the available funds.

The “**Generic budget**” online tool can be used to develop a preliminary budget that covers at least the following categories:

- personnel salary and per diem, including accommodation
- transportation
- equipment and supplies
- storage facilities if required
- laboratory analyses
- selecting clusters, updating maps, and/or carrying out a census of clusters
- social mobilization
- training
- pilot testing
- shipping of specimens and/or samples internationally
- data analysis
- report writing and printing
- meetings, in particular for planning and dissemination.

The preliminary budget should provide sufficient detail for estimating funding requirements for essential survey activities. If the estimated costs exceed the available funds, additional funding will need to be obtained. This may require delaying implementation for a defined period, or adjusting the main survey objectives or survey design (such as the number of strata, population groups, or biomarkers assessed). Different options to reduce costs should be proposed, discussed, and agreed to by the Steering committee and the Technical committee.

The budget should then be revised based on any agreed revisions to the survey design, and additional details should be provided. A detailed budget should be subdivided by the major categories (such as personnel, transportation, training and laboratory analysis) with comprehensive line items developed and refined.
Module 10. Budget and timeline

For each row in the budget spreadsheet, there should be columns providing details about the unit (such as “person” or “item”), the number of units required, the number of days or months (if applicable), the unit cost in the local currency, and the total amount in the local currency. If needed, the costs in local currency should be converted to US dollars or other relevant currency, with a note that specifies the date and the exchange rate used. The spreadsheet may also include columns that identify the sources of funding for specific line items, as well as any restrictions, including time frames, for the use of the funds. The “Generic detailed budget” online tool can be used to develop this more detailed form for costing and tracking of expenses.

Once funding is secured, it is important to define the mechanisms by which money will be distributed to the implementing organizations. The detailed budget should be used to track costs closely and to prevent budget overruns. Where such overruns are unavoidable, early detection is important to identify additional resources or change the scope of work to fit activities and outputs within the available budget.

How to develop a survey timeline

Depending on the complexity of the survey, planning, implementation and reporting can take anywhere from months to years to complete. A key part of the planning process is the development of a detailed workplan and realistic timeline that defines major activities and stipulates who is responsible for each.

Time needed for planning is frequently underestimated, and it is common for those unfamiliar with micronutrient surveys to believe that the lengthy planning process will not apply to their setting. It is recommended to assume a minimum of nine months, often as many as 12 months, to complete all preparatory tasks before the training and fieldwork can begin. Long delays in the proposed timeline may affect the availability of personnel within the Management and/or Field team as well as the effectiveness of the supplies that have been ordered. The Survey coordinator is responsible for developing the timeline and for tracking activities.

To develop a timeline, it is standard practice to start by determining the timing of training and data collection (fieldwork). Fieldwork often needs to be completed during a particular time period to avoid:

- adverse climate (for example the rainy/monsoon season or winter snow);
- political events (such as a presidential election); or
- religious and/or cultural periods where lifestyle and dietary practices may be different than usual (for example Ramadan, or the Christmas holiday season).

It will also be important to bear in mind intervention-related events that link to survey objectives, for example biannual vitamin A supplementation or the start of a pilot project.

Once the fieldwork date is agreed and scheduled (with some flexibility), the remaining activities should be listed in the order to be completed, recognizing that the timing of some activities will overlap with others. For example, supplies can be ordered at the same time the survey questionnaire is being developed.

Timelines should include these elements:

- a comprehensive list of activities organized sequentially, sometimes split into different phases, such as design, planning, implementation, analysis, documentation and dissemination;
- the person or agency responsible for each activity;
- the target date for completing the activity; and
- a space for documenting the date the activity was actually completed.
Module 10. Budget and timeline

See the “Generic timeline” Gantt chart online tool showing examples for specific survey phases.

Tasks that should be started as early as possible include procuring laboratory supplies and equipment (see Module 9: Survey equipment and supplies) and hiring the most appropriate implementing agency for data collection. Hiring is a process that may take several months. Supplies and equipment that are internationally procured also may take numerous months for release from customs and can accrue customs fees, which requires up-front planning. Timelines are working documents and need regular review and updating. It is normal to make changes to a timeline during survey implementation.
Module 11. Data collection tools, field manual, and database

In this module, we will discuss:

- Developing and testing the main survey questionnaire
- Developing other survey data collection tools
- Information about informed consent from respondents and assent from children
- Detailed steps to assess the age of a young child
- An outline and contents of a field manual
- Important features of database development to ensure standardization
Module 11. Data collection tools, field manual, and database

Data collection materials include the main survey questionnaire and other forms (or tools), such as the specimen collection control form, cluster control form, and anthropometry data form. These tools can be paper-based or electronic, or some of each. For example, where the main survey questionnaire is in electronic format, some of the control forms may be paper-based. See Module 4: Survey design for a more detailed comparison of the use of electronic- or paper-based data collection for the main survey questionnaire.

The Survey coordinator and the relevant technical subcommittee need to spend considerable time developing the questionnaire and related survey tools to ensure that they function as intended so that all collected data can be correctly processed and linked with parallel information, such as food samples or biological specimens. The data collection tools should be as concise as possible while ensuring that they measure indicators that meet the survey objectives. This may be a challenge in a situation where the Technical committee has representation from a wide range of disciplines.

The survey questionnaire and other data collection forms will be extensively reviewed, and interview techniques will be practiced during training. Pilot testing all aspects of the survey generally takes place at the end of the training. The questionnaire should be tested:

- in one or more local languages, as appropriate
- using the same data collection method (paper-based or electronic) that will be used during the survey
- in conditions similar to the field to simulate the actual data collection
- in a location that is not going to be visited as part of the survey.

Following the pilot testing, all survey teams should convene and discuss the experience and make any necessary modifications to the questionnaire and other data collection forms. More details can be found in Module 12: Training and pilot testing.

Developing and testing the main survey questionnaire

The survey questionnaire should aim to gather information that will fulfil the survey objectives as well as provide data for monitoring current strategies and planning for the future. It needs to be clear how the results of the questionnaire will be used, how they will relate to other results, and whether the inclusion of a particular indicator can potentially affect overall programme management. In circumstances where the results of a specific part of the survey will be compared with the results from a previous survey, the wording of the questions and the population groups addressed should be as similar as possible across the two surveys.

It can be helpful at this stage to develop empty report tables (called “shells”) to show how final results could be presented. Each table can be accompanied by notes describing how the data will be collected and how they relate to the survey objectives.

The questionnaire is usually designed to collect certain types of information. This may include:

- Information about the main micronutrient-related indicators stated in the survey objectives, for example
  - confirmation that the biological specimens or food samples were collected from respective individuals or households
  - results of any field-based testing (for example, the haemoglobin concentration or the result of a rapid diagnostic test for malaria).
- Information that can facilitate cross tabulations, such as
Module 11. Data collection tools, field manual, and database

- demographic information
- socioeconomic information
- information reflecting respondent knowledge, attitudes, and practices (KAP), usually in relation to micronutrient interventions
- participation in nutrition interventions.

• Information that helps interpret measurements of micronutrient biomarkers (see Module 3: Biomarker selection and specimen handling for further information) such as
  - pregnancy status
  - lactation status
  - use of supplements
  - cooking practices that may affect the micronutrient content of food
  - time of day that specimens were collected (if biomarkers are subject to diurnal variation)
  - approximate time since last meal
  - altitude of the cluster location
  - whether the respondent is a smoker and, if so, the average number of cigarettes smoked per day.

• Information to assess the impact of certain nationally-relevant practices on micronutrient status, for example
  - the frequency of using salt substitutes (such as bouillon), to estimate the potential contribution of this dietary habit to iodized salt intake
  - behaviour related to a culturally specific infant feeding practice, to assess the impact of a targeted behaviour change communication campaign
  - the use of cooking ash, which affects food’s acidity and in turn affects the bioavailability of micronutrients in multiple micronutrient powders.

When questionnaire modules are similar to those included in the UNICEF Multiple Indicator Cluster Survey (MICS) and the Demographic and Health Survey (DHS), they should use similar design and wording. Using the same or similar wording of questions and similar tables for presenting results makes it easier to compare with other surveys conducted in the country as well as with surveys conducted in other countries. The UNICEF MICS questionnaires are available in English, Spanish, French, and Russian (http://mics.unicef.org/). The DHS model questionnaires are available in English and French (https://dhsprogram.com/What-We-Do/Survey-Types/DHS-Questionnaires.cfm). Standard methods for analysing the data to fill suggested table shells are provided in the MICS and DHS documentation, available on the respective websites. Suggested wording for questions used in micronutrient surveys among different population groups can be found online in the example questionnaires from Malawi (men, women, child), Uganda (household, women, child) and Afghanistan (household, children under five, school-aged children, and women).

New questions that have not been part of previous surveys need to be developed systematically, preferably following the cognitive interviewing process described in this module.

The overall format of the questionnaires will vary by survey, depending on the objectives, the number and types of micronutrients being assessed, population groups and interventions of interest. All formats must include standard identifying information for each household and participant so that information for each individual can be related to the respective household data and to samples and specimens collected. Sometimes it may also be useful to link the data for specific household members. Unique barcode labels, which are discussed in more detail in Module 9: Survey equipment and supplies, are the recommended method when collecting this information.
Designing an electronic data collection program, with all the required logic and error checks, can take many months. Developing the electronic data entry systems would ideally take place after the paper-based versions of these tools have been pretested with appropriate target populations (see the sequence of development in Fig. 11.1).

Interviewing should be done in the language most familiar to the participant. This may require translating the main survey questionnaire into several languages. Later sections in this module provide guidance on translating and pretesting questionnaires.

**Cognitive interviewing**

Cognitive interviewing is used to identify and resolve problems with the wording of survey questions and the flow of the questionnaire. This process should be conducted as part of questionnaire development, in parallel to or immediately after pretesting, as it will lead to greater reliability and better interpretation of the final survey questionnaire and outcomes. The process should be applied with each main language into which the tool has been translated.

Cognitive interviewing also helps to identify problems that are not always obvious when designing survey questions, such as:

- differences between cultures or individuals in interpreting questions and the intention of answers provided;
- participants not understanding, or being confused by, certain words;
- participants not understanding, or not knowing, how to answer;
- participants not being willing to respond truthfully to a question;
- ambiguity or poor fit between a question and the options for response;
- redundancy of questions that add no new useful information; and
- problems with measuring the concept the question is trying to address, for example, questions that expect a knowledge of typical serving sizes or defining the intended period of time for a recall question.

Box 11.1 provides examples of how cognitive interviewing can improve micronutrient survey questions.

There are several approaches to conducting cognitive interviews. The approach described here is “concurrent cognitive interviewing”, which are one-on-one interviews where an interviewer asks the survey questions and the participant responds. After each question is asked and answered, the interviewer probes about that question and the participant’s response, seeking information to better understand the participant’s interpretation of the question and the basis for the response. Sample probing questions include “What did you think that question was trying to ask?”, “Can you repeat that question using your own words?” and “How did you come to that answer?” General probes often help identify problems that were not anticipated, and tailored probes can focus on areas that are anticipated to be problematic. Interviewers should write notes during the interview to document responses, problems, and potential solutions.

Initially, five to ten interviews are conducted with a range of people from the intended survey participant groups, then responses are analysed, and the questions are revised if needed. Additional interviews are carried out with new participants to test the revised questions. The process is repeated until the final questions are agreed.
It is important to identify the most essential questions that require testing. These are most likely to be questions that have not been included in previous surveys or not in one or more of the languages of the planned survey, or questions that are challenging to phrase, or those intended to measure prioritized indicators. Box 11.2 provides more detail on the concurrent cognitive interview technique.

The sequence of processes required to finalize the data collection tool is shown in Fig. 11.1.

**Translation and back-translation**

The general process set out by WHO for translating survey tools can be applied to micronutrient surveys. The key steps are:

- Finalize the survey tools in English or another applicable language (such as Spanish, French or Portuguese).
- Develop translated versions in each of the languages needed for the micronutrient survey. The translation should be conceptually equivalent (have the same meaning), culturally appropriate and easily understood.

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Module 11. Data collection tools, field manual, and database

- Back-translate the questionnaires to the original language to ensure that the content and meaning of the questions have been maintained.

Translation and back-translation should be done by two different individuals who are fluent in the language of the original questionnaire, whose mother tongue is that of the translated questionnaire, and who have not been involved in the questionnaire development. Box 11.2 provides more detailed information on the process put forward in the WHO guidance.

**Box 11.2. Tips for the concurrent cognitive interview technique**

Concurrent cognitive interviewing uses a structured interview guide with general and specific probes for each question. The interview guide helps ensure that all probes are asked.

Interviewers should be encouraged to help identify potential solutions to the problems that may arise by, for example, suggesting and testing revised wording during the test process.

At the beginning of the cognitive interview, it is important that participants understand that:

- they are being interviewed to develop the survey questionnaires so that the interviewer can determine if there are any weaknesses or mistakes with the questions;
- they are being asked to describe anything that is difficult to understand, or identify a question that is hard to answer;
- the interviewer is interested in the ways that they decided on their answers, how sure they are about them, and any problems that they encountered;
- the interviewer did not write the questions and they should not worry about criticizing or hurting anyone’s feelings by pointing out problem; and
- there are no “wrong” statements and they should respond truthfully.

**Pretesting the survey questionnaire**

The survey questionnaire must be pretested with a range of people from the intended different survey populations, including people of different educational and socioeconomic backgrounds. Testing each type of questionnaire tool with five to ten people should help identify any major concerns (such as erroneous skip patterns).

Pretests should be conducted by one or more trained interviewers. Ideally, these interviewers would be those who are likely to serve as supervisors or Team leaders for survey implementation. Interviewers should note where the participant hesitates or gives incorrect answers, to indicate where the tool could be made clearer. The pretest experience should be discussed with the Survey coordinator and relevant Technical committee members to suggest improvements when revising each tool. Whenever a significant change is made, such as the structure of a form, a second round of pretesting should be conducted.
Fig. 11.1. Sample sequence for developing the main survey tool

1. Draft questionnaire, including standardized modules where available, for example, from MICS/DHS
2. Qualitative interviews to determine appropriate question and response options for questions, as necessary
3. Review with the Technical committee and revise as needed
4. Conduct cognitive interviewing, revise and re-conduct cognitive interviews to check revisions
   - Repeat as needed
5. If needed, translate and back-translate this version
6. Pretest the tool and make revisions as needed
7. Translate and back-translate any changes that were made
8. Train survey personnel in all aspects of the survey tool
9. Pilot test the survey tool
10. Revise as needed, repeat pilot testing as appropriate
11. Start survey data collection
Other data collection tools

A number of additional tools are needed to support survey implementation and to monitor the progress of the fieldwork. These are described in the following sections. Not all surveys will use all tools.

Anthropometry data collection tool: Anthropometry is usually conducted after having completed the interviews, therefore the anthropometry form often comes at the end of the questionnaire. It is essential to confirm that anthropometry measurements are taken from and recorded for the correct individual. This means that the anthropometry form may include questions about date of birth and about sex even when these were collected at the beginning of the interview. Repeating the questions helps the field team verify that the correct individual is being measured. Any errors would also be picked up during programmed data cross-checks, which may happen in the field with electronic data collection, or at a later stage of data management with paper-based forms. The main data collected on the form should include:

- date of birth and date of assessment (to determine age)
- sex
- height/length (with indication of which was collected) and unit of measurement
- weight and unit of measurement
- response: successful, refused, did not come for measurement
- codes to identify the stadiometer, scale and measurer.

Additional anthropometric data may include:

- a note of bilateral pitting oedema
- mid-upper arm circumference (MUAC)

The format of the tool needs to be pilot tested. The form used for training should be the same as that used in the field.

Specimen collection and tracking tools: Biological specimen collection is also usually conducted after the main interview, so the tool to document this process may be located at the end of paper questionnaires or in another format that can be linked to the main paper or electronic questionnaire. Similar to anthropometry, it is essential to check that the specimen ID matches the respective individual ID number.

The specimen collection tool notes:

- the code of the person collecting the specimens
- the codes for the equipment used (to check for systematic errors)
- the time of data collection, and fasting status if applicable
- whether each of the intended specimens was collected for each individual
- the value of results (if applicable)
- referrals to health facilities (if applicable).

A yes/no question for all intended specimen collection should be included at the end of each questionnaire and should also be included in the summary cluster control form (described later in this module).

Specimen tracking forms should be developed to allow the field phlebotomists and laboratory technicians to match the specimens collected at the household and analysed in the field with the specimens transported to the laboratory. The forms can be modified according to the survey. If necessary, an additional form should be
developed, depending on what laboratory processing is done in the field. The specimen tracking forms should accompany biological specimens or food samples collected (and processed, where applicable) from the field to the laboratory where they will be further processed, analysed or stored. Tracking forms include a summary of the total number of each type of specimen processed and transported, the time and temperature at each transfer from field collection to arrival at the final analysis laboratory, and the signature of the person responsible at each stage.

In addition, a “Laboratory specimen log” should be created to record each specimen that arrives, is stored, and is analysed at that laboratory. To reduce errors in data entry, this would ideally be an electronic spreadsheet with barcode scanning of sample IDs.

Sample “Specimen tracking form for phlebotomist” and “Specimen tracking form for laboratory technician” are found in the online tools.

**Temperature monitoring:** A section to record temperature is included in the “Specimen tracking form for phlebotomist” online tool. Additional forms need to be developed for each level of the cold chain. Thermometers or temperature monitors must be used to check the temperature of each cool box. It is important to set up a system that allows the temperature to be determined in a way that does not cause repetitive opening of the cool box. The “Specimen tracking form for phlebotomist” is best placed inside a plastic covering to prevent moisture damage to the paper forms and secured with tape on the outside of each specimen storage container (cool box, refrigerator or freezer).

**Referral forms:** Some indicators, such as the prevalence of anaemia, can be tested in the field. The Steering committee should agree with the ministry of health on the appropriate action to take if results indicate that a participant has a health condition or nutritional deficiency that needs attention. These conditions are most frequently severe anaemia, malaria or oedema.

In some cases, survey participants may be referred to local health facilities according to criteria determined by the ministry of health. These criteria usually include the availability of clinical guidelines and treatment, the capacity of staff to handle an increased volume of patients, and costs. If survey staff are instructed to refer participants, the ministry of health will need to advise staff in nearby public health facilities about the possibility of participants arriving with survey referrals for specific conditions or illnesses. See the “Referral form” online tool for an example of a referral form.

In all cases, the referral policy should be clear and set by the ministry of health. This policy needs to include a management plan for test results and referral logs that are in accordance with the agreed confidentiality of personal data. Participants need to be clearly informed about how data will be used and by whom. The referral protocols should be tested and practiced in the field. A log of all referrals to health facilities should be maintained by each team. Examples of these can be found in the “Referral log for anaemia” and a “Referral log for malaria” online tools. Additional logs can be developed depending on the specific referral criteria in country.

**Cluster control form:** The cluster control form provides a list of all households that are to be interviewed in the cluster, and is useful to the interviewers, laboratory personnel and supervisors. The main information
Module 11. Data collection tools, field manual, and database

usually includes the items in the list below. In cases with prior selection of households based on an earlier census, other details might be included.

- household and cluster number
- name of the head of household
- status of interview/sample collection (not present, refused, complete, incomplete)
- number of attempts to contact household members
- call-back information if an interview was not completed
- referrals made
- reasons for any non-response.

The information on the cluster control form must be concise and at the same time must include all important information needed to summarize the main data collected. See the “Generic cluster control form” online tool and an example from Malawi (“Malawi cluster control form”).

At the end of each day, the supervisor(s) should check all cluster control forms filled out by the survey team members and produce a “Cluster summary sheet” for each cluster. This form can be either paper-based or electronic. If in paper format, it can be photographed when complete and uploaded along with electronic data collection forms, or it can be transported or emailed to the central data location. The information contained in the form should be entered into a database of monitoring information so it can be cross checked with the laboratories that are monitoring data collection within clusters to ensure consistency.

Informed consent and assent

Most countries require an ethical or scientific review of the survey, and guidelines concerning the rights of each potential respondent, both adults and children, should be followed. This is detailed in Module 1: Planning and designing a micronutrient survey.

Ethical considerations protect the dignity and rights of survey participants. Written or verbal informed consent from participants is conventional. For a young child, the caregiver may give this consent. Separate consent should be requested for the questionnaire, for specimen collection and for anthropometry, and the response to each consent process should be indicated on the questionnaire. Children over a certain age (the specific age varies by country) may be requested to provide assent in addition to the consent from their caregiver. It is important to consult with country-specific ethical review boards before starting any data collection. The “Informed consent form” template can be found in the online tools, and must be adapted using specific guidance available in the country. Additional templates for consent and assent forms can be found at https://www.who.int/ethics/review-committee/informed_consent/en/.

During the consent process, the interviewer explains to the participant (or caregiver of the child) the purpose of the survey, the type of questions that will be asked, and the biological specimens and measurements that are to be taken. Each type of specimen (such as blood, urine or stool) and the procedure for collecting it should be carefully explained during the consent process so that the participant understands the benefits and risks of what they are agreeing to. The consent process should also explain the various tests that will be conducted and what, if any, results the participant will receive. If the participant refuses the entire survey or part of the survey (for example, the collection of biological specimens), his or her choice must be respected.
**Age determination technique and tools**

Most surveys ask for the age and date of birth of each participant. For participants up to 59 months of age, the date of birth and date of visit should be recorded. For individuals 6 years of age and above, age can be estimated in completed years, that is, the age at the time of the person’s last birthday. If the respondent (ideally the main caregiver of the child) knows a child’s age in months or years this should be entered in the appropriate section on the form. If data are being collected electronically, the electronic device should be programmed to calculate the number of years and months from the reported date of birth to the date of the interview. If paper-based forms are used, the interviewer should record the date of birth and the date of the visit on the questionnaire for later use in a tool developed for determining age in months or years. These calculated ages should be cross-checked with the age provided by the caregiver.

The age of a child is crucial for making and interpreting appropriate anthropometric measurements. This underscores the importance of probing for an accurate date of birth. Even where the date of birth is included on a child health card or similar document, it is possible that it was incorrectly recorded or that the writing may make it difficult to determine, therefore the date of birth should always be confirmed with the caregiver. If the primary respondent does not have the necessary information, the interviewer should ask other household members.

These are the steps for estimating the age of a child under 6 years, using a recorded birthdate:

1. Ask the respondent for documentary evidence of the child’s date of birth (such as a birth certificate, child health card or holy book).
2. Record the day, month and year of birth as noted on the documentary evidence, and indicate the type of documentary evidence that was provided. Even if the respondent recalls the child’s date of birth, politely ask to see a copy of the documentary evidence and record the information directly from it.
3. If no document is available, ask the respondent for the date of birth as they recall it and indicate the source on the questionnaire as “respondent’s report”.
4. If the respondent does not know the child’s exact date of birth, then at minimum the month and year of birth should be obtained using a local events calendar. The local events calendar will have been prepared and tested previously, and all anthropometrists should have been trained its use. See the “Age determination for children under 6 years of age” online tool for more guidance on estimating the month and year of birth using the calendar of events and indexing techniques.

Whether using documentary evidence or the respondent’s report, the anthropometric team should record the actual date of birth, if specified. If using the local events calendar, it is probable that it will be impossible to identify the exact date of birth. In this case, anthropometrists should enter “00 00 00” (unknown) for the date of birth and enter the birth month and year as determined by the local events calendar. The source of the information should always be recorded in the questionnaire.

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For children aged 6 to 18 years, follow the same steps as for children under 6 years to get the best estimate of the date of birth. If it is not possible to get the exact date, the month and year of birth are adequate. For adults over 18 years of age, the year of birth is adequate.

**Field manual**

A comprehensive field manual for the survey provides detailed guidelines for every component, including:

1. An overview of the survey objectives, sampling and household selection, population groups assessed, team composition, and indicators to be collected.
2. Data collection processes, data collection forms, consent processes, and interview techniques.
3. Labelling.
4. Anthropometry techniques.
5. Collection methods for biological specimens and food samples.
6. Specimen and sample processing, referrals (for malaria, severe acute malnutrition or severe anaemia), and cold chain.
7. Quality control procedures.
8. Care of equipment and supplies.

The field manual should be developed by the Survey coordinator with input from the Technical committee as soon as the survey protocol is completed and approved. Each member of the field team should have a copy of the manual, which will be used in all stages of training, pilot fieldwork, and data collection. Thorough familiarity with the manual will help ensure a high degree of standardization and consistency across multiple survey teams. Separate manuals for enumerators and phlebotomists/ laboratory technicians might be developed depending on separate roles and responsibilities. **Box 11.3** contains a typical table of contents for a manual. See different survey manuals for examples of field manuals developed for previous national micronutrient surveys.
### Box 11.3. Typical table of contents for a micronutrient survey field manual

#### Module 1: General information
- Introduction and background
- Aims and objectives of the survey
- Population groups
- Household enumeration
- Selection of households
- Fieldwork logistics
- Team composition and training
- Roles and responsibilities of staff for the survey
- Protocol for data collection in the household
- Informed consent process
- Data entry and analysis
- Supervision

#### Module 2: Interviewing
- Consent process, interview techniques
- Completion of the questionnaire
- Recording responses, writing numbers
- Correcting mistakes
- Detailed instructions for questionnaire modules
- Visit schedules
- Editing and checking
- Referral procedures

#### Module 3: Biological specimen collection
- Consent process
- Universal precautions
- Cold chain logistics
- Laboratory personnel
- Labelling
- Preparing for fieldwork and specimen collection procedures in the household
- Transportation of specimens from the field to district or central laboratories
- Specimen processing and storage
- Quality control and assurance
- Packaging and shipping

#### Module 4: Food specimen collection
- Consent process
- Collection and labelling
- Specimen processing and storage
- Quality control and assurance
- Replacement

#### Module 5: Anthropometry
- Consent process
- Measuring children under 24 months of age
- Measuring children 24 months of age or older
- Measuring adults
- Calibrating equipment
- Anthropometry standardization
- Tips for successful measurements
Module 12. Training and pilot testing

In this module, we will discuss:

- Developing the training agenda
- Planning the training: venue, trainees and trainers
- Training sessions
- Testing and evaluating trainees
- Training supervisors and team leaders
- Pilot testing
- Refresher training
Module 12. Training and pilot testing

This module provides an overview of issues to consider for planning and conducting training for the survey. It also includes links to examples of training manuals used around the world. The details of each national training course will be specific to the survey and will be based on the survey objectives and design, as well as the number and composition of survey teams.

The quality of survey data is largely dependent on the quality of fieldworker training. Preparing high-quality training can take several months and includes developing the training agenda, identifying a suitable training venue, identifying the trainees and the trainers, developing training materials and sessions, organizing standardization exercises for anthropology and practical opportunities to collect biological specimens, and planning the pilot exercise.

Developing the training agenda

In general, training for a micronutrient survey takes about two weeks. The specific duration depends on various factors, including the number of trainees, their prior experience, the complexity of the survey, the indicators to be assessed, the length of the main questionnaire, the number of working hours per day and the number of working days per week.

The agenda should include a mix of classroom and practice sessions throughout the day, and should be flexible to allow for additional days in case trainers find that fieldworkers are not yet ready to begin the pilot, or if the pilot highlights issues that need review and additional practice before actual data collection. The “Generic training agenda” online tool contains a sample agenda as well as examples of training agendas from Nepal and Ethiopia.

In a typical training course, the first day consists of overview sessions for all trainees together. These sessions familiarize the trainees with the rationale, objectives, and design of the survey, and on the respective roles and responsibilities of the survey team. On subsequent days, trainees may be divided into groups according to their role in the survey. For example, the interviewers are trained separately from the phlebotomists and field laboratory technicians. Depending on which staff are assigned to anthropology, those sessions may be scheduled and conducted with one large group or limited to certain team members. Box 12.1 presents some general guidelines for planning the training.

Planning the training: training venue, trainees and trainers

Training venue

The training venue needs to be big enough to accommodate the larger plenary group and have suitable smaller spaces for the skill-specific groups. In addition, it should have spaces for smaller breakout groups to role-play and practice new skills. The venue should have electricity, access to food and refreshments, restroom facilities and comfortable seating for all participants, preferably at tables or desks. Most venues need to be booked well in advance with a flexible arrangement to allow for potential delays in the start date for the training.

Training on biological specimen collection will also require:

- work benches or tables
- electricity
- a source of clean water
- secure storage for laboratory supplies and equipment.
Ideally, different training sessions can be conducted in parallel at a single training site. This helps to maximize the consistency of information, allows for answers to queries that may arise during the course of training and builds team relationships. Depending on fieldwork procedures, this also facilitates cross training as needed. If it is necessary to use separate sites, try to use sites that are easily accessible to each other, with reliable and frequent contact between them. Costs for transportation between sites should be included in the budget.

Trainees

In general, the fewer the trainees, the better the quality and shorter duration of the training. The number of trainees in each skill-specific group should be limited to 40. Initial plenary sessions should involve a larger number of trainees prior to separating them into these skill-specific groups.

In addition to the fieldwork teams, people to consider for selected parts of the training should include:

- Household listing and mapping teams. If the mapping has not yet been conducted, these teams could benefit from understanding the survey objectives for the purpose of community engagement when they visit the selected clusters.
- Data managers and data entry supervisors (where data collection is paper-based). Data managers and data entry supervisors need to have prior in-depth understanding of the questionnaires and of characteristics such as data limits and skip patterns. In order to be able to brief data entry staff on the questionnaires, they should participate as trainees in the sessions on detailed questionnaire review, interviewer training and questionnaire completion.

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1 “Cross training” refers to a situation where the same person may be trained to carry out several different tasks, for example in some surveys, all team members are trained on anthropometry.
Module 12. Training and pilot testing

- Central laboratory staff. Training central laboratory staff will allow them to contribute to and understand what to expect from fieldwork procedures for specimen collection, processing, transport, storage and tracking.

Detailed training sessions for these roles are usually conducted separately from the main training, thus separate materials will need to be developed. The exact content and duration of these sessions vary, depending on the complexity of the tasks and the previous experience of the trainees. Sample materials can be found in the online tools by searching for instructions.

**Trainers**

Trainers need to be identified and involved early on in the planning process. They should have expertise in their assigned topics and understand the expectations and commitment required to prepare for and lead relevant sessions. Examples are staff from ministry of health or from a national nutrition organization who can train others on anthropometric measurements, and Technical committee personnel from the national statistics office who can conduct sessions on household mapping and listing.

It is critical that all trainers have detailed knowledge of the survey protocol, including methodologies and planned fieldwork procedures, and that they understand how the different sessions of the training fit together so that contradictions and potential confusion can be avoided. The person responsible for the overall training (usually the Survey coordinator) should brief all trainers ahead of time so that they are aware of the objectives and procedures to be followed in the survey.

A lead trainer should be assigned to prepare, review and coordinate the overall training process (including presentation materials and activities for each training session). Presentations should be appropriate for adult learning and should be focused on the assigned topic, reviewed for consistency and unnecessary duplication, practiced in advance of the training and fit within the allocated timing for the session. Including more than one trainer for major sessions may help maintain interest and improve the variety for both trainers and trainees.

**Training sessions**

The main training sessions to be organized are Mapping and household listing, Anthropometry, Biological specimen collection and processing, Interviews, Electronic data entry and Data quality.

The types of presentations for each of the main sessions or topics on the agenda varies depending on the survey, but often micronutrient surveys include the presentations shown in Table 12.1. The main content of these presentations will also be covered in the appropriate survey manuals. Sample micronutrient survey training presentations for anthropometry, biospecimen and food sample collection can be found in the online tools.

All training materials should be obtained and available before starting the training course. These items should be included in the supplies list and in the budget. Box 12.2 presents a list of minimum supply needs for different personnel involved with the training.

**Mapping and household listing:** As described in Module 7: Selecting households and participants, separate teams tasked with this job should be trained by experts, usually from the national statistics office. The teams may start their work prior to the main micronutrient survey. In these cases, the main micronutrient survey
teams undergo a brief training and orientation (usually around two to four hours), to learn about the overall sampling methodology and the mapping and household listing exercise, so that they know what to expect in terms of maps and what to do upon arrival in the enumeration area (EA). If the main micronutrient survey team is to conduct the survey mapping and household listing, extra days need to be built into the main training agenda to accommodate this.

**Table 12.1. Presentation topics for micronutrient status survey training**

<table>
<thead>
<tr>
<th>Presentation topic</th>
<th>Content to be covered</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Introduction to the survey</strong></td>
<td>Aim and objectives, Team structure and respective roles</td>
</tr>
<tr>
<td></td>
<td>Indicators to collect, Timeline</td>
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<tr>
<td></td>
<td>Population groups, Communication</td>
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<tr>
<td></td>
<td>Survey design</td>
</tr>
<tr>
<td><strong>Household listing and mapping (assuming that this is conducted by a separate team)</strong></td>
<td>Example maps and map key – how to use</td>
</tr>
<tr>
<td></td>
<td>Example household listing and selection of household and different population groups for different indicators – how to use</td>
</tr>
<tr>
<td></td>
<td>Eligibility criteria</td>
</tr>
<tr>
<td><strong>Data collection</strong></td>
<td>Questionnaires</td>
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<tr>
<td></td>
<td>Other data collection and log forms</td>
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<tr>
<td></td>
<td>Consent process</td>
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<tr>
<td></td>
<td>Labelling</td>
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<tr>
<td></td>
<td>Interview techniques and quality control of the process</td>
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<td></td>
<td>Navigating and linking the questionnaires</td>
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<td></td>
<td>Standard writing techniques for numbers or letters</td>
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<tr>
<td></td>
<td>Collection of global positioning system (GPS) coordinates</td>
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<tr>
<td></td>
<td>Using and maintaining electronic devices (for electronic collection)</td>
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<tr>
<td></td>
<td>Saving and sharing/uploading data (for electronic collection)</td>
</tr>
<tr>
<td><strong>Biological specimen collection and processing</strong></td>
<td>Best practice in specimen handling</td>
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<tr>
<td></td>
<td>Specimen collection, testing (as applicable), and processing</td>
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<tr>
<td></td>
<td>Labelling, storing and transport</td>
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<tr>
<td></td>
<td>Completion and maintenance of specimen forms</td>
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<td></td>
<td>Quality control in the field</td>
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<td></td>
<td>Equipment and supply management</td>
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<tr>
<td></td>
<td>Cold chain, as applicable</td>
</tr>
<tr>
<td><strong>Collection of household food samples</strong></td>
<td>Food sample collection and testing (as applicable)</td>
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<tr>
<td></td>
<td>Labelling, storing, and transporting</td>
</tr>
<tr>
<td></td>
<td>Completion and maintenance of specimen forms</td>
</tr>
<tr>
<td></td>
<td>Household food replacement (as applicable)</td>
</tr>
<tr>
<td><strong>Anthropometry</strong></td>
<td>Methods and best practice for anthropometry assessment (depending on which measurements are included)</td>
</tr>
<tr>
<td></td>
<td>Anthropometry standardization</td>
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<tr>
<td></td>
<td>Digit preference</td>
</tr>
<tr>
<td><strong>Data quality</strong></td>
<td>Quality checks in the field</td>
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<tr>
<td></td>
<td>Review of key quality control-related parts of the questionnaires as well as data and specimen collection forms and labelling</td>
</tr>
</tbody>
</table>
Box 12.2. Training supplies and reference materials

All trainees
- Official identification badge and a letter from the government that authorizes the survey, if needed
- Survey field manual
- Questionnaires in each language (in sufficient quantity if paper-based)
- Questionnaire labels (if pre-printed)
- Consent forms for each type of consent required and in each language
- Household listing form, cluster control form and cluster summary sheet
- Local event calendar (if needed)
- Electronic data collection devices with all necessary software preloaded
- Accessories for the electronic devices
  - Power packs
  - Chargers and adapters, as required
  - USB or microcards for backing-up data
  - Mobile wireless internet devices (if needed)
- Pens
- Note pad
- Food sample kits including field testing kits and labels for pilot, as applicable
- Backpack (usually provided at the end of the training, once teams have been selected)
- Field supplies for the pilot (usually provided at the time of the pilot)

Laboratory technician trainees
- Laboratory field manual
- Daily supply list for specimen collection, processing, and transport
- All forms (for example, temperature logs, referral forms, and specimen tracking sheets)
- Supplies for specimen collection, processing and storage (including labels for pilot)
- Snacks and olive oil if modified relative dose response (MRDR) is part of protocol

Team leaders
- Maps and household listing forms
- Team leader field manual
- Team leader assignment sheets and cluster control forms
- Replacement food items, if applicable
- Other incentives for the pilot test, if applicable

Household listing teams (where applicable)
- Area maps and household listing forms
- Household mapping and listing manual

Teaching materials
- Flipcharts and paper
- Colour markers
- Projector(s)
- Laptops for presentations
- Height/length measuring boards
- MUAC tape, if applicable
- Scales
Module 12. Training and pilot testing

**Anthropometry:** Anthropometry assessment and training require extra advance planning to ensure that equipment is available and to make appropriate arrangements for practice and standardization exercises. All data collection forms, equipment and materials including dolls and props for practicing measurement should be obtained well ahead of the training. All anthropometry equipment must be stored in a secure location during training, piloting and fieldwork.

Arrangements should be made to have a sufficient number of children of different ages present during the training sessions. Children and caregivers (or daycare staff) should be invited in advance of the training. This may involve certain permissions and logistics, for example transport and refreshments.

Training should ideally take place just before the start of data collection. It is recommended to schedule seven days for anthropometry training. The exact amount of time needed will depend on the number of trainees, their prior experience, the populations being measured, the number of children and adults available on whom to practice measurements, as well as the outcome of the standardization exercise. A minimum of four days is required to teach high-quality measurements: one classroom day for identifying households and participants and completing questionnaires, one day for using and maintaining equipment and taking measurements with dolls, plus two days of hands-on training. These are followed by the two-day standardization exercise and one day of field testing.

It is expected that classroom training on how to identify households, participants, and properly collect date of birth has already been covered.

All survey team members serving as the “main measurer” anthropometrist during fieldwork are required to undergo and pass the standardization exercise, which ensures that they are able to take accurate and precise measurements of child height and length. The “assistant measurer” should not act as the “main measurer” unless he or she has also passed the standardization exercise.

**Anthropometry standardization exercise:** Working in teams of two (one “main measurer” and one “assistant measurer”), the trainees measure the height and weight of at least 10 children. Half of these children should be under 24 months of age and the other half between 24 and 59 months. Each child remains in an assigned station while the teams move from one station to another. Each team measures each child twice. It is recommended to schedule the first measurement in the morning before the young children’s usual nap and meal times, then take a break and do the second round of measurements later so it does not interfere with the children’s usual routines. If caregivers and children are brought to the training site for a half or full day then, at a minimum, transportation, food, and beverages should be provided for them. It may also be necessary to provide a small incentive. For all exercises, the Survey coordinator should send reminders one or two days in advance to ensure that the activity can go ahead as scheduled.

An expert anthropometrist will serve as the “gold standard” measurer with his or her assistant. The measurements of the trainees will be compared to the expert’s measurements. It is recommended that the technical error of measurement (TEM) be applied. A TEM cutoff value for length/height of <0.6 cm for

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Module 12. Training and pilot testing

precision and <0.8 cm for accuracy may be considered as acceptable. A height standardization tool to determine the TEM is available online from the DHS Program.¹

Trainees who do not perform adequately will need to be retrained until their measurements fall within the acceptable TEM. Time for this should be planned when designing the training process. Presentations on anthropometry, and the WHO/UNICEF 2019 ADQ guidance on anthropometry, can be found in the online tools “Anthropometry training” and “Anthropometry training for the Malawi survey”.

The space for the anthropometry standardization exercise should be big enough for ten stations and for teams to move easily around the room. Fig. 12.1 provides a diagram of how a standardization exercise may be set up. Although there may be fewer than 10 teams measuring children, there should always be 10 children in the standardization exercise, and a few additional children in the event that replacements are required.

Fig. 12.1 Example of standardization exercise set-up Source: WHO, UNICEF 2019.²


Module 12. Training and pilot testing

**Biological specimen collection and processing:** Depending on the complexity of the biological component of the survey, four to five days are usually required for training on biological specimen collection and processing. An additional one to two days may be needed for visits to local hospitals, clinics, or other suitable settings to practice specimen collection and processing. Through presentation and practical exercises, the training should cover:

- best practices and universal precautions for handling biological specimens
- informed consent
- specimens to collect and method of collection
- specimen processing procedures
- field tests (biological and food samples)
- explanation of test results to survey participants
- referrals to health facilities for conditions such as anaemia, as agreed with the ministry of health
- labelling of specimens
- forms (paper and/or electronic) and how to complete logs
- maintaining the cold chain and transportation of specimens
- informing respondents of their results and clinic referrals.

You can find sample training presentations on field laboratory procedures in the online tools.

**Interviews:** Training on interviewing will require several days and will cover such topics as:

- good interview techniques
- the process of obtaining informed consent
- questionnaire content
- a question-by-question review of the questionnaire
- how to complete the questionnaire
- interview practice
- food sample collection (where applicable)
- data collection forms and log files
- age determination.

The field manual will provide useful information on each question: its intended meaning, whether, how, and when to probe and how to legibly record (paper) or enter (electronic) responses. During the training sessions, there may be some refinement of the questionnaire and the skip patterns, and some discussion about the exact wording, especially if the questionnaire has been translated into more than one language. Plenty of time needs to be available for demonstrations, role-plays, and practice.

The paper-based version of the questionnaire should be used initially, so that interviewers become familiar with the tool structure and skip patterns in full. Only then should trainees move to practicing and using the electronic version if applicable.

If interviews need to be administered in a language that is not written or that enumerators are not as accustomed to reading, more time will be needed during the training. Enumerators need to practice in the stated language and to agree on standardized verbal translations. Time might also be needed to work with local translators.
Module 12. Training and pilot testing

You can find more on interviewing techniques in the “checklist for interviewer training” and “tips for interviewer training” tools, and a “Malawi interview skills” presentation.

Electronic data entry: Training for surveys that collect data electronically should include comprehensive explanations and practice on using the electronic devices. Sufficient time should be allocated to ensure that all field staff are familiar with and practiced in:

- device functions and maintenance (including keeping the devices charged)
- completing, editing, and sharing of questionnaires
- global positioning system (GPS) data collection tools
- barcode scanning
- any other features, such as taking photos of items in the household.

The total amount of time required for specific device-related training depends on the complexity of the survey, the familiarity of staff with electronic data collection, and the number of different population groups included with separate and/or relational questionnaires. Prior to training data entry staff, extra time (anywhere from one to three days) will be needed so team leaders and supervisors can learn and practice responsibilities specific to the use of electronic devices.

Electronic devices may be assigned and checked out to trainees on a daily basis at the beginning of the training, with serial numbers matched to signatures of the trainees. This will allow trainees to practice outside of the training hours if they so wish or are instructed.

All devices should be collected before the start of fieldwork to check that they:

- function properly
- have the correct software (and updates) installed
- are fully charged
- do not have unnecessary data files that may have been uploaded as part of the survey training and pilot
- have assigned IDs that are paired with survey enumerators, with a master list of which individuals and teams have which devices (including spares).

Data quality: The training agenda must include time to cover issues related to data quality and supervision. All trainees should understand that their performance will be monitored throughout fieldwork and that supervisors will periodically spot-check households, review all completed paper-based questionnaires, and conduct checks of electronic questionnaires. Team leaders will observe full interviews by all enumerators during the first few days, and will recheck predetermined questions for about 10% of interviews during the period of the fieldwork. Specimen logs (such as for temperature, and transfer) should be checked every day against expected and actual specimens collected.

Testing and evaluating trainees

Trainees should be assessed based on observations of practice in classroom and field settings. In addition, it is useful to consider giving short unannounced quizzes each day or at key points during the training. This helps trainees focus on what they have learned so far and helps trainers identify areas that need additional attention. The quizzes should be straightforward and focused on key content relevant to the trainee’s role. Five to eight questions should be enough. Marking and feedback should be done within 24 hours. A mix of
Module 12. Training and pilot testing

written tests, oral questions and group discussion on varying days might be considered. Sometimes it is useful to have trainees swap tests and grade each other’s work, followed by a group discussion of the answers.

The criteria for selecting trainees should be objective and documented for each person. Having written tests and marked performance criteria for practical classes during the training can help survey staff document the reasons why certain trainees are or are not selected. It may also be helpful to administer a graded test at the end of the training. The score of the trainee can be taken into consideration when selecting the field teams. Final decisions on hiring fieldworkers should be based on a pre-determined overall evaluation of individual performance, including assessment of the trainees’ performance during class sessions, field practice, and test scores. Good interview techniques, a high level of skill in collecting and processing blood and a demonstrated ability to work as part of a team may be more important than high test scores.

Training for supervisors and team leaders

As described in Module 8: Survey supervision and personnel, the fieldwork support team usually includes team leaders and regional supervisors (field and laboratory, as applicable), who are in turn supported by specialists within the Technical committee.

In some surveys, some or all of the team leaders and supervisors are pre-identified based on their skills and prior field survey experience. In other situations, these positions are filled from the pool of trainees. Whichever process is used, the selection of team leaders and supervisors should be based on objective criteria that include experience and expertise, skills in leadership and management and the ability to carry out high-quality supervision.

Pre-identified team leaders and supervisors can assist during the field staff training. They should receive several days of specialized training before the general training course begins. This is an opportunity for the team leaders and supervisors to receive training in appropriate skills, for example, setting up electronic devices and checking and uploading data files, and to gain experience in the leadership role before the fieldwork.

If team leaders and supervisors are to be selected during the general field staff training, then it is useful to observe the performance of potential candidates in various roles. After team leaders and supervisors are selected, they will need one to two days of additional training. The agendas for training and piloting should reflect this time commitment. The skills to reinforce will depend on the pool of trainees from which the leaders and supervisors and selected. For example, if team leaders are selected from the pool of interviewers, then they may need additional training on the biological specimen collection processes, whereas if they are selected from the laboratory trainees, then they may need more exposure to the interview process. They should also be familiar with the key points in relation to anthropometry.

A manual specific to team leaders and supervisors should be prepared for the training. In addition to the topics covered for all field staff, team leaders and supervisors should receive further instruction in the following areas:

- Interpretation of maps and household listings. When mapping and household listing are not conducted in advance, more intensive and time-consuming training on sampling and map reading is required. This should include a visit to a sample segment to practice reading the map and locating selected households and participants.
- Observing interviews, checking questionnaires and labelling (paper and electronic), and providing feedback to field staff.
Module 12. Training and pilot testing

- Electronic device setup, charging and management, including downloading survey questionnaires, uploading questionnaires, checks to complete on electronic questionnaires, GPS measurements and reporting, device maintenance and troubleshooting.
- Collecting and processing biological specimens, managing the cold chain, labelling, transportation, and completion of log forms.
- Strategies for monitoring data quality.
- Logistics.
- Team leadership, mobilizing communities, maintaining team morale, supportive supervision, dealing with problems and communicating with other teams and regional or national supervisors.

It may be useful to challenge team leaders by asking them to review and amend questionnaires (paper or electronic) and laboratory forms that have been incorrectly completed. This is a good way to evaluate their ability to find errors and deal with them appropriately. Team leaders should be observed during the training period to review their performance and identify areas where additional input may be required.

**Box 12.3** provides an example of responsibilities related to the use of electronic-devices for which a team leader/supervisor may need additional training. Examples of team leader and supervisor manuals can be found in the “Ghana supervisor manual” and the “Ghana training manual iodine survey ODK section for interviewers” online tools.

**Pilot testing**

All survey procedures must be tested before survey implementation begins. Pilot testing at the end of the training helps to estimate the amount of time it takes to complete the survey in each cluster and to identify any potential remaining concerns with the survey questionnaire, forms and protocol.

A pilot test usually takes several days, however the time needed will vary depending on the complexity of the survey. Each field team should have the opportunity to conduct the pilot in a number of households from both an urban and a rural setting to provide a good range of experience. Urban and rural settings can be quite different and have different challenges.

The pilot test needs to be organized well in advance, and must not take place in enumerated areas selected for the main survey. Ideally, the location should be relatively close to the training venue to minimize travel and maximize implementation time.

The entire fieldwork process should be piloted, from organizing supplies and freezing gel packs to entering data and processing samples. After each day of the pilot there should be a debriefing session with the whole team to discuss their experiences and resolve any challenges faced. Trainers may need to provide some refresher training for any major problems identified. If the first day of the pilot raises major issues that require time to rectify, for example significant changes to the survey questionnaire or the specimen collection process, then the subsequent days of the pilot may need to be delayed. For electronic data collection, programmers should be involved and available to troubleshoot in as close to real time as possible.

**Logistics:** The sites for the pilot test should be identified one to two months in advance, and permission should be requested from the local administrative body and community leaders. Transport from the training venue to the pilot sites needs to be organized. The number of pilot sites required will depend on the number of field
teams participating. To simplify logistics, several teams may work within one practice cluster if the cluster is large enough.

The practice cluster needs to be mapped. This may be done as practice during the training sessions for the mapping and household listing team, or it may form part of the pilot test if that is part of the field team’s role. Once mapped, team leaders should work with the Survey coordinator to decide which households in a particular cluster will be allocated to each team, according to the survey protocol.

**Final field preparation:** After the pilot test has been completed and systematic challenges have been identified and corrected, the teams are ready to start fieldwork. A short time period should be allotted between the end of the pilot test and the start of fieldwork. This will allow for any additional training or pilot testing, finalization of the survey questionnaire (paper-based or electronic) if needed, checking and cleaning electronic devices where used, preparation and inventory of all field supplies, and travel of field teams to their initial clusters.
Module 12. Training and pilot testing

Before the fieldwork begins, the final field teams should be selected and allocated to the various clusters. All trainees, even those not selected, may be provided with a certificate for completing the training course. A “Generic certificate of participation” is available in the online tool.

When possible, the initial fieldwork should begin in clusters nearest the training venue. This ensures that all the teams are working as near to each other and to the central coordinator as possible, and allows for close supervision and easier communication during the initial stages when concerns are more likely to arise.

Refresher training

Sometimes surveys take several months to complete, and it may be necessary to schedule a refresher training part way through the field work. This is particularly relevant if issues arise with data collection or if morale wanes during the field work. Sometimes refresher training may be scheduled around a break, such as a religious holiday, where field work is temporarily suspended. It can provide a great opportunity to gather everyone together for motivation and for discussion and troubleshooting any specific difficulties being faced in the field. In some countries, especially large countries, it may make sense to have several regional refresher training courses to save on travel time and cost.

Refresher training should be brief, one to two days at most, so that teams are able to get back to the field quickly and resume data collection.
Module 13. Field logistics

In this module, we will discuss:

- Managing supplies and specimens in the field
- Field team supervision and monitoring
- Coordination and communication in the field
- Arranging for travel and accommodation
- Data management in the field
- Cold chain maintenance and quality control
- Shipping specimens
Managing supplies and specimens in the field

Managing the large quantities of supplies and specimens in the field requires rigorous record keeping, tracking, and monitoring at every level. Data collection forms for these management tasks are described in Module 11: Data collection tools, field manual, and database.

Distribution of items to field teams

After training and pilot testing, and immediately prior to departure to the field, all supplies and equipment for the main survey should be assembled and issued to teams. Depending on the field plan and scheduled meetings with regional coordinators, teams should receive the equipment that will be used throughout the survey and enough supplies for the first three to five clusters. The remaining supplies and spare equipment should be split among the regional coordinators, who will need to store them securely for later distribution. This approach reduces the risk of loss or theft during fieldwork, and it also uses less space in the already-crowded survey vehicles. A schedule needs to be drawn up for providing supplies to the field teams and for collecting specimens and survey-generated waste.

As described in Module 9: Survey equipment and supplies, extra equipment and supplies should be purchased in case items get lost, broken, or stolen. These additional items need to be managed in such a way to allow quick dissemination to teams as required. Each team should carry spares of things such as batteries, mobile devices, stationery and plastic bags. Team leaders should also have cash to buy some of these items in the field if needed and available.

It is not uncommon for vehicles to break down or become damaged during fieldwork, and a backup plan should be in place in case this happens.

Daily list of supplies and equipment

Every team member should have a checklist of the type and quantity of supplies and equipment required for their daily fieldwork. The checklist should be reviewed in the evening to account for all supplies used, to estimate additional supply needs for the following day, to check that all equipment is present and functioning and that devices are fully charged, as applicable.

An example of a Daily supply list per team can be found in the online tools.

Inventory and storage of laboratory-related supplies during data collection: Every evening, the laboratory technician should review the supply stocks and the list of expected supply needs, and make sure that there are sufficient supplies for several days of fieldwork. When there are only about five days’ worth of supplies left, the technician should contact the Regional supervisor to coordinate another delivery. Supply stock-outs could delay fieldwork, with related implications on costs and logistics.

The laboratory technician should also work with the Team leader to ensure that there is sufficient freezer space for the expected number of new specimens, that there are enough frozen gel packs for the next day’s work, and that transport to the central laboratory is scheduled as needed.

Equipment maintenance and transportation during fieldwork: All equipment must be carefully maintained during fieldwork. One team member should be designated to check the working condition of all
Module 13. Field logistics

equipment at the end of each day and to report any concerns to the Team leader. Field equipment items (such as scales and portable photometers) should be stored in their carrying cases when not in use to help protect them from dust and humidity. Special care must be taken to protect equipment from excessive movement during transportation in vehicles. Funds may be set aside in the budget to hire help to transport items when necessary.

Transfer of specimens in the field: Any specimens that need to be transferred must be logged and accounted for at every point of transfer. This includes transfers from the household to the field laboratory, from the field laboratory to the regional laboratory, from the regional laboratory to the central laboratory and, when indicated, from the central laboratory to an international laboratory. The “Specimen transfer form” should have spaces to mark when samples have arrived at the next stage of transfer, and the same checks should be used at each transfer point between the cluster and the regional and central laboratories (as appropriate), with space to note the person responsible for the transfer at each stage. The cluster log form is the document that ensures accountability and prevents specimen loss in transit.

All specimens should be accompanied by a specimen tracking form that indicates the barcode. In some surveys there are two specimen tracking forms: a “Specimen tracking form for the phlebotomist” and a “Specimen tracking form for the laboratory technician” The barcode contains the unique ID for the specimen that includes a code for the household and cluster number. The total number and type of specimens collected from each household within a cluster should be recorded on the Cluster control form, and then summarized on the Cluster summary sheet for each cluster. These three types of forms are explained in Box 13.1.

When specimens are received at the field laboratory, they should be checked against the transmittal form (cluster log form) for matching barcodes. When they do not match, the Team leader should work with the individuals who did the collection or processing to find and correct errors. Any remaining discrepancies between the transfer form and the specimens should be noted on the cluster summary sheet and explained.

Field team supervision and monitoring

The Team leader provides overall quality control for the team by monitoring the performance of each member and ensuring that he or she is doing the job according to protocol and within expected timelines. The Team leader guides the team members respectfully and helps solve problems when they are not performing adequately. Roles for team members are clearly defined in their terms of reference, performance standards are set by the Survey coordinator, and expectations should be clearly communicated during training. The “Cluster control form” can be used to monitor the tasks of each individual (such as the number of interviews completed or blood draws conducted) and can help the Team leader assess and monitor individual and team progress.

As described in Module 8: Survey supervision and personnel, supervision should take place at multiple levels. Field visits by major stakeholders should be encouraged. They can help motivate teams to maintain a high quality of data collection despite potentially challenging conditions, and reinforce the importance of their work. These visits should be made mainly by people who participated in developing the survey protocol or in the training. Members of the Technical committee are likely to be able to detect and resolve any issues with survey implementation. Anyone making survey site visits should give feedback to the Survey coordinator. Where issues are identified, the Survey coordinator can then ensure that all teams are aware of the issues and that uniform corrections can be made as needed.
The following paragraphs describe specific tasks for supervising and monitoring interviewers, phlebotomists and field laboratory technicians.

**Box 13.1 Forms for specimen transfer**

<table>
<thead>
<tr>
<th>Specimen tracking form (for phlebotomist and lab technician)</th>
<th>The specimen tracking form is used to provide detailed information on the number of aliquots that were generated from each biological specimen. It indicates where they are stored during transport, by cryovial box number and by a unique identifier such as the barcode. The specimen tracking form contains individual-level information, while the cluster log form contains summarized information. Depending on the design of the forms, they can be a single form.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cluster control form</td>
<td>The cluster control form tracks the number of interviews, the number of biological specimens and the number of food samples that are collected by target population (or per household) per household. This information is consolidated in the cluster summary sheet.</td>
</tr>
<tr>
<td>Cluster summary sheet</td>
<td>The cluster summary sheet tracks the number of interviews, the number of biological specimens and the number of food samples that are collected by target population (or per household) per cluster.</td>
</tr>
</tbody>
</table>

**Supervising and monitoring interviewers:** Supervising and monitoring interviewers require a number of tasks: observing each interviewer regularly, re-conducting interviews with participants when needed and reviewing the quality of all data collected. These actions are critical throughout the training, the pilot test, the beginning of the survey implementation, and during data collection. In the beginning of the data collection period, interviewers are still learning how best to collect data and conduct their interviews, and may need extra support to ensure a high level of quality. At the end of the survey, data quality risks decreasing due to fatigue.

When observing interviews, the Team leader should sit close to the interviewer without disturbing the participant. The Team leader needs to assess whether the interviewer is interpreting the responses correctly, recording the correct responses, following skip patterns and following the recommended rules of conduct. The interview should not be interrupted unless there is a serious mistake or a problem with the equipment. Feedback, both positive and negative, should be provided to the interviewer in private, away from the interview setting and after the interview is over.

Spot-checks are useful to ensure that interviewers actually interviewed all selected members in a household. Following up and re-interviewing appropriate household members by asking certain critical questions (such as the number of individuals in the household, dates of birth, and other questions that could falsely create skip
patterns to make the interview time shorter) can help identify instances when interviewers are not interviewing a particular household member selected for interview. Reasons for this error could include accidentally interviewing the wrong household member, entering the wrong age for a household member (thus making that individual ineligible for interview) or making other data entry errors. Re-interviews should be done in approximately 10% of households. If possible, the re-interview should be conducted on the same day or at least while working in the same cluster to ensure that the household members and any visitors to the house are still present. If omissions are found, the Team leader should discuss the mistake with the interviewer, who should then return to conduct interviews with any respondents missed. Errors made by an interviewer should be followed by a verbal or written warning, and the case should be documented and reported up the line of command. In order to prevent additional errors, the Team leader should observe the interviewer more frequently. In cases where the interviewer repeats errors, the Team leader should discuss the situation again with the interviewer and may decide that the errors are grounds for dismissal.

If using paper-based surveys, every questionnaire must be thoroughly checked in the field prior to leaving the community so that any errors can be corrected. This is a long process and may require that a data editor be included in each field team. Small errors can cause significant problems during analysis.

Editing paper questionnaires in the field must be done with a pen of a different colour than was used by the interviewer. Missing responses, unreadable responses, and inconsistent or incorrect responses should not be corrected by the editor. Instead, using the different colour, a question mark should be put next to the item concerned. The page number or question number should be written on the front or back of the questionnaire for ease of finding the problem. The error should be discussed with the interviewer, and feedback (both positive and negative) provided. Each error should be brought to the attention of the Team leader, who will decide how to proceed. It may be that the interviewer can make a correction immediately without contacting the respondent, otherwise, the interviewer needs to return to the household to obtain the correct data. Errors that come from multiple interviewers need to be addressed by the whole team. The interviewer, field data editor or Team leader should never make up an answer.

**Phlebotomists and field laboratory technicians:** The Laboratory supervisor (or Team leader, if there is no Laboratory supervisor) needs to observe the performance of all phlebotomists and laboratory technicians to ensure that they are following the protocol and standard operating procedures. The most critical times to observe phlebotomy and to provide quality control on specific tasks is during the training and the pilot test, as well as at the beginning of implementation. These tasks, known as quality control points, include labelling correctly, taking appropriate time to draw blood, demonstrating the ability to collect blood, collecting the correct amount of blood, properly maintaining the cold chain, and storing and transporting blood and other specimens correctly. If the number of specimens is not consistent with the number of household members who were eligible for specimen collection, the reasons for the inconsistencies need to be verified.

Accidental errors should be corrected. Purposeful errors are more difficult to identify, but if the same phlebotomist has a higher frequency of problems with specimen collection or field readings, it could indicate a problem with their performance. Spot-checking and observing work in the field are the best ways to detect mistakes.
Coordination and communication in the field

Communication is used to share field experiences, give feedback to teams, obtain permissions at different levels and mobilize communities. Regular communication is essential, within field teams and between the field teams and the Regional supervisors and Survey coordinator. Before fieldwork starts, a communication plan should be established for routine coordination and for emergencies. The rules should specify who should communicate with whom and on which topics. A reliable means of communication, usually mobile phones, should be provided. SIM cards for multiple networks may be needed where coverage is known to be irregular. The rules will vary by survey and by the national context related to expected network coverage and ease of communication.

Box 13.2 describes examples of routine communication.

**Box 13.2. Examples of routine communication**

Routine communication between Team leaders and Regional supervisors usually concerns:

- managing timely payments to team members;
- managing transport of supplies and equipment to the field as well as transport of paper-based forms and of specimens from the field;
- providing a daily progress update, for example the cluster, number of households surveyed, and the number and type of specimens collected; and
- raising questions about any issues experienced.

The Regional supervisor manages the information received in accordance with his or her role and provides an overall update on a regular basis to the Survey coordinator.

Routine communication between the Team leader and team members usually includes verifying progress and safety, including alerts from team members when:

- they are moving to another location
- difficulties arise, such as problems in locating a household or in using equipment.

In general, telephones should not be answered during an interview, as this disrupts the flow of the conversation. It is recommended that communication from Team leaders be done by text messaging, requesting a call back when the interview is finished.

Sharing field experiences through messaging and chats: Communication between teams is also essential, and can help teams troubleshoot common problems that arise. Fieldwork experiences can be shared with the entire survey field team in real time using a forum set up on a mobile messaging or chat application. All field team members can be encouraged to share their observations, challenges, and questions. Feedback can then be provided by all members of the technical team and by other field teams, with summary recommendations and clarification from the supervisors or coordinators. Applications can be used to set up various forums that are specific to each field team, to different roles within a team (such as interviewers or laboratory personnel), and for different levels of survey management.
Feedback to teams: Regional supervisors and the Survey coordinator should provide frequent, helpful feedback to teams. Feedback should always be constructive, even when discussing challenges or improvements that need to be made. Feedback to a specific team member should be done directly in person or over the phone, and not in a public forum.

Permission at national, regional, and district levels: Official permissions to conduct a micronutrient survey should be obtained at national, regional, and district levels by the appropriate members of the Steering committee and the Technical committee. This approval should first come through any specific institutional review board or ethical clearing committee in the country, as discussed in Module 1: Planning and designing a micronutrient survey. The Principal investigator or Chair of the Steering committee should be responsible for officially informing various levels of government about the survey objectives and the implementation plan, and for obtaining approvals from all levels. In administrative areas where the fieldwork will take place, letters of support from the ministry of health should also be obtained to facilitate fieldwork.

Permission at cluster and household levels: Prior to entering a community, and where mapping and household listing are being conducted in advance, the listing team should seek permission from local officials to conduct the survey. The appropriate local community leader should be provided with a letter of support from the district-level health office, with any accompanying letters from other administrative level.

The listing team should also meet with other community leaders to brief them, with clarity and sensitivity, on the aims and objectives of the survey and on the types of information the survey team will collect. These leaders may include local elders, health administrators and personnel from the office of statistics, as well as field focal points including, in some cases, members of households selected for interview. Their understanding and collaboration are crucial for access to households and to maximize consent, especially for the collection of biological specimens. Each interviewer and phlebotomist needs to be courteous and tactful when entering a household. In many settings, team members need an official letter, or a badge and identification from the government to justify the data collection.

The listing team should record contact information for all local leaders. This will allow the survey field team to call in advance to inform the community when they will be arriving and to finalize any necessary local logistical arrangements. This information will also be important in case of any emergency.

Before starting fieldwork, the Team leader should visit local health facilities to discuss the referral process agreed previously with the ministry of health. In this way, preparations can be made to manage referrals as needed.

Mobilizing communities: Team leaders should have a list or map of survey households that, if possible, includes the names of the head of household (see the “Household listing form” template). Team leaders should contact the identified local leaders and health facilities at least one week ahead of the fieldwork to share the plans and to request their presence and assistance. The visit should be confirmed, or amendments made, two days before the expected date. This process also provides the Team leader with advance notice of any community events that might affect data collection, for example, a market day or a wedding, and allows local leaders to plan for the team’s arrival. Local leaders may be requested to alert the selected households about the field team’s arrival and the objectives of the data collection exercise. If the listing includes a phone number for the household head, Team leaders can also call respondents directly to arrange interviews. The first visit of a data collection team into a community is best when accompanied by a local community leader.
**Arranging for travel and accommodation**

In consultation and coordination with the Regional supervisor, each Team leader is responsible for making travel arrangements for his or her team. This includes ensuring that every vehicle is maintained by the driver, is safe, provides adequate transportation to the work site and is used only for survey purposes.

The Team leader is also responsible for arranging accommodations in a secure location near the survey clusters. Ideally, the accommodations should also provide a central location for team meetings, equipment storage and equipment charging. Logistics are simplified if the lodging can be near the field laboratory processing facility.

Transport and accommodation plans should be shared with the Survey coordinator or administrative assistant, in accordance with the survey communication protocol.

**Data management in the field**

**Electronic data:** The Team leader is responsible for managing data collected in the field. Electronic data should be backed up to protect records from accidental loss. Data entry should be set up so that interviewer data are backed up on the device on which they were entered, and uploaded onto the Team leader’s device at the end of each day. Where possible, data should also be uploaded to the server. USB sticks with adapters to fit the device, as needed, can also be used to back up data. This may be an important option in areas where it is not possible to upload data to the server for several days.

All teams should have a global positioning system (GPS) to assist them in finding selected clusters and households. Most mobile phones and tablets come with these systems installed, and they should be used whenever possible to verify the location of data collection and allow for cross-checks with the team number and number of data files received.

**Paper-based data collection forms and other records:** The most important paper-based forms collected in the field (for example, cluster control forms, specimen tracking forms and questionnaires) should be tracked as they move from the household to the cluster to the region and then to the central data entry point. Where electronic devices are being used for the main questionnaire, any paper-based forms should be photographed and the image uploaded along with the completed questionnaires from the cluster. All forms should be stored securely, using methods to protect confidentiality during fieldwork, while in transit, and at the final data entry point.

Where paper-based data collection is used, there should be a central data entry system to track the data on all types of forms. Typically, information from the cluster control form, which includes the total number and types of specimens collected, is entered by the same personnel who enter the questionnaire data.

Detailed specimen tracking data are typically entered by the central laboratory. Where barcode labels are used, a hand-held barcode reader should be used at the laboratory to record incoming specimens onto a spreadsheet and compare specimens received with the specimen tracking form. The Survey coordinator should be made aware of any inconsistencies. This spreadsheet should be shared with the relevant laboratories for later entry of analysis results.
Labelling: The unique ID should accompany participants throughout the data collection process. For paper-based data collection, a label should be placed on the household and individual questionnaire forms at the time of interview. For electronic data collection, a code is scanned or entered by hand into an electronic collection device. At the time of any sample or specimen collection, a corresponding label should be placed on the food sample or biological specimen and on the specimen control forms, and this number should be checked against the label on the household or individual form. These label codes can be scanned into an electronic device to ensure that they match the ID of the household or survey participant. If there is a discrepancy between a scanned ID (for example it does not match to any household or individual) then the data collection system will generate a warning and block further data entry until the ID does match a household or individual. It is imperative for the quality and reliability of survey results that each team member responsible for handling the labels reads them very carefully so that they are not mixed up or used interchangeably.

Procedures should be in place to ensure that barcode data and linkages are checked and corrected where necessary before leaving any cluster. The unique ID can be scanned by the electronic device used in the field (note that this may require downloading an easy-to-use scanner onto all devices, which is then linked to open at the appropriate point in the data collection modules). For paper-based data collection, the label barcode for questionnaire data (double) entry and for recording sample analysis results at the laboratory can be entered manually. However, barcode scanners that plug into a computer and can insert a scanned code into, for example, a selected database entry field or cell on an electronic spreadsheet are not costly, and these are the recommended option.

**Cold chain management and quality control**

It is essential to monitor the temperature of cool boxes and freezers and oversee custody of the biological specimens. If specimens are stored at the wrong temperature, even for a short period of time, they may no longer be useable and all the work to collect them could be wasted.

Cold chain management and quality control during specimen transportation and storage ensure that specimens are kept within the correct temperature range. All survey team members must be aware of the planned cold chain logistics, so that they can work together to ensure that the cold chain is properly maintained at all times. The “Overview of cold chain logistics” online tool provides an example of a good cold chain management system. The example is from a survey that had a comprehensive list of indicators and related biological specimen types (blood, urine and stool) that needed to be kept at temperatures varying from −20°C to approximately 8°C, in field conditions where electricity was not regularly available.

**Shipping specimens**

In many surveys, selected analyses will take place outside the country. Prior to shipping, specimens need to be properly prepared, which includes labelling and using a reliable system and carrier that will guarantee that the cold chain is maintained. In most cases, dry ice is used to keep specimens frozen. Sometimes obtaining dry ice can be complex and it is important to consider the source well in advance.

More information on instructions for packing specimens, as well as guidelines for labelling and shipping, can be found in the “Guidelines for shipping specimens”, “Labels and markings required for proper shipment”,...
Module 13. Field logistics

“Packaging/shipping specimens using cold packs instructions”, and “Packaging/shipping specimens using dry ice instructions” online tools.
Module 14. Data entry and cleaning

In this module, we will discuss:

- Ensuring the quality of data
- Processes for data entry, examples of data entry software and management of the database
Confidence in survey results is heavily dependent on the quality of data collection and analysis. Data quality is affected by such factors as database design, data entry, cleaning and processing. There is usually an expert subcommittee to manage and provide oversight for tasks related to data management and analysis. More information is provided in Box 8.2 of Module 8: Survey supervision and personnel and in Module 15: Data processing and analysis.

Ensuring the quality of data

The procedure for entering and managing data, whether collected electronically or on paper, should include guidance on:

- key staff and supervisors responsible for each stage (database design, data entry, cleaning, management, processing and analysis);
- the process for entering data;
- software and equipment to be used;
- developing the data entry screen with appropriate validation checks and skips;
- the flow and tracking of data from the field to the final format; and
- data confidentiality and security.

The choice of paper-based or electronic data collection must be made early on in the survey planning process. Electronic data collection is the preferred method, as it reduces time and improves data quality. In addition, electronic data can be backed up every day while teams are still in the field, whereas paper forms risk being lost or misplaced before the data are entered into an electronic database. Further discussion of the advantages and disadvantages of each method can be found in Module 4: Survey design. When paper-based questionnaires are used, sufficient entry staff are needed to perform double data entry (entry of each questionnaire by two independent staff).

Each survey team member, from the interviewers to the Survey coordinator should have a clear task related to data quality in his or her roles and responsibilities. During field work, the responsibility for data integrity and quality starts with the interviewers. Information should be entered in a standardized, legible format onto paper questionnaires and other data collection forms. Ultimately, team leaders are responsible for the consistency and completeness of the team’s data. Team leaders in the field need to review all data on the questionnaires to ensure that they are clear and complete, and check for potential data errors or mismatching between specimens and individual or household questionnaires. See Module 11: Data collection tools, field manual, and database, Module 13: Field logistics and the “Survey field manual” online tool for more information on these checks.

Processes for data entry, examples of data entry software and management of the database

Constructing the data entry system

A strong data entry system is required to ensure high-quality data, whether data are collected electronically in the field or are entered from paper-based questionnaires. To improve data quality, the data entry program should have preprogrammed skips, correctly formatted fields for variables such as dates, and validation checks that set appropriate limits for certain variables, such as dates of birth for children under 5 years of age and values for haemoglobin levels. It should also include cross-checks for consistency between related variables,
such as the ID code of a woman of reproductive age compared with the household ID and the stated number of women in this age group in the household. In this way, the system rejects any unexpected values and the variable is flagged for further review.

Construction of a data entry system requires a complete data collection tool (questionnaire). It is common to develop the electronic data collection system or data entry system for paper-based tools after the cognitive interviewing process (see Module 11: Data collection tools, field manual, and database). This version can be used to train data entry staff (where applicable) who can practice with completed forms during the training and pilot test. Minor adjustments to finalize the tools may still be expected during the training and piloting process, and it is important to make sure that all changes are made to the final software version, whether uploaded for electronic collection or used to enter data from paper-based forms.

For double data entry of paper-based forms, a discrepancy check program needs to be developed to compare the independent entries.

The steps required to develop the system and enter data are illustrated in Fig. 14.2.

With electronic data collection, there is no need for double data entry, nor for the related discrepancy checks and reconciliation. It is possible to move straight to data checks, cleaning, and analysis. This is one of the principal advantages of electronic data collection.

**Fig. 14.2. Steps for developing the data entry system and for entering data**

1) Finalize survey questionnaire  
2) Develop data dictionary  
3) Construct data entry system  
4) Pilot test data entry system  
5) Receive completed paper questionnaires from field  
6) Start double data entry  
7) Check discrepancies between double data entries  
8) Reconcile any differences  
9) Conduct data checks for unexpected values  
10) Review original forms to resolve issues  
11) Final data set available for analysis

**Paper-based data collection: completed forms sent to central data management office.**

**Electronic data collection: completed forms uploaded to central server for data management.**

5) Start main survey data entry  
6) Conduct data checks for unexpected values  
7) Reconcile errors where possible  
8) Final data set available for analysis
Choosing software

Software for data entry from paper-based forms: Programs that can be used for data entry from paper-based questionnaires include Epi Info, Epi Data, and Census and Survey Processing System (CSPro). Several Microsoft® Office programs, including Microsoft Access, offer additional options.

Software for data entry for electronic data collection: Programs available for electronic data entry include Epi Info and Open Data Kit (ODK), a frequently used free and open access software. Factors to consider in choosing software include cost, capacity to generate relational (hierarchical) data files (for example, linking a woman of reproductive age to the household she is in and to a child she may have) and whether open access is an important feature.

In either case (paper-based or electronic), a program that can be modified by others relatively easily should be used in case the primary developer becomes unavailable.

Developing a data dictionary

A data dictionary defines all variables included in the survey questionnaire. It is required for developing the data entry program so that type, field width and validation checks (agreed upon acceptable values) can be programmed for each variable. The data dictionary also needs to define all variables created from the original data, for example, the variable “anaemia” may be defined from the result of the haemoglobin test together

<table>
<thead>
<tr>
<th>Variable</th>
<th>Variable name</th>
<th>Variable type</th>
<th>Variable width</th>
<th>Example values/notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Participant number</td>
<td>ID</td>
<td>Numeric</td>
<td>3</td>
<td>001–999</td>
</tr>
<tr>
<td>Household number</td>
<td>HHID</td>
<td>Numeric</td>
<td>2</td>
<td>01–25</td>
</tr>
<tr>
<td>Residence</td>
<td>URBAN_RURAL</td>
<td>Numeric</td>
<td>1</td>
<td>1 = Urban, 2 = Rural</td>
</tr>
<tr>
<td>Region</td>
<td>STRATA</td>
<td>Numeric</td>
<td>1</td>
<td>1–3</td>
</tr>
<tr>
<td>Cluster number</td>
<td>CLUSTER</td>
<td>Numeric</td>
<td>2</td>
<td>01–30</td>
</tr>
<tr>
<td>Age in months</td>
<td>AGE</td>
<td>Numeric</td>
<td>2.1</td>
<td>06.0–59.9</td>
</tr>
<tr>
<td>Date of birth</td>
<td>DOB</td>
<td>dd/mm/yyyy</td>
<td></td>
<td>[values set according to survey date and expected age of respondent]</td>
</tr>
<tr>
<td>Sex</td>
<td>SEX</td>
<td>Numeric</td>
<td>1</td>
<td>1 = Male, 2 = Female</td>
</tr>
<tr>
<td>Date of survey</td>
<td>SURVEY</td>
<td>dd/mm/yyyy</td>
<td></td>
<td>15/06/2004–20/08/2004</td>
</tr>
<tr>
<td>Haemoglobin</td>
<td>HB</td>
<td>Numeric</td>
<td>2.1</td>
<td>04.0–18.0</td>
</tr>
<tr>
<td>Urinary iodine</td>
<td>UIC</td>
<td>Numeric</td>
<td>4.1</td>
<td>0000.0–1000.0 µg/L</td>
</tr>
<tr>
<td>concentration</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Retinol binding protein</td>
<td>RBP</td>
<td>Numeric</td>
<td>2.2</td>
<td>00.00–90.00 µmol/L</td>
</tr>
<tr>
<td>concentration</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iodine in salt based on</td>
<td>SALT_RTK</td>
<td>Numeric</td>
<td>1</td>
<td>1 = Yes, 0 = No</td>
</tr>
<tr>
<td>rapid test kit</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iodine level in salt</td>
<td>SALT_QUANT</td>
<td>Numeric</td>
<td>3</td>
<td>000–120 mg/kg</td>
</tr>
<tr>
<td>based on titration</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Note: These may not be correct minimum and maximum values for use in populations living at high altitudes.*
Module 14. Data entry and cleaning

with the individual’s age and pregnancy status. A data dictionary is also essential for developing the data analysis syntax. **Box 14.1** provides an example of a data dictionary.

**Testing the data entry system**

The data entry system requires extensive testing, preferably by a number of people entering different options that will, for example, test different skip patterns. After this testing, the system should be piloted among different groups, to assess:

- validation checks (expected data ranges/exclusion of implausible values and cross-checks with values for other entered, related variables)
- data entry formats
- skip patterns
- logical, user-friendly variable names, labels, format and flow.

Results of the pilot test may reveal that the data dictionary needs adjustment. Piloting of the data collection and data entry system should be done prior to training, so that enumerators are using the most optimal system during the training.

**Data entry requirements**

Data entry should start as soon as possible after the initiation of fieldwork. This will allow common errors to be identified early, reasons for errors to be determined and corrective action to be taken.

- A micronutrient survey may require that a large amount of data be entered. For paper-based survey questionnaires, data can be entered into the electronic database either:
  - At the end of the day by the survey team. This approach requires significant time in the field that could otherwise be spent on data collection. On the other hand, it allows for the quick correction of erroneous data by allowing the team to return to a cluster. It also enables data to be backed up onto a separate device to avoid loss of information that could result if the paper version of the completed questionnaire was lost.
  - By double data entry at the central data management location. This is the most commonly used method for when data collection is paper-based. It may improve data quality by reducing the rate of errors and inter-individual variability because a limited number of experienced data entry personnel enter the data. This approach requires strong supervision and detailed checks in the field to ensure the legibility and quality of the data entered. This method requires:
    - a minimum of two data entry staff assigned by the database manager to enter the data;
    - entry of information from each questionnaire by each of these two people (double data entry);
    - comparison of the two data files by the database manager using the discrepancy check program;
    - reconciliation of any differences based on the paper version of the questionnaire; and
    - monitoring of personnel performance and retraining where needed.
- For accountability, the final and complete set of data files should include:
  - A clean final master version of the data to be used for data analysis. The final master dataset will have a data dictionary with variable labels that link to specific questionnaires.
- The two sets of raw entry (to confirm double entry).
- A log of any discrepancies found. The log of discrepancies per variable could be presented as a table with the following headings:

<table>
<thead>
<tr>
<th>Variable</th>
<th>Data entry staff n° 1 value</th>
<th>Data entry staff n° 2 value</th>
<th>Resolved value</th>
</tr>
</thead>
</table>

**Cleaning data to finalize the database for analysis**

Whether collected on paper or electronically, data require checks and cleaning. Data cleaning is intended to identify potentially erroneously recorded data. For paper-based collection, checking and cleaning take place after data entry errors have been corrected by comparing and reconciling double-entered data. For electronic data that are continually uploaded to the server, checking and cleaning can be done on a regular basis during the field work period.

With electronic data collection, a data review exercise can be done several times a week. This allows feedback to be sent to the Survey coordinator on progress toward the expected numbers of interviews and specimens. For example, it might be found that consent for blood collection among children under 5 years of age is lower than expected, which may prompt follow-up to find the cause and advise teams accordingly. Again, any changes to procedures should be documented to avoid biasing the sample.

**Duplicate entries:** Unexpected duplicate entries need to be fixed immediately. Household and individual ID numbers are unique and should occur only once in a data file. Duplicate ID numbers may have several causes, for example a single questionnaire may have been entered twice, two different individuals were assigned the same ID number or one of the two ID numbers was entered incorrectly. This last issue can be avoided by using barcode labels and barcode readers for unique ID numbers in the field and at the laboratory.

Dates and other identifiers are very useful in data cleaning processes. Data tracking documents that describe the clusters, dates and individuals should be used as management tools to help disentangle duplicate data entries and additional types of irregular data findings. Within the data tracking system, there can be a list of ID numbers and the date that data were collected for each individual. For example, if you know that person 1234 belongs to cluster 567 and you have two entries for person 1234, then you can check the cluster number to determine which data was entered incorrectly. This part of data cleaning—determining which of the duplicate entries is correct—requires time and attention to detail.

**Implausible values:** The most common method of checking data is to produce a frequency table for every variable and to identify values that fall outside of a normally acceptable range. This range should be defined in the data dictionary (see Box 14.1). Where outlying values are found, they should be traced back to the original questionnaire to see if it could be a simple data entry error, due to handwriting that is difficult to read or to an incorrect decimal place. In general, valid data entry types and ranges are pre-set in any electronic data collection form in order to reduce the likelihood of “out of normal range” errors. Where there appears to be a consistent unexpected value for a specific cluster, the Team leader should be notified and he or she should verify whether the value reflects something unusual about that cluster. If the checks are being conducted on an ongoing basis during data collection and there is a consistently unexpected value produced by one interviewer, the Team leader should follow up and monitor the performance of that person. Often the
outlying values cannot be verified and corrected, and a decision needs to be made regarding changing the variable outcome to a ‘missing’ value. Any such findings and changes need to be documented.

Logical errors found during the data cleaning should be investigated and, when possible, corrected. This is relevant for electronic or paper-based data collection.

Examples of logical errors include:
- the date of birth is recorded as after the survey date;
- the date of birth does not fit with the expected age of the individual, for example the age calculated from the date of survey and date of birth is not the same as (or within an acceptable range of) the stated age;
- the designation of “urban” and “rural” is inconsistent among households within the same cluster;
- body mass index (BMI) values indicate that the height and weight measurements may have been entered in the wrong boxes, or that a decimal place has been entered incorrectly.

Logical error checks should be pre-set in electronic data collection forms. By correctly programming the electronic data collection system, it is possible to ensure that these errors cannot be entered, and such values are immediately flagged and can be rectified. For example, if a BMI is outside of the expected range, the participant’s weight and height can be measured again.

All errors must be either corrected or deleted from the database, and the process should continue until the data are considered “clean.”

**Missing data:** Missing data may have been entered as 99.9 or 999.99, depending on the questionnaire instructions. Missing data, including refusal codes, need to be appropriately recoded so they do not skew the summary statistics. In addition, the number of missing responses for each variable needs to be investigated. If there are many missing values, check that these are not a result of a database or data entry error.

**Merged data:** Laboratory data that are not measured during the data collection period (for example, haemoglobin levels or the presence of malaria) usually become available well after the final database has been approved. These laboratory data will need to be merged with the questionnaire data, using the household or individual’s unique ID number.

Here is an example of how to verify merged data: If the survey data file shows 800 women of reproductive age eligible for specimen collection, and the corresponding laboratory data file includes only 700, you would expect that 100 eligible women refused consent to provide a specimen or that the specimen volume was insufficient. However, after merging data by unique ID, it might be that only 650 lines of data match. In this case investigations to resolve the discrepancy may include:
- reviewing the “Specimen transfer form” to compare the IDs of women of reproductive age against specimens collected and specimens sent to the laboratory; or
- verifying the use of a barcode reader to enter ID numbers at the laboratory (on arrival and during recording of analysis results). If the barcode reader was not used, it is possible that IDs were incorrectly entered at the laboratory and that some specimen ID numbers do not match with a corresponding ID of women of reproductive age;
Module 14. Data entry and cleaning

- reviewing the response to specimen collection for all individual IDs where laboratory data are missing to assess the reason. Reasons include a declined test, inadequate specimen collected, unable to be measured (for example if the blood was haemolysed), or lost specimen.

Creating individual-level and household-level data sets

There will be individual data sets and household data sets. The individual data sets may need to be cross-linked, for example, mother and child pair. They will also be linked to the household. During the planning stages, unique IDs and linking variables were ideally created in the data entry form to enable linking at the data management stage. Software should be selected that allows for hierarchical linking of data where needed.

Managing the database

All data, collected on paper or electronically, should be entered and maintained securely in a central database. Typically, a Database manager is responsible for developing the database and maintaining backups. However, there will be multiple people that work on managing the survey database, depending on the complexity of the survey. A Data coordinator needs to work with the Database manager as well as software programmers, statisticians, and other specialists to ensure that the data are entered, linked, and maintained in a secure, organized way. Saving data in two different networks or servers, with different access permissions, helps ensure that there is no loss of data or risk of files being deleted or manipulated by error. The Database manager and Data coordinator need to have strong experience working with large databases.
Module 15. Data processing and analysis

In this module, we will discuss:

- Data processing
- Analyses for surveys designed with stratification and clustering
- Additional information for interpreting of nutrition
- Standard analysis and checks for anthropometry data
Module 15. Data processing and analysis

Because this micronutrient survey manual focuses on the standard micronutrient survey design (a multi-stage cluster survey with or without stratification), this module focuses on processing and analysing data from surveys with this design. In specific situations, such as a survey in a refugee camp, where the simple random sampling (SRS) method is selected, analysis should be conducted using SRS-specific procedures. This usually means that there is no need to apply survey weights or a stratum or cluster variable when running frequency tables and generating mean estimates.

Analyses of micronutrient survey data should be conducted using software that accounts for multi-stage complex survey design with stratification. Such software includes Epi Info, SAS version 8.0 or later, SPSS with the optional SPSS Complex Samples module, Stata, Sudaan, and R.

Data processing

Before data analysis can begin, data may need to be prepared or processed to accommodate the statistical software or methods being used.

Categorizing variables: For data on biological specimens and food samples, there may be a need to create new variables to interpret the results of analysis. For example, haemoglobin needs to be divided into a categorical variable with two levels, one for anaemic and one for non-anaemic. Anaemia can be further categorized into none, mild, moderate, and severe, according to WHO guidance. Additional information on cutoffs and the need to adjust for such factors as altitude, smoking and inflammation can be found in Module 3: Biomarker selection and specimen handling.

Calculating anthropometry Z-scores: The recommended standard approach for anthropometry data analyses uses the WHO Child Growth Standards as the reference. Analyses can be done using standard software, such as the Anthro software, or macros (SAS, SPSS, STATA and R) that can be downloaded from the WHO website and applied directly to the data. WHO recently developed an online tool for anthropometric data analyses. This tool updates the Anthro methodology to provide more accurate estimates of standard errors and confidence intervals for prevalence and mean Z-scores. The WHO Anthro Survey Analyser, based on the “R and R Shiny package” provides interactive graphics for data quality assessment. It also provides a summary report template offering key outputs (such as Z-score distribution graphics) for various grouping factors and nutrition status tables with accompanying prevalence and Z-score statistics. The software is currently available either online or offline.

Analyses for surveys designed with stratification and clustering

Survey weights are calculated to estimate the probability of selected households and individuals being included in the survey sample compared with the probability of each household or individual in the entire population being included in the survey sample. These survey weights are then used in the analysis to adjust the survey data to represent the larger sampling frame of households that all having an equal probability of being sampled.

Weighting for stratification

A stratified sample is produced when the population is divided into distinct, independent strata. Using PPS to select PSUs followed by systematic random sampling of an equal number of households within clusters means
that each household within the stratum has an equal probability of being selected. This is described in more
detail in Module 4: Survey design, Module 4: Sample size and Module 6: Selecting clusters.

Stratum-level data can be used to produce nationally representative estimates by adjustment or weighting for
the proportion of the national population in each stratum. Table 15.1 shows an example of calculating stratum
weights, where regions A, B and C represent survey strata. The respective populations are indicated in two
ways: as numbers and as percentages of the national population. The number of specimens tested for
haemoglobin is also stated, along with the proportion of the total test specimens within each stratum.

Table 15.1. Example of calculating stratum weights for use in analysis of estimates based on
haemoglobin samples

<table>
<thead>
<tr>
<th>Region (stratum)</th>
<th>Population</th>
<th>Haemoglobin specimens tested</th>
<th>Stratum weight I a</th>
<th>Stratum weight II b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>A</td>
<td>372 978</td>
<td>62.9</td>
<td>334</td>
<td>34.5</td>
</tr>
<tr>
<td>B</td>
<td>127 841</td>
<td>21.6</td>
<td>324</td>
<td>33.5</td>
</tr>
<tr>
<td>C</td>
<td>92 117</td>
<td>15.5</td>
<td>309</td>
<td>32.0</td>
</tr>
<tr>
<td>Total</td>
<td>592 936</td>
<td>100.0</td>
<td>967</td>
<td>100.0</td>
</tr>
</tbody>
</table>

a Column 1 divided by column 3
b Column 2 divided by column 4

To derive a correct national estimate based on haemoglobin specimens, stratum weights need to be applied
to each stratum to account for differences in population size. There are two approaches for calculating the
stratum-specific weights. Table 15.1 describes them as “stratum weight I” and “stratum weight II” to
differentiate the two methods. Whichever method is used, it must be applied consistently for all survey data.

The calculation of stratum weight I requires the approximate number of individuals that each survey sample
represents. This is determined by dividing the population number in the stratum by the number of participants
sampled in that stratum. In Table 15.1, the stratum weight I for Region A is 372 978 ÷ 334 = 1116.70. In other
words, every individual sampled for haemoglobin can be considered to be representative of 1116.70
individuals in the region.

The calculation of stratum weight II requires dividing the percentage of the population in each stratum by the
percentage of the specimens collected in each stratum. In Table 15.1, the stratum weight II for Region A is
62.9 ÷ 34.5 = 1.823. This alternate approach can be useful when the total population size per region is not
available, for example if a census has not been updated or if there is conflicting information between the
census and other reliable estimates of population size.

Once the stratum weights are calculated, they should be added to the data file. In the example shown in Table
15.1, if the method for stratum weight II were used, then each individual sampled from Region A would have
the weight 1.823, each individual sampled in Region B would have the weight 0.645 and each individual
sampled in Region C would have the weight 0.484.

Depending on the survey sampling design and factors affecting non-response and missing data, different
weights may need to be calculated for different variables. More information on adjusting sampling weights
Important considerations related to clustering

As described in Module 5: Sample size, the design effect (DEFF) and intra-cluster correlation (ICC) are important indicators of the effect that using a cluster survey has on the data, when compared with a survey based on SRS. Both the DEFF and ICC can be estimated from the data during the analysis phase of the survey. The DEFF is equal to the variance accounting for the complex survey design, divided by the variance assuming SRS.

The following sections focus on analysis of data from cluster surveys that used the PPS approach to selecting PSUs.

Calculating percentages for stratified cluster data

Calculating percentages for stratified cluster survey data is very similar to calculating percentages for data collected using SRS, with the exception that a stratum weight variable is included in the calculation. Table 15.2 presents the number of individuals with haemoglobin samples who were found to be anaemic along with the unweighted percent prevalence for each stratum.

### Table 15.2. Example of calculating stratum weights for analysis of anaemia estimates based on haemoglobin results

<table>
<thead>
<tr>
<th>Region (stratum)</th>
<th>Population n</th>
<th>Haemoglobin specimens tested</th>
<th>Specimens categorized as low haemoglobin (anaemia) n</th>
<th>Unweighted survey result for anaemia %</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>372 978</td>
<td>334</td>
<td>112</td>
<td>33.5</td>
</tr>
<tr>
<td>B</td>
<td>127 841</td>
<td>324</td>
<td>185</td>
<td>57.1</td>
</tr>
<tr>
<td>C</td>
<td>92 117</td>
<td>309</td>
<td>119</td>
<td>38.5</td>
</tr>
<tr>
<td>Total</td>
<td>592 936</td>
<td>967</td>
<td>416</td>
<td>43.0*</td>
</tr>
</tbody>
</table>

*The national percent estimate here is unweighted and incorrect, see text below.

Samples within a stratum are generally self-weighted, assuming PPS selection of PSUs and systematic selection of households and individuals within households within the cluster, with similar non-random non-response between clusters. Therefore, the unweighted prevalence of anaemia for each of the three regions is likely to be fairly reliable, unless there was a significantly different pattern of non-response or missing values between clusters within a stratum.

An incorrect approach to obtaining the national estimate for anaemia prevalence would be to add the total number of people with anaemia (416), and divide this by the total number of samples tested (967), and then multiply by 100 to obtain the percentage \((416 \div 967) \times 100 = 43.0\%\). This unweighted percentage gives the

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percentage of tested samples with low haemoglobin but does not provide a correct estimate of the percentage of the national population with low haemoglobin. The unweighted estimate ignores the fact that the population size in Stratum B is about one third as large as the population in Stratum A and about 1.4 times larger than in Stratum C.

The calculation to determine the correct weighted prevalence of anaemia among the population must take into consideration the population size in each region. For this example, the correct population estimate for anaemia would be:

$$\frac{(62.9 \times 33.5) + (21.6 \times 57.1) + (15.5 \times 38.5)}{100} = 39.4\%$$

In the above calculation, the percentage of the population that lives in each region is multiplied by the percentage of individuals with anaemia in the same region. These values are added together and divided by 100.

Specific statistical software with the capacity to handle complex survey design should be used to calculate the results. Such programs use weights that have been generated by the analyst and account for the survey design used on the basis of information entered into the software. Applying these design variables, the software can be used to calculate the weighted 95% confidence intervals (CIs) around the estimate and the DEFF.

When it is not possible to perform a survey using PPS sampling of PSUs, for example, if PSUs were selected using a simple random or systematic sampling methodology with a sample of participants interviewed in each cluster, then the population size of each sampled cluster is required to correctly analyse the data. The approach to weighting is similar to that described above, with the difference that the population size in each cluster surveyed would be used to calculate the sample weight.

**Calculating sample weights for non-response**

If the response of households and individuals is similar (and considered as random) across clusters, then weighting for non-response may not be needed. If the response for a primary outcome of the survey differs dramatically by cluster or stratum, then sampling weights may reduce the potential bias for the national estimates. If weighting for interview non-response for households is considered necessary, this adjustment may need to be repeated for each of the different population groups selected from households in the survey. Household response rates may differ due to such factors as a high proportion of the population in a cluster that works away from home, or in situations where a cluster has a wide range of socioeconomic status (SES). Lower SES households are typically more likely to participate, which may bias results toward a higher prevalence of anaemia and micronutrient deficiencies unless the household weight is adjusted for the higher refusal (non-response rate) among high SES households. **Box 15.1** shows an example of how a household non-response weight is calculated. Adjusting for non-response for issues such as SES requires additional information.
For individual response rates, most surveys assess responses separately for interviews and for biological specimen collection. The level of non-response for specimen collection is commonly higher than that for interviews. Where non-response differs significantly between clusters or strata, the sample weight should be adjusted to avoid potential bias of results towards locations with higher response rates. Therefore, in some surveys, for each population group, there may be one sampling weight applied to the questionnaire and another sampling weight used for biological specimens. This may apply at the cluster or the stratum level.

Household or individual sampling weights may also need to be adjusted if specimens are misplaced during transfer to the laboratory for analysis. For example, if samples of salt collected at the household level for later laboratory analysis of iodine content go missing before reaching the laboratory, this must be considered as a non-random non-response and the household sampling weight for the salt iodine analysis results need to be adjusted accordingly.

**Statistical analyses for micronutrient surveys**

Survey data, descriptive data for the sample population, household characteristics, and micronutrient-related indicators are usually presented as weighted percentages with 95% CIs. Where the data are skewed, meaning they are not normally distributed, then the weighted median or geometric mean is presented, along with 95% CIs where these can be generated. It is crucial to avoid reporting a weighted mean and an unweighted CI.

**Box 15.2** provides information about the importance of presenting 95% CIs.

The scope of this manual does not allow detailed information about statistical tests or the syntax for defining the sample design and the tests themselves. It is recommended that country teams work with statisticians experienced in processing and analysing data from complex surveys. In the interpretation of data, it is critical to remember that sampling errors, measurement errors and the skills of the survey team members all influence the precision of results.
Module 15. Data processing and analysis

**Box 15.2. The 95% confidence interval**

It is important to include the 95% CI around any estimate for primary survey outcome indicators, where calculation of this variance is feasible. Surveys present data from a random sample of households or a specific population group that are intended to represent all households or the entire population group in the country. Therefore, all calculations are considered to be estimates of the “true” values for the entire population.

The 95% CI around a prevalence or coverage estimate provides a range of values that includes the "true" estimate, with 95% confidence. Confidence limits are the numbers at the upper and lower end of a confidence interval, and they determine the width of the interval. The width of the CI provides a measure of the precision of the survey estimate: the narrower the confidence limits, the greater the precision.

**Outcome results tables**

Results tables should specify the indicator measured, the population group and age range(s) in which it is being measured, the unweighted sample size, a measure of central tendency (mean, geometric mean or median), precision (95% CI) and/or variability [such as standard deviation (SD) or standard error, interquartile range (IQR) with reported units (for example g/L)] and the prevalence above or below a specified cutoff point, where appropriate. Data should be presented as stratified (by region, age or sex) and as national estimates for the population group. If the data have been adjusted for any other factors (for example inflammation, smoking, or elevation above sea level), this should be noted in a footnote to the table. **Table 15.3** shows a sample table layout.

**Additional information for interpreting nutrition indicators**

**Inflammation:** The concentrations of certain biomarkers are altered during times of infection or inflammation. For example, ferritin is a positive acute phase protein whose concentration increases during inflammation, while retinol binding protein (RBP) is a negative acute phase protein whose concentration decreases during inflammation. This means that measured concentrations may need to be adjusted for inflammation. The concentrations of acute phase protein inflammatory biomarkers, namely C-reactive protein (CRP) and α-1-acid glycoprotein (AGP) indicate the presence of inflammation. The relative elevation in concentration of these biomarkers indicates the stage of the inflammatory response. For this reason, CRP and AGP are commonly measured in micronutrient surveys. More information on this can be found in the “**BOND and inflammation**” online tool, in **Module 3: Biomarker selection and specimen handling** and at **www.BRINDA-nutrition.org**.

**Fasting status and time of day at blood collection:** The time since food was last consumed and the time of day that blood is collected can affect blood concentrations of key micronutrients. For example, zinc concentration is sensitive to the time of day that blood was collected, to when the last meal was ingested and to the use of hormonal birth control. The cutoff values for zinc deficiency are adjusted for the time of day (morning versus afternoon) and fasting status.

For some micronutrients, it is not certain whether the time of day or fasting status affects the interpretation of results. It is recommended to include data entry fields about the time of day, time since the last meal,
### Module 15. Data processing and analysis

#### Table 15.3. Sample mean haemoglobin and prevalence of anaemia in children 6–59 months of age

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Sample size (unweighted)</th>
<th>Haemoglobin (g/L) Mean</th>
<th>SD</th>
<th>95% CI</th>
<th>Prevalence of anaemia % haemoglobin &lt;110 g/L</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
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<td></td>
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<tr>
<td>Male</td>
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<td>Female</td>
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<tr>
<td><strong>Age group</strong></td>
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<td></td>
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<td></td>
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<tr>
<td>6–11 months</td>
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<td>12–23 months</td>
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<tr>
<td><strong>Residence type</strong></td>
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<td>Urban</td>
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<td>Rural</td>
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<td><strong>Strata (region)</strong></td>
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<tr>
<td>North region</td>
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<td>South region</td>
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<td>East region</td>
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<tr>
<td>West region</td>
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<tr>
<td><strong>Poverty</strong></td>
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<tr>
<td>Poor</td>
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<tr>
<td>Non-poor</td>
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<tr>
<td><strong>Total (National)</strong></td>
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</tr>
</tbody>
</table>

Data are weighted to account for survey design
Haemoglobin values are adjusted for elevation above sea level
This table does not include IQR

See **Module 3: Biomarker selection and specimen handling** for more information on adjustments specific to target groups.

Physiologic (pregnant or lactating) status, use and timing of hormonal birth control, and other potentially relevant information. This allows for comparison of methods and results with subsequent surveys to see if there may be a physiologically relevant characteristic that could have influenced any difference in results.

**Pregnancy:** Haemodilution, which occurs during pregnancy, alters the concentrations of circulating micronutrients. Kidney function and relative urine volume also change during pregnancy. As such, for pregnant women there are separate cutoff values for some biomarkers, in particular median urinary iodine and
haemoglobin (see **Module 3: Biomarker selection and specimen handling**). Most indicators of nutrients such as iron, vitamin A, zinc, folate and vitamin B12 do not have established cutoff values for defining deficiency during pregnancy, and few surveys are designed to have a representative sample of pregnant women. Sample size determination and data analysis for pregnant women need to be separate from those for non-pregnant WRA.

**Elevation above sea level and smoking**: Haemoglobin levels are affected by elevation above sea level and by smoking, thus values need to be adjusted to account for these factors prior to classifying for anaemia.\(^1\) Biologically implausible haemoglobin values need to be defined as adjusted (for elevation above sea level and smoking) or non-adjusted (if elevation and smoking variables are not available), then abnormal values should be flagged and potentially set to missing. This is discussed in more detail in **Module 11: Data collection tools, field manual, and database**.

**Standard analysis and checks for anthropometry data**

Using the raw data collected in nutrition surveys, the WHO Anthro software developed by WHO can calculate weight-for-height/length, height/length-for-age, weight-for-age, head-circumference-for-age, mid-upper arm-circumference-for-age, and other anthropometric indices. The calculator also uses these data to generate the associated Z-scores based on international standards. The WHO Anthro software has been adapted for statistical software such as STATA, SAS, and R. It includes macros that can generate Z-scores for each anthropometric indicator for which data were collected and link these to the individual assessed. The WHO Anthro software and macros are available on the [WHO website].(http://www.who.int/childgrowth/software)\(^2\)

Additionally, the WHO Anthro Survey Analyser, based on the “R and R Shiny package”, is a tool for analysing anthropometric data that updates Anthro methodology to provide more accurate estimates of the standard errors and confidence intervals for prevalence and mean Z-scores. It provides interactive graphics for data quality assessment and a summary report template offering key outputs (such as Z-score distribution graphics) for various grouping factors and nutrition status tables with accompanying prevalence and Z-score statistics. This software is currently available either [online](http://www.who.int/childgrowth/software) or [offline](http://www.who.int/childgrowth/software). It also allows for estimating prevalence and mean Z-scores by area (urban, rural), education, wealth and other categories of interest that were measured in the survey.

Z-scores are considered outside of the normal range when they fall outside of a pre-specified range of standard deviations. The ranges differ based on the measurement (see below):

- Weight-for-age Z-score (WAZ) < -6 or WAZ > 5
- Length/height-for-age Z-score (LAZ/HAZ) < -6 or LAZ/HAZ > 6
- Weight-for-length/height Z-score (WLZ/WHZ) < -5 or WLZ/WHZ > 5

In these cases, the value needs to be flagged and set to missing. You can find more information at the [“Anthropometric indices and exclusion flags”](http://www.who.int/childgrowth/software) online tool.

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2. Macros are available at [http://www.who.int/childgrowth/software](http://www.who.int/childgrowth/software). UNICEF Stata Macro is available upon request via email to data@unicef.org. Note SAS and SPSS macros do not calculate confidence intervals for estimates to consider complex sample designs. An update is under development at the time of this publication.
When reporting on anthropometry findings, some signs of quality (data quality checks) should be listed to identify trends in the data that could indicate inaccuracies in the measurements. Examples include: ¹

- completeness/incompleteness, meaning the proportion of children where information is missing on variables by geographical region and by team, age group and sex;
- sex ratio and age distribution;
- number of cases and proportions of mismatches between length/height measurement position and recommended position, by age group;
- digit preference charts, for weight and length/height, by geographical region, by team, whole number digit preference for weight, whole number digit preference for length/height;
- implausible Z-score values;
- standard deviation; and
- checks of normality (for example, whether the data are normally distributed).

Examples of anthropometry reporting can be found in the “Template for reporting on the quality of anthropometric measurements” online tool. Sample tables of how to report anthropometric findings are presented in the “Graph to plot anthropometric indices against reference population” online tool.

Module 16. Survey reports and dissemination

In this module, we will discuss:

- Types of reports and documents to produce
- Disseminating and using survey results
- Including survey results in global databases
Module 16. Survey reports and dissemination

It is essential above all that results are used to interpret the programme situation and inform future nutrition strategies, policies and programmes.

The amount of time allocated for data management, analysis, report writing, review, revision and finalization is frequently underestimated. Depending on the complexity of the survey, the method of data collection, and the number of stakeholders involved, these processes may take a year or more. Simply writing, finalizing, and printing may take up to 18 months and may require clearance at the organizational or national level. There is often a summary report for wider dissemination, in addition to the comprehensive final survey report that may exceed 200 pages.

Because of the long timeline, it may be useful for programme management purposes to develop a preliminary report that highlights key available survey results. This can be done within a few months of completing the data cleaning and processing.

The final results and recommendations of the survey are typically presented in national and sub-national dissemination events, following extensive data review meetings with the Steering committee and other stakeholders. These events provide information for policy makers, programme implementers, donors and potential donors, and the media. They aim to generate support for any recommended strategic and policy changes. More details on planning and implementing a dissemination event are presented later in this module.

Types of reports and documents to produce

Preliminary reports

A preliminary report can be produced after data cleaning and checks have been completed and the main results tables are available. This is usually around six months after completing data collection. Time-consuming factors include reconciling data where paper-based data collection was used and completing specimen analyses for the main biomarkers.

The preliminary report may include:

- An executive summary, which is a high-level summary of the survey objectives, methods, and principal available results.
- An introduction that includes the objectives of the survey, how the survey results fit into the national health and nutrition development plan and an acknowledgement of institutions and funding agencies involved in the survey process.
- A methods section that gives a short overview of the survey methodology, the survey design, indicators, sampling and ethical considerations, survey personnel and training, survey implementation, specimen collection and data management and analysis.
- Key available results for micronutrient indicators if available, response rates, main demographic characteristics, appendices and references. This section may also provide some limited information on the details of the biological indicators measured, design effects for the main results highlighted and references used in the report.
The Malawi 2015-16 Micronutrient Survey key indicator report \(^1\) is an example of a preliminary report.

**Final report**

Final reports serve numerous functions, including:

- providing detailed results of the survey
- allowing external evaluation of the methods and quality of the survey implementation and specimen analysis
- providing a guide for future surveys
- informing policy and programme action for follow up.

The section on discussion and recommendations in a final report needs to be developed following a data review meeting with stakeholders and technical experts. Sometimes a final report will not include a discussion and recommendation section because the data review meeting may be planned for a time after the final report is complete. In those instances, it is more common for the discussion and recommendations to be part of a policy brief or summary report developed later.

The final report may take the form of a comprehensive full report, or another format as deemed necessary or useful by the country. There are many ways of organizing a report, however it is usually divided into chapters with headings similar to those in **Box 16.1**. There are examples of national survey reports in the online tools for **Malawi, Kyrgyzstan, Zambia, Nepal**, and **Tanzania** \(^2\).

**Box 16.1. Example structure of a final report**

- Foreword
- Acknowledgments
- Acronyms and abbreviations
- Map
- Executive summary
- Introduction
- Methods
- Results, by topic (such as demographic characteristics, anthropometry, iron and anaemia, vitamin A)
- Tables and figures that accompany the results section
- Discussion or Conclusion (optional)
- Recommendations (optional)
- Appendices

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Module 16. Survey reports and dissemination

**Summary report**

A summary report highlighting the principal results and recommendations from the survey could be made available electronically, with paper versions as needed. It may be useful to share this report widely among all nutrition-related partners in the country, including government and nongovernmental organization (NGO) partners in health, education, water and sanitation, and economic development, as well as with the media.

**Additional reports and manuscripts**

Additional manuscripts may be developed later, for example, investigation of trends in comparison to data from previous surveys or other sources of data, or the outcome of more in-depth data analyses. These manuscripts could be mainly for national use, presented at conferences, or published in journal articles. The type of document and time for developing it should be agreed during the survey planning phase and refined over the course of the survey and during partner data review meetings.

Data access and availability should also be clarified at the start of the survey. Releasing data publicly may require addressing issues related to privacy and confidentiality. Data files may be made available to other users to perform more detailed investigations, but only after a period assigned for nationally led initiatives and manuscript development. For example, Demographic and Health Surveys (DHS) and Multiple Indicator Cluster Surveys (MICS) are open access once the main report is released. Anyone can access the data from the web after registering and describing the intended use of the data.

Responsibilities for producing the reports, further data analysis and data access should be clearly documented in any memorandums of understanding developed by Steering committee members during the planning process.

**Disseminating and using survey results**

The purpose of conducting a survey is to provide evidence that will guide decisions on existing or new policies and programmes. To do this effectively, survey results and their interpretation in terms of status, progress, and challenges need to be shared broadly in the country, at multiple levels and with a range of partners. Box 16.2 shows some common formats used for dissemination.

Time and resources for dissemination events need to be built into the timeline and budget at the survey planning stage. Official dissemination of results is often first conducted with the main agencies and institutions involved, then presented by high-level government officials in a formal press conference. Meetings with policy makers should also be held to implement or strengthen a cross-cutting programme response as needed. These meetings usually entail the development of short- and longer-term plans that address cross-cutting issues, identify responsibilities for programme follow-up, and agree on timelines for improving intervention coverage as well as methods for monitoring and evaluating interventions.

All dissemination activities require a clear explanation of the results and their implications, and the involvement of local stakeholders in planning and preparing for the dissemination. The main points include:

- highlighting the main results in an accessible language, with technical terms simplified for ease of understanding;
- using maps or other graphics to increase the perceived relevance of the results and ease of understanding;
Module 16. Survey reports and dissemination

- guidance on interpreting tables, figures, and other materials where needed;
- presenting the data in comparison to other surveys;
- showing national and subnational comparisons and trends over time (where possible);
- comparing results to other countries in the region; and
- linking the data to programmes and interventions.

An example of a dissemination event can be found in the “Generic nutrition survey dissemination agenda” online tool.

Box 16.2. Formats typically used to disseminate results and other survey information

Full, detailed report
- This is an important record of the survey methodology and detailed results, often in tabular form. The full report needs to be produced and maintained for the record, however, it is usually not easily readable for a wide audience due to its length and level of technical detail.
- In addition to the widely distributed electronic version, a limited number of hard copy versions are usually made available to each organization, institution and funding body involved with the survey planning and implementation process.

Summary report
- A summary report may include a graphical representation of the data to illustrate the main results. Having descriptive figures that stand alone with appropriate labelling makes the results more accessible to a wide range of partners. Better understanding of the results will help integrate them into broad national health, nutrition, and development plans, and may improve the accountability of stakeholders who implement follow-up activities.

Brochures
- A brochure is a very short presentation of a summary of the survey objectives and findings, in an attractive format.
- Several brochures may be produced with different content, depending on the intended audiences.

Slide presentations
- Electronic slide presentations are produced for presenting to policy makers, potential donors and the media. These are most frequently presented by senior government or organization officials, usually members of the Steering committee.

Follow-up documents
- Short- and long-term action plans based on the survey recommendations.
- Policy documents to reduce the prevalence of micronutrient deficiencies and to introduce, maintain or improve the implementation of interventions.
- In-depth analysis document, often with a regression analysis to examine factors associated with certain deficiencies or trend analyses to compare results with previous surveys or other sources of information.
Including survey results in global databases

The **WHO Micronutrients Database** ¹ summarizes data published in reports and manuscripts concerning the micronutrient status of representative populations at the national, regional (within country) and first administrative levels (for example, canton, state or province). These data are used to provide national, regional, and global assessments of the magnitude of micronutrient deficiencies and to track progress made towards the international goal of eliminating the major micronutrient deficiencies. They are also used to monitor and evaluate the impact of strategies to prevent and control micronutrient malnutrition.

Survey implementers are encouraged to send their final data reports to WHO at nutrition@who.int for inclusion in the database. In addition to the data, the submission must include details that describe the representativeness of the survey, calculation of sample weights, type of specimen(s) collected, whether these were collected under fasting conditions, type of laboratory assay and method used for micronutrient status analysis, as well as information about adjustments that were made to the results (for example for elevation above sea level, smoking or inflammation).

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¹ This is part of the WHO Vitamin and Mineral Nutrition Information System.
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