MICRONUTRIENT DATA GENERATION INITIATIVE

STRATEGIC PLAN

Micronutrient FORUM

Strategic plan for increasing the availability and utilization of reliable data on population micronutrient (MN) status globally

Strategic plan for increasing the availability and utilization of reliable data on population micronutrient status globally

Kenneth H Brown

Sophie E. Moore

Sonja Y Hess

Christine M McDonald

Kerry Jones

Sarah Meadows

Mari Manger

Jennifer Coates

Silvia Alayon

Saskia Osendarp

Preferred citation: Brown KH, Moore SE, Hess SY, McDonald CM, Jones K, Meadows S, Manger M, Coates J, Alayon S, Osendarp S. Strategic plan for increasing the availability and utilization of reliable data on population micronutrient status globally. The Micronutrient Forum, Washington, DC; 2020.

Table of Contents

Executive Summary

This document was prepared under the auspices of the Micronutrient Forum to address broadly shared concerns about the detrimental effects of the limited availability of data on the vitamin and mineral (micronutrient, MN) status of human populations, especially in low- and middle-income countries (LMICs). This scarcity of information thwarts the timely recognition of specific MN deficiencies and impedes the implementation of appropriately designed, cost-efficient, public health nutrition programs to address these problems. The limited information that is available – as derived from national food balance sheets, dietary intake data, clinical case reports, and biochemical assessments of non-representative population samples, as well as the relatively few population-based surveys that have been completed – suggests that several MN deficiencies are likely to be quite common. These deficiencies are estimated to cause hundreds of thousands of preventable childhood deaths annually in addition to widespread physical and cognitive disabilities and reduced physical performance of both children and adults. To determine the true prevalence of these deficiencies and to design coherent, safe and sustainable MN deficiency control programs, high quality, timely, and representative data, based on biomarkers of MN status, are needed. This information is lacking for most nutrients of public health concern in the majority of countries. Even for those nutrients, like vitamin A and iodine, for which more information is available, the data are often outdated; and many countries still have no information at all.

To confront these issues, the MN Forum convened a Core Working Group of nutritionists with experience in nutritional status assessment, survey design and implementation, and public health nutrition programs to develop a strategic plan to correct this situation. A broader Multi-Stakeholder Advisory Group comprised of experts in specific MNs and representatives of national governments in LMICs, bilateral and multi-lateral technical assistance agencies and private foundations, was also formed to review a preliminary draft of the current document and provide critical inputs to the final strategy presented herein. The document is intended for use by nutritionists, epidemiologists, analytical chemists, clinicians, educators, public health professionals and policy makers with an interest in human nutrition and related health outcomes.

This strategy document describes the current data landscape, the reasons for the paucity of relevant information, and the steps required to rectify this situation, with emphasis on a subset of MNs of public health concern, namely vitamin A, folate, vitamin B12, iodine, iron, zinc, and in some settings thiamine and vitamin D, based on their likely high prevalence and profound health implications. According to the World Health Organization's Vitamin and Mineral Nutrition Information System (VMNIS), since 1980 only 77 of 138 LMICs (55.8%) have provided data on the prevalence of vitamin A deficiency among pre-school children (PSC); and only 38.4% of LMICs reported information on their iron status, 15.2% on zinc, 5.8% on vitamin D and 5.1% on vitamin B12. Just 17.4% of countries contributed data on women's folate status. According to the Global Scorecard of Iodine Nutrition, iodine status of school-age children has been assessed more commonly; information is available for 113 LMICs (82%) within the past 15 years. Among countries that reported any data on the population's status for MNs other than iodine over the past 40 years, most conducted just one survey. Thus, much of the information is already outdated.

Key informants from LMICs and agencies that support national nutrition surveys stated that the main reasons for not collecting biomarker information on MN status are the high costs of the laboratory analyses; limited access to laboratories that are able to complete the analyses and/or restrictions on shipping specimens outside the country; complexities in the collection, processing, shipping and storage

of the necessary biological specimens; and a lack of knowledge about the importance of this information (both in national governmental agencies and among program officers of technical and financial assistance agencies). The main factors that favored inclusion of MN biomarkers in national surveys are the presence of supportive government authorities; an in-country "champion" (in a national government, academic institution, or donor agency) to advocate for the information; the presence of an ongoing MN deficiency control program for which baseline or follow-up information is desired; and availability of external experts to provide technical assistance to the local survey team.

Considering these obstacles and facilitators for obtaining the desired information, we developed a theory of change to indicate the steps needed to carry out this agenda, as well as the personnel, infrastructure and other items required to achieve each of the intermediate goals. We also reviewed the recommended biomarkers for each of these MNs and related laboratory tests, so as to determine the types of laboratory equipment needed to complete these analyses; and we examined the field procedures used for collecting and processing relevant clinical specimens. Based on these sets of information, the Core Working Group prepared the following set of recommendations.

Main recommendations:

- Launch a multi-component initiative to increase the availability and utilization of reliable information on population MN status through targeted advocacy and provision of technical assistance, laboratory services, professional training, and financial support. Establish a management team to carry out selected components of the initiative, including mobilization of resources to support these activities, and to achieve greater coordination among stakeholders.
- Create a new Project Partners Team comprised of representatives of global and national agencies involved in generating data on population MN status or supporting these efforts financially or technically to coordinate activities and avoid unnecessary duplication of effort. Establish a Technical Advisory Committee to review selected technical aspects of the project periodically and provide critical feedback.
- Develop an advocacy and communication plan targeted to key national decision-makers and program officers of donor agencies to describe the importance of generating and utilizing data on MN status and to motivate the inclusion of MN status biomarkers in national nutrition, health, or other population-based surveys.
- Establish a multi-donor, central fund and appropriate governance structure to support this initiative, and leverage funding from international public and private donor organizations. This fund should be used to support technical assistance efforts, create and maintain resource laboratories, as described below, help defray costs of specimen collection and laboratory analyses, and support the overall management of the initiative.
- Provide technical support to countries for the design and implementation of national surveys to include assessment of population MN status, including how to select target groups (and develop related sample size estimates) and MNs of interest, and best practices for the collection, processing, and transport of clinical specimens for laboratory analysis.
- Establish regional resource laboratories in sub-Saharan Africa and Asia that can receive specimens from national surveys and complete analyses of the full set of recommended MN status biomarkers, based on the list of key MN status biomarkers and related laboratory analysis

methods described herein. The same laboratories can serve as regional hubs to provide training for national scientists and technicians from other countries in these key analytical methods.

- Expand existing External Quality Assessment systems, or create new ones as needed, to confirm the accuracy of laboratory results. In some cases Certified Reference Materials need to be developed or made more accessible to assist with inter-laboratory standardization.
- Expand and support the existing WHO VMNIS data repository, ultimately to include individual-level data, and the clearinghouse for information on MN status assessment surveys. Support periodic analyses of existing information to update regional and global estimates of the prevalence of MN deficiency and disseminate information on these results.
- Track progress of the initiative in terms of resources leveraged, specimens analyzed, data generated (and quality of this information), individuals trained in laboratory analysis and data analysis and interpretation, and ultimate utilization of this information for program design.

Acknowledgements

The initial drafts of this document were prepared and reviewed by members of the Core Committee on Micronutrient Data Generation and Utilization: Silvia Alayon, Kenneth H Brown, Sonja Y Hess, Maria Elena Jefferds, Christine M McDonald, Sophie E Moore, and Saskia Osendarp. Other co-authors – Jennifer Coates, Kerry Jones, Mari Manger and Sarah Meadows – were invited to contribute to specific sections of the document. The document was subsequently reviewed by members of a Multi-Stakeholder Advisory Committee, who provided comments on a preliminary draft. The full names and institutional affiliations of all committee members and co-authors are listed in **Appendix 1**.

The Micronutrient Forum greatly appreciates the contributions of the members of the respective committees. Included in these groups are representatives from academic, NGO, governmental, non-profit and for-profit organizations and foundations. This diversity of perspectives was sought to ensure the report would be as informed as possible. The final recommendations included in this report reflect the consensus of these groups, although individual committee members may disagree with selected aspects of the recommendations. Thus, participation in these groups does not imply that all members fully support each recommendation. Furthermore, participation in these groups shall not influence the ability of individuals or organizations to participate in future efforts by the Micronutrient Forum to implement these plans.

We also appreciate the administrative support and thoughtful comments from Reed Atkin of the Micronutrient Forum, and the logistical support provided by Ramela Carrion of the International Food Policy Research Institute.

Funding for this activity was provided by a grant from the Bill & Melinda Gates Foundation (BMGF) to the Micronutrient Forum.

1 Introduction

Vitamins and specific mineral elements are collectively referred to as "micronutrients" (MNs) because the dietary requirements for these essential nutrients are relatively small (typically <100 mg/d) compared with carbohydrates, fat, and protein, which are consumed in quantities >1 g and are regarded as "macronutrients". MNs are critical for specific aspects of metabolism and for maintaining tissue structure and function. MN deficiencies result in a broad array of serious health and functional consequences, including physical disabilities, increased morbidity, impaired growth and neuro-cognitive development, and in extreme cases death. Public health policies and programs have been developed to address these deficiencies; but full understanding of the extent of the problems and design of effective, efficient, safe and sustainable intervention programs require reliable information on population MN status based on biochemical indicators specific to each MN. Regrettably, representative national and sub-national MN biomarker data are very limited, both with regard to the number of countries that have generated relevant information, as well as the frequency of data collection, the number of MNs considered, and the analysis, interpretation and utilization of the data once they become available. As a result, programs are not always deployed where and when they are needed; and these programs are often less cost-effective and sometimes riskier than they could be.

There are many reasons for this lack of information, including limited understanding by policy makers of the importance of MNs for human health and the usefulness of information on MN status for program planning; insufficient professional capacity to explain the need for this information, advocate for data generation, and design and implement MN status surveys; poor access to adequately equipped and staffed laboratories to complete the analyses reliably; costs and logistical constraints involved in specimen collection, transport, storage and laboratory analyses; and inadequate downstream capacity to interpret and apply this information for program design and evaluation. Nevertheless, there is evidence to indicate that generating and applying this information would lead to more coherent, cost-effective and safer MN deficiency control programs. In other words, investing in better data would ultimately yield cost-savings as well as healthier populations and safer programs.

This document provides the rationale and justification for generating more high-quality information on population MN status, the entities currently engaged in pursuing this agenda, and the steps needed to expand this effort.

2 Objectives of the MN Data Generation Initiative and the present strategy document

The ultimate objective of this initiative is to increase the availability and utilization of reliable information on population MN status, so as to motivate policy makers and program planners to consider programmatic interventions where appropriate, and to provide them with the information needed to design and implement coherent and safe programs to prevent and control MN deficiencies and assess their impact. This objective is aligned with the United Nations' Strategic Development Goals (SDGs) 2 and 3, namely to end hunger and achieve food security and improved nutrition (SDG 2), and to ensure healthy lives and promote well-being for all (SDG 3).

There are three primary objectives of the present document:

- To describe the importance of producing more high quality data on population MN status for justifying, planning and evaluating programs to improve population MN status and ensure the safety of these programs
- To identify information gaps (including national and sub-national data availability, timeliness of data, and range of MNs considered)
- To propose the steps that should be taken to promote and support the collection, interpretation and dissemination of more high-quality information on population MN status

3 Scope of the document and intended audience

This document presents background information on MN nutrition and recommended actions to promote and support the generation of more extensive, high-quality, population-based information on MN status. The document is conceived as a critical step to help catalyze effective efforts to collect, interpret and apply relevant information in different settings. The document is intended for nutritionists, epidemiologists, analytical chemists, clinicians, educators, public health professionals and policy makers with an interest in human nutrition and related health outcomes.

4 Background information and rationale for generating better data on population MN status

4.1 MNs that are considered to be of public health importance

All of the essential MNs are required to maintain health, although some are more widely recognized as being of public health importance, especially in low-and middle-income countries (LMICs), because of the likely high prevalence of their deficiencies and/or the severity of the clinical responses to poor status. The MNs currently considered by the Core Working Group to be of greatest public health concern in LMICs are vitamin A, folate, vitamin B12, iodine, iron, zinc, and in some settings vitamin D and thiamine, because severe deficiencies of these MNs may result in physical disability, sensory impairments, restricted physical growth and neuro-cognitive development, or death. Other MNs, like riboflavin, niacin, pyridoxine, and selected mineral elements, such as selenium, may be equally important; but these MNs have not yet been recognized as being of major public health importance, either because of the paucity of information available on population status or an incomplete understanding of the health implications of deficiency. Calcium is also considered to be a key MN of public health importance, both for the prevention of rickets in children and pre-eclampsia in pregnant women. However, calcium is not addressed in the current document because there are no easily measured biomarkers of calcium status, and assessment of the risk of deficiency is based primarily on dietary intake data. The dietary reference intakes of different MNs and the health effects of their deficiencies are summarized briefly in **Table 1**.

Notably, some of these MN deficiencies contribute importantly to child and maternal mortality worldwide. For example, deficiencies of vitamin A and zinc each increase the susceptibility to and severity of common infections, like diarrhea and pneumonia, which are responsible for a large proportion of child deaths in LMICs (Brown et al. 2009; Imdad et al. 2017; Stevens et al. 2015). Maternal folate insufficiency increases the risk of infant neural tube defects (NTDs, including anencephaly and spina bifida), resulting in both stillbirths and post-natal deaths (Blencowe et al. 2018). Maternal iron deficiency anemia during pregnancy is associated with an increased incidence of low birth weight (Pena-

Rosas et al. 2015; Figueiredo et al. 2018), thereby contributing to infant mortality; and severe maternal anemia of all causes during pregnancy increases the risk of maternal death (Daru et al. 2018).

According to the 2013 Lancet Nutrition Series (Black et al. 2013), each year more than 425,000 deaths of children less than five years of age are attributable to maternal or child MN deficiencies. Another estimate, using a broader range of MN deficiencies than considered in the Lancet Nutrition Series and somewhat different sets of assumptions, concluded that as many as 745,000 under-five deaths may occur each year due to MN deficiencies (Brown et al. 2015). Even this latter figure may be an under-estimate of the actual mortality burden, as it does not include an updated estimate of NTD-related deaths or the increasingly recognized problem of deaths due to thiamine deficiency and infantile beriberi (Myanmar Ministry of Health and UNICEF 2014; Barennes et al. 2015; Qureshi et al. 2016).

In addition to the impact of MN deficiencies on child mortality, selected deficiencies adversely affect neuro-behavioral and cognitive development. For example, both iron deficiency anemia and iodine deficiency impair children's cognitive development, as well as adult cognitive function (Greig et al. 2013; Bath 2019). Other MN deficiencies, like thiamine and vitamin B12 deficiencies, affect neurological function and social and educational performance (Mimouni-Bloch et al. 2014; Black 2008). Addressing these MN deficiencies is imperative for maximizing human performance.

4.2 Rationale for generating data on MN status and specific population sub-groups of interest

There are five major reasons why greater availability of high-quality information on population MN status is critical for establishing coherent MN deficiency control programs. First, reliable, population-level information is needed to define whether a deficiency problem exists in a particular population, and whether the prevalence is of sufficient magnitude to justify a public health preventive program rather than just individualized treatment of sporadic cases of deficiency. Second, data are needed on the population sub-groups most affected to enable appropriate targeting of interventions to maximize program efficiency and avoid unnecessary (and costly) program outreach. Third, data are needed to gauge whether the programs are achieving their desired outcomes. Fourth, for those MNs that produce adverse effects when consumed in excessive amounts (see **Table 1** for ratios of average requirements to safe upper intake levels for individual MNs), it is important to monitor for any possible risks of toxicity imposed by these programs. Finally, the information is useful for research purposes to determine the relationships between MN status and a variety of health outcomes.

Infants and young children and women of child-bearing age (especially during pregnancy and lactation) have relatively high MN requirements for their body size, either because of rapid tissue synthesis or MN excretion in breast milk and menstrual blood, and a correspondingly higher risk of deficiencies. For these reasons, public health programs typically focus on these population sub-groups; and information on their MN status is particularly important. Adolescents may also have an elevated risk of MN deficiencies because of increased requirements for rapid growth during this period, but very little information on MN status is available for this segment of the population.

There is ample evidence to indicate that the availability of data can instigate programmatic action, drive program modifications, and contribute to greater cost-effectiveness and safety of these programs. To cite just a few examples, availability of information on population median urinary iodine concentration (UIC) and/or newborn thyrotropin levels, has inspired multiple countries to initiate (or re-initiate) iodine

intervention programs and has provided countries with the rationale for increasing or decreasing the level of salt iodization, as needed, to maximize program impact and ensure safety (Zimmermann et al. 2005; Zou et al. 2014; Kavishe et al. 2019; Richards and Colleagues 2020). Similarly, data on the prevalence of vitamin A deficiency in Guatemala motivated research on vitamin A fortification, which lead to a national sugar fortification program (Arroyave and Mejia 2010). Data on RBC folate status from the US National Health and Nutrition Examination Survey have allowed the CDC to identify population sub-groups with sub-optimal RBC folate concentrations who need to be targeted with additional interventions beyond just wheat flour fortification to prevent NTDs (Tinker et al. 2015).

With regard to potential opportunities for cost savings, a recent set of analyses using vitamin A biomarker and dietary data and vitamin A program delivery costs in Cameroon found that it was possible to reimagine the existing vitamin A intervention programs and achieve the same level of effective coverage by expanding food fortification nationally and reducing the scope of vitamin A supplementation (VAS) in areas with better vitamin A status (Brown et al. 2015; Vosti et al. 2015; Vosti et al. 2020). These program modifications could save more than sixteen million dollars in program costs over ten years, whereas the data that enabled this modeling were collected for less than one million dollars. Thus, investment in data could more than pay for itself through greater program efficiency. Likewise, data on improved vitamin A status in Guatemala following revitalization of the sugar fortification program have allowed the government to scale back VAS, thereby reducing the risk of vitamin A toxicity while lowering program costs (Wirth et al. 2017). Similarly, findings on adequate UIC following salt iodization in Nepal allowed the country to discontinue the iodized oil capsule program in 1999 (Paudyal et al. 2020). Thus, generating data on population MN status has not only presented opportunities for MN intervention programs and improvements of these programs, but has led to safer and more coherent interventions and considerable cost savings overall.

4.3 Types of information used for assessing the risk of inadequate MN intake and MN status, and justification for the current focus on MN status biomarkers

The risk of inadequate or excessive MN intakes and the actual MN status of a population and can be assessed in several ways, including through measurement of food availability, dietary intake, physical examination, and biochemical analysis of specific blood, urine or breast milk biomarkers of MN status. Information on the coverage of intervention programs is also helpful to determine when it reasonable to assess the impact of the program on the population's MN status and to help interpret this information. The choice of which approach or approaches to use depends in large part on the purpose of the measurement effort and locally available professional capacity and financial resources.

An expedient first step for designing population-based MN interventions is to review the availability of key MNs in the national food supply relative to population requirements (Wessells and Brown 2012; Arsenault et al. 2015), using national food balance data compiled by the Food and Agriculture Organization of the United Nations (FAO) (see the **FAOSTAT platform).** An advantage of reviewing national food balance sheets for MN adequacy is that the data are already available, so no new data collection is required for this initial assessment step. Though sufficient MN availability in the food supply does not guarantee adequate intakes across the population, it can indicate whether food availability is a first-order limiting factor. In other words, if the amount of nutrients in the food supply is less than the population's theoretical requirements, some individuals in the population will be likely to have inadequate intakes and an elevated risk of deficiency.

Dietary assessment involves the collection of individual or household food intake data by means of direct observation and weighing of foods consumed or by eliciting respondent recall of foods consumed (Gibson and Ferguson 2008). After converting food intake data to nutrients using food composition tables, the results are then compared with theoretical MN requirements to quantify the risk of inadequate intake for individuals and various population subgroups. Dietary data are particularly useful to elucidate the underlying dietary causes of MN deficiencies, to inform MN program design, and to monitor dietary response (or non-response) to MN interventions. However, dietary data have a fundamental weakness in that they only indicate a possible risk of MN deficiency (or excess), and they do not provide direct information on MN status. For example, a person may consume apparently adequate intakes of a MN, but still be deficient because of poor bioavailability (possibly due to inflammation, dietary absorption inhibitors, or food matrix issues), intestinal malabsorption, or because the individual has particularly high requirements because of underlying genetic polymorphisms. For MNs like vitamin A, which can be stored in the body, individual intake on a particular day or days does not necessarily correlate with status. Thus, dietary data have important limitations, and additional clinical or biochemical studies are needed to assess actual MN status.

Clinical assessments rely on the appearance of specific symptoms or signs of deficiency, such as anemia, skin or oral lesions, rickets, or night blindness. However, clinical information may not always be specific to a particular MN. For example, a number of different MN deficiencies (as well as other non-nutritional conditions) can contribute to the appearance of anemia, several B vitamin deficiencies can cause oral lesions, both vitamin D and calcium deficiencies can cause rickets, and both vitamin A and zinc deficiencies can cause night blindness. Moreover, clinical signs appear fairly late in the progression of deficiency, and the prevalence of these signs is usually fairly low, thereby necessitating large survey sample sizes to determine their prevalence with a reasonable degree of precision.

Because of the limitations of dietary and clinical data, as described above, measurement of population MN status must rely primarily on biochemical assessments completed in representative samples of the population of interest and selected sub-strata within these populations (defined in terms of age group, sex, geographic region, urban-rural residence, socio-economic status, etc.). Laboratory analysis of specific biomarkers of MN status is the most rigorous, reliable, and efficient method to determine the prevalence of MN deficiencies in a population. However, proper interpretation of these laboratory results requires some understanding of MN metabolism. For example, the concentration of some biomarkers circulating in blood or plasma may be affected by the presence of inflammation; some markers may respond to intervention only if the baseline values are low, whereas others respond across the full spectrum of baseline status; and different biomarkers may be useful for detecting deficiency versus toxicity of a particular MN. These issues need to be understood to decide which biomarkers to include in a survey and how best to interpret the results.

It is important to note that to achieve a comprehensive understanding of the causes of MN deficiencies and the full range of options for their prevention, ideally both dietary intake assessments and measurement of biochemical indicators of MN status should be completed. Dietary data, like biomarker data, are not often collected at national scale, though initiatives are already in place to overcome the barriers to conducting dietary assessments. For this reason, the current document focuses primarily on what will need to be done to scale up biochemical assessments of population MN status.

4.4 Agencies and individuals involved in collecting, compiling and interpreting biomarker information on population MN status, and existing repositories of this information

A number of international agencies, bilateral aid agencies, non-governmental organization (NGOs), technical assistance groups and academic institutions provide support to governments in LMICs to collect and interpret information on population MN status. For example, UNICEF and NGOs like Nutrition International (NI), the Global Alliance for Improved Nutrition (GAIN) and Helen Keller International (HKI) often provide technical and financial support for implementing and analyzing surveys. The US Agency for International Development provides technical assistance for survey design, implementation, and data use through the DHS program, which is implemented by ICF and its partners (USAID 2020b). The US Centers for Disease Control and Prevention's International Micronutrient Malnutrition Prevention and Control Program (IMMPaCt) also provides technical support for MN status assessment surveys, as does the French Research Institute for Development (IRD). Private firms, such as GroundWork, offer assistance in survey design and implementation.

Individual MN expert groups, like the Iodine Global Network (IGN), the International Zinc Nutrition Consultative Group (IZiNCG), the Nutrition Program of the New York Academy of Sciences (NYAS), and the Folate Task Force of Nutrition International advocate for the inclusion of specific biomarkers in nutrition surveys and extend technical assistance to ensure optimal specimen collection and appropriate laboratory analysis. The World Health Organization (WHO) develops guidance on the assessment of hemoglobin and indicators of micronutrient status at the population level and curates and compiles representative information on population MN status in its Vitamin and Mineral Information System (VMNIS), as described in greater detail below (**Section 6.9.1**).

4.5 Gaps in data, including % of countries lacking information, MNs not considered, and timeliness of available information

The MN-related biomarkers that, historically, have been examined most commonly are markers of vitamin A and iodine status, as well as anemia as an indicator of several possible MN deficiencies and other diseases. However, as described below, many countries are lacking data even for these biomarkers; and for many countries the data are not sufficiently up-to-date or adequately disaggregated to support program decisions. In recent years, a few countries have begun to assess indicators of iron, zinc, folate, vitamin B_{12}, vitamin D and thiamine status, but these latter data are still relatively scarce.

The WHO VMNIS is the best publicly accessible source of information on the MN status of representative population groups, although some national survey data are not included in this data base, either because WHO is unaware of a particular survey or has not yet curated and published the data, or because the country has decided not to provide the information for public dissemination. Nevertheless, the VMNIS data base is reasonably complete, so this information is used herein to assess the current availability of data on MN status in LMICs. The World Bank classifies countries ("economies") as upper-income if the annual gross national income (GNI) per capita is >US$12,375, and all countries with a GNI less than that figure are considered LMICs. As of June 2019, there were a total of 138 LMICs. Using this classification system, we have examined how many of these LMICs have data on anemia and specific MN status biomarkers in the VMINS data base. The IGN updates information on iodine status separately, so the IGN "scorecard of iodine nutrition" was also consulted for this analysis (Iodine Global Network 2020).

Anemia assessment is frequently included in health and nutrition surveys because hemoglobin analysis can be completed relatively easily using capillary blood obtained by fingerstick and analyzed with a portable device at the site of collection. Anemia is not a specific marker of MN status, but several MN deficiencies can cause anemia, so the data are often used to generate inferences on possible MN deficiencies. As shown in **Figure 1**, according to the VMNIS, 107 LMICs (77.5% of all LMICs) have provided data since 1950 on hemoglobin concentration to assess anemia among preschool-age children (PSC).

The amount of information available in the VMNIS on specific MN deficiencies among PSC in LMICs is generally less abundant (**Figure 2**). From 1988-2018, 77 LMICs (55.8%) reported data on vitamin A status, using either serum retinol or retinol binding protein concentration; 53 (38.4%) reported on serum ferritin as an indicator of iron status; 21 (15.2%) reported on serum zinc; eight (5.8%) reported on vitamin D; and seven (5.1%) reported on vitamin B_{12}. As information on folate status is more critical for women of child-bearing age, we examined the availability of information on folate status among non-pregnant women of child-bearing age (NPW) in the VMNIS. Data on red blood cell or serum folate among NPW are available for a total of just 24 LMICs (17.4%). Although a handful of LMICs have generated information on thiamine, riboflavin, or selenium status of PSC, these data are not yet reported in the VMNIS.

To assess the frequency of MN status data collection for those countries that have produced data, we focused on two MNs for which there is relatively more information, namely vitamin A and iron. In 2015 Stevens *et al*. published a paper on the global prevalence of vitamin A deficiency, in which they reported that only 83 countries produced relevant information for PSC during the years from 1990 to 2013, of which just 29 conducted more than one survey during this 23-year period (Stevens et al. 2015). This amounts to an average of approximately six surveys to assess vitamin A status completed globally each year. Stevens *et al.* further noted that 55 LMICs generated no representative data on vitamin A status of PSC during these years. We also reviewed the VMNIS data base and found that of the 52 countries that provided data on PSC iron status from 1988 to 2018, 39 countries (75%) completed just one survey during this 30-year period, 12 countries completed two surveys, and one completed three. The average year of the last survey for those countries that completed a single survey was 2005, and the average number of years between surveys was nine years for those countries that completed two surveys. In summary, not only is there a paucity of information for most MN status biomarkers, but the information that is available is often outdated.

The situation with regard to data on iodine status is somewhat different. The iodine status of populations is generally assessed by measuring urinary iodine concentration among school-age children, although recently more attention has also been directed to WRA. According to the IGN, a total of 126 LMICs (91%) have produced this information since 1994, and 113 of these countries (82% of all LMICs) have generated data within the past 15 years (Iodine Global Network 2020).

With the help of IZiNCG and inputs from the aforementioned technical agencies involved in survey implementation and other key informants from governments and research institutions in LMICs, we also compiled information on the most recently conducted or currently planned surveys in LMICs that include MN status assessment. Specifically, we focused on surveys that were completed during the past five years or are currently in the planning or implementation stage. This review found 21 surveys that met these criteria, of which nine took place or are soon to be carried out in Africa, three in South or

Southeast Asia, three in the Western Pacific, three in the Eastern Mediterranean, and one each in Europe and Central America (**Figure 3**). All of the 20 surveys for which we were able to obtain final or provisional lists of MN biomarkers included assessment of hemoglobin, as well as markers of iron and vitamin A status. Folate status was assessed or scheduled for assessment in 18 countries (90%), vitamin B_{12} in 17 countries (85%), iodine in 16 countries (80%), and zinc in 13 (65%). Eight countries (40%) completed or planned assessment of vitamin D status, and two countries each (10%) assessed selenium or thiamine status.

The number of recent surveys that were identified suggests that only about four new surveys that include MN status biomarkers are now being conducted annually in LMICs, with sample sizes generally ranging from 490-6840 women and a similar number of PSC (mean = ~2000 women and ~2000 PSC per survey), except for India and Pakistan, which each enrolled more than 20,000 participants (**Figure 3**). Some countries have also collected specimens from school-age children (particularly in the case of iodine assessments), adolescents, and adult males, so the total number of specimens actually collected in each country may be greater than the total number of specimens shown in **Figure 3,** which just reflects the analyses planned for women and PSC. The recent surveys included a broader range of MN status biomarkers than was the case previously; and they generally included biomarkers of inflammation (90% of surveys), which as noted above is helpful for interpreting some of the MN status biomarkers. Based on the average sample sizes for women and pre-school children included in these national surveys (excluding India and Pakistan), the recent surveys generated a mean of ~4000 sets of specimens from these two population sub-groups combined. This number multiplied by an estimated four surveys completed annually indicates that the total laboratory throughput each year is currently as much as ~16,000 for each biomarker analyzed. This figure can be used to project the number of specimens that might be generated in the future as efforts to amplify the annual number of surveys become more successful.

By way of comparison, we also compiled the number of selected other health surveys completed during the same period (2015-2019). A total of 63 standard or special, nationally representative Demographic and Health Surveys (DHS) surveys or Malaria Indicator Surveys, both of which may have the capacity to collect blood specimens, were completed under the DHS Program during this period (USAID 2020d). A total of 45 nationally representative Multi-Indicators Cluster Surveys (MICS) were completed with UNICEF support during this same period (UNICEF 2020). In other words, ~21 nationally representative health surveys supported by either DHS or UNICEF were carried out each year. These and other health surveys could provide a platform for collecting information on MN status, so there are multiple opportunities for generating information on MN status in countries that could potentially benefit from this information. Adding MN status information to other survey platforms, like annual agricultural surveys or household income and expenditure surveys could further expand potential data collection opportunities, although, with few exceptions, these latter surveys usually do not collect biological specimens.

4.6 Reasons for the current paucity of information

IZiNCG and the MN Forum have collaborated to collect information on barriers and enablers for collecting information on MN biomarkers in the context of national nutrition and health surveys (Manger and McDonald 2020). A series of interviews and e-mail exchanges were completed with 11 key informants involved in six nationally representative surveys that included measurement of at least some

MN status markers (Cambodia, Malawi, Pakistan, Uzbekistan, Ghana, and Uganda) and with three representatives of key agencies that support surveys that include nutrition data (UNICEF, US Centers for Disease Control, and ICF). The characteristics of the surveys are summarized in **Table 2**, and the full report will be available from IZiNCG (Manger and McDonald 2020).

Based on responses to a pre-formulated list of questions, the authors of the report categorized factors that served as barriers or facilitators for including MN biomarker assessments in the surveys. The most important barrier cited by the country representatives was the cost of the laboratory analyses. Other barriers that were mentioned less frequently were the lack of reliable, experienced, in-country laboratories; the inability to export clinical specimens due to government regulations; complexities in specimen collection and processing; time pressure to complete the other components of the survey; and concerns that attention to MNs could undermine the data quality of other aspects of the survey. Several of the external experts also noted that a lack of knowledge (both among the in-country decision makers and within the donor and technical support agencies) about the importance of MNs for human health and the usefulness of MN status data for justifying and planning intervention programs is an important obstacle to leveraging the necessary funding. The main factors that favored inclusion of MN biomarkers in the surveys were the presence of supportive government authorities; an in-country "champion" (in a government, academic or donor agency) to advocate for the information; the presence of a planned or ongoing MN deficiency control program for which baseline or follow-up information was desired; access to suitable laboratories; and availability of external experts to provide technical assistance to the local survey team.

Because more information is available for iodine biomarkers than for other MNs, it is useful to explore the reasons for this relative degree of success. Iodine programs initially relied on physical examination to assess for the presence of goiter as a means of detecting iodine deficiency; but because of the difficulty in standardizing these assessments and the fact that goiters often respond slowly or not at all to iodine interventions, these assessments were ultimately displaced by the use of urinary iodine concentration (UIC) as a marker of iodine exposure (Zimmermann and Andersson 2012). As noted above, UIC is most commonly measured in school-age children (SAC, 6-12 years of age). Because most children in LMICs attend primary school, the specimens can be obtained at a common collection site, thereby facilitating specimen acquisition. Moreover, the ability to use urine rather than blood specimens reduces the level of invasiveness and simplifies the specimen collection and processing. Also, spectrophotometric analysis of UIC is fairly simple; and global quality assessment programs have been developed. Finally, technical support has been available from an experienced international community of clinicians (endocrinologists), nutritionists and public health specialists through the IGN; and a data tracking system is publically available. These facts, coupled with the availability of a low-cost, effective intervention (universal salt iodization), have made population assessment of iodine status fairly common. This experience, although not completely transferrable to other MN biomarkers, provides some insights into what will be required to achieve a similar degree of success.

5 Theory of change for generating more high quality information

The ultimate objective of generating more high quality data on population MN status is to enable the planning and implementation of more coherent, effective, efficient, safe and sustainable MN deficiency control programs. Considering the aforementioned obstacles and facilitators for obtaining the desired

information, we have developed a theory of change to indicate the steps needed to carry out this agenda, as well as the personnel, infrastructure and other items required to achieve each of the intermediate goals **(Figure 4)**. In many cases, there has been considerable progress in achieving these intermediate goals, as will be discussed subsequently.

The first step in this sequence of efforts is to: 1) inform national policy makers and donors about the importance of MNs for human health and the current scarcity of information on the prevalence of MN deficiencies, and 2) promote greater availability and use of this information in MN deficiency control programs. This will require a deliberate and adequately resourced communication plan to deliver these messages. At the country level, available information from prior surveys, as well as suggestive information derived from food balance sheets, dietary intake surveys, clinical case reports, and biomarker surveys using convenience samples, should be summarized to determine the MNs of possible concern in each setting and the population sub-groups likely to be affected. Appropriate biomarkers of the selected MNs and related laboratory methods should be described, along with an explanation of how this information can be applied to support program decision-making. As part of this process, national champions and technical experts should be identified, as well as the need for any external technical and financial support.

Once there is consensus on the need for the data and which types of information should be collected, the next step is to plan for obtaining the biological specimens in the context of a dedicated or shared survey platform. This requires access to the survey team, planning the sampling design, working through the logistics of specimen collection and processing, and sensitization of the study population. After the specimens are collected, they need to be transferred to a reliable laboratory for analysis. This, in turn, requires access to laboratories that are appropriately equipped and staffed to store samples, carry out the analyses, and participate in related quality assessment schemes to guarantee the accuracy and reproducibility of the results. Finally, the data must be analyzed, interpreted and applied for policy formulation and program design and evaluation. The remaining sections of this document will review the current status of each of these steps and what more needs to be done to achieve the desired outcomes.

6 Steps for promoting data generation

The actions needed at the global level to produce better data on MN status involve multiple stakeholders, including national governments, international technical agencies, regional political and economic entities, MN technical support groups, academic institutions, civil society organizations, private companies, and public and private donor organizations. The order in which specific steps are implemented will depend on their likely cost, technical feasibility, political support, and predicted impact. This document will not attempt to prioritize among the different steps, as this will require input from the broad range of stakeholders indicated above. Instead, the document will simply list the steps in what is deemed to be a logical order, so that individual stakeholders can develop their own priorities for implementing these actions, ideally in a coordinated manner. The steps that are listed in this section include both actions that have already been completed or are in progress, as well as those that need to be initiated or expanded.

6.1 Establish working groups to develop, review and implement a strategy for generating reliable data on population MN status

Two working groups were engaged to develop the strategy proposed herein. The members of the Core Working Group and the larger Multi-stakeholder Advisory Group are shown in **Appendix 1**.

6.1.1 Core Working Group to review the current situation and develop a strategy for generating better data on population MN status

To kick-start this series of actions, we identified individuals and groups who are already committed to executing selected components of this agenda, and we invited them to participate in the overarching strategy development. A seven-member, Core Working Group was formed to include individuals from the MN Forum, IZiNCG, OpeN-Global, CDC/IMMPaCt, and a member of USAID's Advancing Nutrition project, as well as several individuals affiliated with academic institutions and collaborating with the MN Forum. The Core Working Group members drafted an outline of the current strategic plan and contributed to writing specific sections of the document.

6.1.2 Multi-stakeholder Advisory Group to review the proposed strategy and coordinate future implementation efforts

We also recruited members to serve in a larger, Multi-Stakeholder Advisory Group, either as experts in particular technical domains or as representatives of international agencies and donor groups or LMIC government agencies. This larger group reviewed and commented on the draft strategy document and offered feedback on the overall process. The advisory group also provided a mechanism for sharing information on the individual groups' current activities and identifying specific entities that could assume responsibility for developing and implementing selected components of the strategy in the future.

6.2 Create a communication plan to disseminate information on the importance of MN deficiencies and the need for more high quality information on population MN status

A strong communication and advocacy plan is needed to interrupt the vicious cycle whereby micronutrient malnutrition remains unaddressed because of the lack of data and countries fail to generate the necessary data because of limited understanding of the importance of this information and insufficient financial and professional resources to produce it. The communication plan should focus on two primary target audiences: 1) policy makers and program planners in LMICs, and 2) leadership and program officers of agencies that advise governments and/or contribute funding for nutrition programs.

The key components of the communication and advocacy plan that need to be considered are:

- The main messages for the two principal target audiences
- Prioritization of target countries, with a primary focus on those countries with a high risk of MN malnutrition and a lack of recent data on MN status (e.g., no survey in the past five years or lack of information on specific MNs of potential public health concern)
- Identification of key global, regional and national advocacy events and/or communication channels to disseminate the messages. Examples of relevant global events include the UN General Assembly, the International Nutrition Congress, the MN Forum Global Conference, Nutrition for Growth (N4G) and the Global Food Systems Summit. Exemplar regional events include the African, Asian, and Latin American Nutrition Conferences, regional meetings of the

UN agencies, and meetings of regional economic organizations. National events consist of national nutrition days and other national health conferences, as well as meetings of national professional societies. Additional communication channels include webinars, peer reviewed publications, (mass) media publications and, importantly, face-to-face interviews with major decision makers.

- Identification of key national influencers in the target countries – including the main decision makers for nutrition and health policies and programs, the SUN focal points, and local champions of nutrition and health – who can be approached directly with appropriate information tailored to the local situation.

The principal messages to be disseminated should focus on basic information about the MNs of public health importance, how they are assessed, and the rationale for collecting this information to help shape possible programmatic responses. In particular, the messages should cover current knowledge of the importance of specific MNs for human health and their roles in meeting the Sustainable Development Goals. For example, reduction of anemia in women is one of the Key Performance Indicators of SDG 2, so adequately addressing several MN deficiencies among women is essential for meeting SDG 2. Indeed, indicators of specific MN deficiencies should become performance indicators of SDG2. Global and country-specific information on the prevalence, time trends, and high-risk groups for MN deficiencies should be summarized when available. When national information on MN status is non-representative or completely lacking, information on the risk of inadequate MN availability in the food supply, the % of the population that cannot afford a healthy diet, or the prevalence of inadequate MN intake based on existing dietary data can be used to justify action. After making the case for why MNs are important and why information is needed on the prevalence of deficiency for program planning and monitoring progress, general information should be provided on how to assess MN status and resources that are available to support data collection and interpretation. Finally, country-specific estimates of the costs and cost-benefits of collecting data on MN status should be discussed.

As part of the country-specific advocacy efforts, an inventory of key (government) decision-makers, influencers and key allies (e.g., local or regional scientists who support the need to collect more MN status data) should be prepared in target countries. National nutrition plans should also be reviewed to help in positioning messages in the context of existing national priorities. As a next step, local and regional communication plans, including key advocacy events to publicize the need to collect MN status data, will need to be developed in collaboration with local and regional support scientists. The communication plans should include virtual or face-to-face meetings with the key influencers to inform them about the implications of the data gap and the need to collect more and better data on MN status.

6.3 Achieve consensus on biomarkers of MN status and related modifying factors (e.g., inflammation)

During the past decade several groups, such as the Biomarkers of Nutrition for Development project, the Nutrition Program of the New York Academy of Sciences, OpeN-Global, the International Atomic Energy Agency, and the World Health Organization, have invested considerable effort to review existing information on biomarkers that can be used to assess MN status, factors that affect their interpretation, and corresponding reference values for each biomarker. These entities are described further in **Appendix 2,** and the main biomarkers recommended by these groups are summarized in **Table 3.** The cutoffs for each biomarker that are used to define deficiency are listed in **Table 4** by age, sex,

physiological status, and other categories (e.g., fasting status), as appropriate. The latter table also shows whether the cutoffs are based on clinical/functional data or are defined statistically. Additional research needed to better define these cutoffs is described in **Section 6.3.2**.

6.3.1 National decisions on which biomarkers to include in a survey and for which population sub-groups

The need for data to justify and support MN intervention programs should be imbedded within national nutrition plans and information systems. Individual countries will need to decide which MNs are of possible concern in their setting and which biomarkers should be measured. These decisions should be based on the likelihood that a certain MN deficiency may exist in the population or because a particular MN deficiency control program has already been initiated. Appropriate background information for these decisions includes clinical case reports of deficiency, information from food balance sheets or dietary intake studies that suggest that the food supply or food consumption may be inadequate to meet the dietary requirements for a given MN, and prior research studies using information from representative surveys and non-representative convenience samples. Certain conditions, like folate insufficiency and vitamin D deficiency, appear to be common in many of the small number of LMICs that have been assessed, so these should probably be considered everywhere. Likewise, markers of inflammation should be included in all surveys if iron, vitamin A and zinc biomarkers are being measured, as these biomarkers can be affected by inflammation (Namaste, Rohner, et al. 2017; Larson et al. 2017; McDonald et al. 2020).

Detailed discussion of the survey sample sizes and population stratification is outside the scope of the current document. Nevertheless, it is worth reemphasizing that young children, WRA, and possibly adolescents seem to be most vulnerable to MN deficiencies, so these population sub-groups should be prioritized for assessment. It is also worth noting that the risk of deficiencies may vary by geographic regions within countries, urban-rural residence, and socio-economic status, so these strata may need to be assessed separately, depending on the situation in particular countries. However, there is little justification for assessing individual political divisions (provinces or districts) separately if the food environment is reasonably consistent across those divisions.

6.3.2 Pending issues and steps needed to resolve them

More research is still needed to expand our understanding of existing MN biomarkers and how they are best applied and interpreted, and to develop new ones. In some cases, clinical or functional criteria are needed to establish a physiological basis for setting the cutoffs that indicate deficiency or excess status. For other biomarkers, such as 25(OH) vitamin D, normal ranges are still being debated, in which case results should be reported using multiple cutoffs until consensus is achieved. To avoid confusion, while allowing for cross-survey comparisons, a single cutoff might be chosen for the survey report, with other cutoffs presented in an appendix. When more than one biomarker is available to assess a particular MN, research is needed to determine how best to harmonize the different sets of results. It is possible that new lines of "-omics" research will be useful for discovering novel biomarkers that might eventually complement or supplant the existing ones. Despite any limitations in the currently recommended biomarkers, these have multiple advantages, as described above, and should continue to form the basis for assessing population MN status.

6.4 Review field specimen collection procedures, integration into existing survey platforms, and scope for innovation

Implementation of population-based surveys that include assessments of MN status biomarkers requires strict adherence to best practices for collecting, processing, transporting and storing the specimens while maintaining a cold chain to ensure the integrity of the specimens. The design of these surveys and decisions regarding whether they are conducted as a stand-alone effort or integrated into another population-based survey platform require careful consideration of the options in any given situation. These issues are discussed in the following sections and in **Appendix 3**, both to describe the challenges that must be confronted and to illustrate the resources that are needed to complete the field work. Much of the information presented in these sections has been adapted with permission from the Micronutrient Survey Manual (CDC et al. 2020) published by the Centers for Disease Control and Prevention (Atlanta, USA), World Health Organization (Geneva, Switzerland), and UNICEF (New York, USA).

6.4.1 Specimen collection and processing, cold chain logistics, related costs, and recent innovations

The sequence of procedures required to deliver well preserved specimens to the lab begins with their collection and initial processing in the field, followed by transport to a central facility and storage until the time of analysis. Typically, specimens of blood, urine and/or breastmilk are collected to assess MN status. The volumes that are collected need to allow for backup specimens in case of problems during shipment or if the laboratory needs additional material for duplicate analyses. Venipuncture and/or finger prick can be used to collect blood specimens in the field, depending on the volume of blood needed and the MN biomarkers to be measured. The phlebotomist must be experienced with collecting specimens from both children and adults so as to avoid undue discomfort on the part of the participants and to minimize any failed attempts.

Field processing of blood specimens involves careful labeling of the sample collection and storage containers, separation of serum or plasma from blood cells, and apportioning samples to separate vials for each set of laboratory analyses. Typically, these steps are performed in a temporary field laboratory or nearby formal health facility or laboratory. During each of these steps, cold chain logistics are essential to ensure the integrity of the specimens. Once specimens are collected and processed in the field, they are transported either to regional laboratories for temporary storage until final transportation to the main central laboratory or directly to the central lab. One example of the costs of supplies and equipment required for specimen collection, processing and cold chain maintenance for a national MN survey in sub-Saharan Africa can be found in **Appendix 3**. As personnel and transportation costs vary markedly across countries, we have not attempted to estimate these items, which would also need to be considered as part of the total cost of implementing a MN status survey.

There are multiple challenges and inefficiencies related to the foregoing procedures that affect the ability to collect MN status data. In most cases, venous blood specimens are required because of the volume needed to analyze multiple biomarkers and the fact that micro-methods using capillary blood are not available for all analytes. Further, the challenges related to maintaining a cold chain at each stage of specimen collection, processing, transport and storage have been noted. Collection of smaller volumes of blood through capillary (finger stick) sampling would be advantageous, as the procedure is simpler to teach and to carry out; but capillary sample volume is typically insufficient to cover the full set

of MN biomarkers. Moreover, with current technology, analysis of iron and vitamin A biomarkers from dried blood spots (DBS) is not considered reliable (Lynch et al. 2018; Tanumihardjo et al. 2016). All these factors introduce logistical challenges, increase costs, and may introduce the need for external technical experts to work with countries to support high-quality data generation.

Key innovations needed to reduce barriers and costs for population-based surveys include developing new blood collection and novel testing methodologies. For example, developing point-of-collection (POC) devices that can assess MN status in the household would simplify logistics, as would the availability of reliable assays that require only small blood volumes and could be analyzed from DBSs, which might eliminate the need for a cold chain or relax the temperature range requirements. Innovations in DBS technologies may increase their reliability, which currently limits their use for MN biomarkers. New assessment methods that rely on saliva and urine would also be useful, as their collection is less invasive than blood draws and generally more acceptable to survey participants. All such innovations will require independent, external validation.

6.4.2 Stand-alone vs integrated surveys

Information on MN status may be collected either through an independent, stand-alone nutrition survey or as a fully integrated or separately conducted, but linked, component of another population-based survey. It is important to assess during the earliest stages of planning whether other surveys that cover the same population group(s) and geographic area are being planned around the same time frame of interest, and whether they plan to use requisite survey design and sample sizes of target population groups to meet the objectives of the MN survey. If so, stakeholders should discuss whether it would be possible to expand the scope of the other survey to include a MN module and share resources in a way that reduces overall costs and burden, while maintaining high quality data collection. It should not be

assumed that the organizers of the other survey will agree to incorporate a MN module. Further, it may not be the case that survey integration will provide substantial cost savings; and realistic budgets need to be calculated based on the survey design, sample size, and other factors. Some advantages and

Box 1. Advantages and disadvantages of incorporating a MN component into another planned survey[1]

Advantages:

- A sampling frame may have already been developed by the lead agency of the other survey.
    - If the sample size (for the same population group of interest) for the planned survey is larger than that needed for the micronutrient component, a subsample of clusters, households or participants could be systematically selected for the micronutrient component.
- There may be access to additional data variables collected through the other survey that would increase the scope for subgroup analyses in relation to micronutrients and micronutrient interventions.
- Skilled survey staff would already have been identified and trained.
    - It may be necessary to complement the other survey team with additional enumeration staff, laboratory staff, and a technical field and logistics team. These staff would need to be trained in the specifics of specimen collection and transport required for the MN module.
- Logistics support would already be available.
    - Additional logistical requirements may need to be put in place, for example, cold chain facilities.
- The potential burden of conducting more than one large-scale survey in the same country during the same time frame would be avoided.
    - Integration into health, infectious disease and agriculture surveys may also strengthen weak collaborations and communication among these communities and nutrition, increasing the interest in MN status.
- Use of a survey platform that is standardized across multiple countries and over time provides access to well-established systems for survey implementation and may increase country buy-in and trust

Disadvantages:

- The survey may not include a sufficient number of households per cluster to obtain stable estimates for some population groups of interest, for example young children.
- Incorporating a MN component may require additional time for data collection in the field compared to the surveys being conducted independently.
- There may be complications in ensuring that the objectives of both surveys are met.
- There may be a lower participation rate or higher rate of incomplete questionnaires if the questionnaire becomes significantly longer as a result of combining two surveys.

[1]Adapted with permission from CDC, WHO and UNICEF (2020).

disadvantages of incorporating a MN component into or linking with another planned survey are presented in **Box 1**. 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 of this is that the first-stage sampling may have been performed and the maps and information to identify households and subjects may already be available.

6.4.3 Related data collection instrument

Data collection materials include the main survey questionnaire and other forms (or tools), such as a tracking sheet that indicates how many specimen types were collected per day (or per cluster) as well as how many households or individuals were surveyed in a cluster. These tools can be paper-based or electronic, or a mixture of both. For example, where the main survey questionnaire is in electronic format, some of the specimen tracking forms may need to be paper-based. The types of information that should be considered for collection are described in **Appendix 3**.

6.4.4 Issues to be resolved, and potential areas for future technology development and related next steps

As described in the foregoing sections, multiple challenges must be addressed to collect high quality MN status biomarker data in national surveys. Most of the difficulties related to maintaining a cold chain can be overcome using currently available technology, although strict attention to details is necessary. Challenges remain in collecting sufficient volumes of blood and analyzing the specimens in the laboratory. Advancements in the development of blood collection and novel testing methodologies would simplify procedures and reduce burden. Innovations in these areas could substantially increase countries' ability to conduct MN assessment surveys independently, as well as reduce barriers to integrating or linking MN status modules to other surveys. Reliable assays that require only small blood volumes and methods that can be applied to assess MN status using biomarkers in saliva and urine would greatly facilitate specimen collection and would be more acceptable to survey participants.

6.5 Compile list of recommended analytical methods for key biomarkers, laboratory equipment required to complete these analyses, and acceptable limits of accuracy and precision

The previously mentioned BOND reports, WHO recommendations, and individual expert committee publications have described the biomarkers that are currently available to assess MN status and the cutoffs used to define deficiency and excess status, as summarized in **Tables 3** and **4**. The OpeN-Global web site https://open-global.kcl.ac.uk/ provides information on the analytical procedures that can be used to measure each of these biomarkers. These procedures are also summarized in **Appendix 4,** along with information on the availability of certified reference material for each biomarker and any existing external quality assurance systems. This information can be used to determine what laboratory instruments and other infrastructure would be required to establish a comprehensive MN assessment laboratory, and to estimate the cost of outfitting a laboratory, as described in **Section 6.5.9**. Because personnel costs and expenses for reagents and supplies vary considerably across countries, the costs of completing individual laboratory analyses must be calculated separately for a given context. Although we believe that it would be useful to summarize the expected accuracy and precision of individual laboratory assays, against which newly developed assays or laboratory instruments could be compared, we have not completed this task as part of the current strategy document. This pending task should be completed during a later stage of this initiative.

6.5.1 Equipment needs for MN biomarker resource laboratories

To determine what equipment would be required to establish a resource laboratory capable of measuring all of the recommended biomarkers using the aforementioned methods, we have summarized the specific equipment needs for analyzing each biomarker, as shown in **Table 5**. In particular, the major pieces of required equipment are a high performance liquid chromatograph (for serum and breast milk retinol and vitamin D), an ELISA plate reader (for multiple protein-based assays), a specialized plate reader for ETK assays of thiamine status, a spectrophotometer for multiple assays, an AAS or other instrument (for analysis of zinc and other mineral elements), and other miscellaneous laboratory equipment that may already exist in many labs. Ideally, a liquid chromatograph, tandem mass spectrometer (LC-MS/MS) would also be available for measuring vitamin D, specific folate metabolites, and possibly other B vitamins. We also list general laboratory infrastructure items, such as freezers, refrigerator, centrifuge, balances, and pipetters, which are required for all or most assays.

As the number of specimens submitted increases over time, it may be necessary to purchase more than one piece of some of the equipment items. By that time, revenues generated through the routine analyses should be used to offset the cost of any new equipment needs. We assume that any institution selected to host a resource laboratory will already have some of these equipment items available, so the total cost of setting up a refurbished, comprehensive laboratory is likely to be less than the estimated total cost of approximately one million dollars per laboratory that is shown in **Table 5**. Moreover, depending on the throughput of clinical specimens, some of the items listed, such as the automated pipette and combined 96-well plate washer and reader, may be considered optional initially.

6.5.2 Innovations in laboratory analyses

In the course of compiling the foregoing information, several unresolved issues related to specimen collection, processing, and analysis have been identified; targeted innovations could improve our ability to generate robust data on population MN status across contexts. Particular domains for such innovations might include:

- Sample volumes and specimen type – the full range of biomarker analyses must be completed on sample volumes that are small enough to be collected easily in the field, including from young children. Laboratory analysis methods need to be developed with attention to sample volume and ideally using a single or limited number of sample types.
- Specimen processing – some clinical specimens are sensitive to sub-optimal ambient conditions, such as high temperature, repeated freeze-thaw cycles, and UV light. Innovations around utilization of more robust samples types, e.g. DBSs, hair and nails is needed, as well as simpler, low-cost methods to maintain the cold chain.
- Assay optimization – some assays, such as the RBC folate microbiological assay, require more than one day to complete. Conversion of these multiple-day assays to simpler methodologies would be desirable.
- Multiplex approaches – Currently, many MN biomarkers are measured in stand-alone assays. A move towards 'multiplex' approaches, whereby groups of analytes can be measured in small volumes, could simplify laboratory procedures and reduce costs. Multiplex assays have the additional advantages of lower training requirements for lab staff, lower infrastructure/equipment needs, fewer consumables, and less waste. However, there are several challenges related to the use of existing multiplex assays: 1) these are only appropriate

for plasma/serum (although DBS is being piloted); 2) they require further harmonization against reference methods, and appropriate quality controls and quality assurance programs are not yet fully available. Also, such methods may not be amenable to certain biomarkers based on enzyme activity or because extraction steps required for measurement of one analyte in a panel affect measurement of other analytes in the sample.

- 'Omics' approaches -- Finally, although there is a move towards 'omics' approaches (proteomics, lipidomics, metabolomics) within the field of nutritional epidemiology, these methods are not yet applicable for assessing MN status and they are unlikely to replace direct measurement of the recommended MN biomarkers in the near future.

6.5.3 Issues to be resolved and potential areas for future technology development

As we move towards implementing laboratory support services to enable population-based MN status assessment, there are a number of issues that require further consideration and consensus. These include:

- Assay accuracy and precision vs. utility: For certain biomarkers (e.g. immunoassays and HPLC vs. LC-MS/MS method for plasma 25-hydroxyvitamin D) there remains a trade-off between accuracy and precision of methods and their practicality (cost, analytical ease, infrastructure requirements). We currently propose that comprehensive, centralized laboratory facilities for global use should employ optimal reference methods whenever possible. For individual countries that decide to analyze specimens in national labs that are not able to carry out the recommended analytical methods, we have also listed alternative methods, noting that appropriate steps should be taken to enable cross-comparison between methods.
- Need for more affordable external quality assurance schemes: In some cases, the cost of participating in existing EQA systems precludes participation by some LMIC laboratories, so donor support may be required to expand their use.
- International standardization programs and laboratory accreditation schemes: To help ensure between-laboratory quality and standardized results, and to assist central laboratories gain recognition for being able to offer high quality analyses, development of an international laboratory accreditation scheme/s is essential, so that laboratory performance can be regularly evaluated. Further, each laboratory needs to have easy access to assay standardization programs for each biomarker, supporting between-laboratory quality assurance.
- Need for improved biomarkers of status: Given the lack of a single sensitive and specific biomarker that is informative across the full range of status for some MNs, further research is still needed to identify and validate new biomarkers of MN status. In some cases, such as with thiamine metabolites and thiamine-dependent enzymes, cutoffs to define deficiency have not yet been established; and in other cases, such as with vitamin D, there is not yet consensus on the appropriate cutoff. In the latter case, it is advisable to present survey results using multiple different cutoffs, so they can be compared widely with other data sets.

6.5.4 Next steps to resolve these issues.

In response to the issues highlighted above, the following next steps are required:

- Based on the currently recommended analytical methods for each MN biomarker, standardized laboratory procedures should be published, and external quality assurance schemes should be

publicized. All resource laboratories should use the recommended methods and participate in the same EQA schemes, and national laboratories should also be strongly encouraged to do so.

- Where laboratories are unable to implement the method of choice, simpler or lower cost alternative methods should be recommended, alongside appropriate quality assurance programs.
- For MN biomarkers for which there are no existing quality assurance schemes and/or certified reference materials, these will need to be developed and made readily available to the global community.
- It would be useful to develop consensus on the acceptable accuracy and precision of the existing reference methods, so as to provide a "target product profile" to guide the development of novel laboratory methods or analytical devices, including POC analytical methods.
- Research should be encouraged to continuously search for better biomarkers of MN status.

6.6 Identify and support resource laboratories

Currently, there are no internationally recognized resource laboratories that are capable of analyzing the full range of MN biomarkers and able to receive specimens from multiple countries. Moreover, individual countries sometimes insist that all laboratory analyses be completed in country, which can create an insurmountable barrier for generating reliable information in a cost-effective manner. The following sections address these issues and propose possible solutions.

6.6.1 National vs regional/global laboratories; individual laboratories for selected biomarkers vs comprehensive laboratories

Understandably, many countries want to achieve or maintain autonomy in clinical laboratory capacity; and some governments may be uncomfortable relinquishing direct control over clinical specimens and resulting data. However, using regional or global resource laboratories for biomarker analysis is almost certainly more cost-efficient than establishing and/or certifying individual national laboratories for each assay in every survey. In situations where national laboratory capacity does not currently exist or has not been appropriately vetted using widely accepted laboratory certification schemes, there are likely to be considerable delays in equipping the national laboratories, procuring supplies, training staff members, developing analytical procedures, and achieving certification. Moreover, the cost of establishing independent laboratories for each country is much greater than the cost of supporting one or more central laboratories to receive specimens from multiple countries. National laboratories often remain idle or under-utilized between surveys, sometimes resulting in staff turnover and continuous training needs, whereas central laboratories could operate continuously. Therefore, the current working group strongly advocates for establishing a small number of regional or global resource laboratories that have the capacity to analyze specimens generated anywhere in the world. Initially, one laboratory could be established in sub-Saharan Africa and one in South or Southeast Asia, pending accrual of experience regarding the demand for services over time and the possible need for additional labs.

To address issues of national sovereignty and control of biological specimens, several options are proposed. First, the regional resource laboratories could be established under the supervision of a trusted international organization, like WHO, international health or agricultural research centers, or regional scientific societies or academic bodies. Second, national laboratory scientists/technicians could accompany specimens to the resource laboratory and observe or participate in the specimen handling and analysis. As such, the resource laboratories could also serve as training and technology transfer

centers for national scientists; and the national professionals could share their experiences and perspectives with the resource labs and participate in the performance verification processes. Data could be segregated in nationally controlled data bases, accessible only to authorized national personnel and central laboratory authorities. Finally, once all analyses are completed and confirmed, any unused specimen portions could be returned to the country of origin for storage or further analysis. Alternatively, specimens could be stored in a central biorepository for future use under the control of national authorities.

Very few laboratories currently measure all of the recommended biomarkers. In most high-income countries and institutions within those countries, independent laboratories have evolved with expertise in selected classes of biomarkers or analytical methods. However, as discussed above (**Section 6.5.1**), a reasonably small number of analytical instruments and related infrastructure are needed to complete all of the recommended analyses. Thus, establishing a small number of central resource laboratories that are capable of completing all the analyses should be feasible. This would circumvent the need to equip national laboratories in all countries planning a MN status assessment survey or to transfer specimens to multiple different laboratories for the desired array of biomarker analyses, with the attendant additional shipping costs, logistical complexity and greater risk of specimen losses or deterioration. Issues of laboratory throughput in relation to the anticipated number of specimens generated annually are discussed below.

6.6.2 Specimen transfer, data confidentiality, and open access to data

When transferring biological material or data from one researcher or institution to another, it is important to prepare a prior agreement on the terms of this transfer, regardless of the nature of the institutions (i.e., non-profit or commercial). In recent years, material transfer agreements (MTAs) have become the most commonly used tool to regulate the transfer of research materials, including, for example, blood and plasma specimens for MN status assessment. Cervo et al. (2016) recently proposed a model MTA for biobanks and biorepositories, in which they identified several essential components of the agreements, namely: 1) permissions, liability and representations, 2) custodianship and distribution limitations, 3) appropriate use of materials, including biosafety concerns, 4) confidentiality, non-disclosure, and publications, and 5) intellectual property protection for both the provider and recipient. Similarly, in an effort to harmonize MTAs in South Africa, the National Department of Health recently developed a model MTA of Human Biological Materials (Labuschaigne et al. 2019). To facilitate research and collaboration and avoid administrative delays, several researchers have recommended simple MTAs that focus primarily on the purpose of the sample exchange (Whitton et al. 2019; Bubela et al. 2015).

MTAs can also govern data sharing, so that the conditions of the data use are agreed upon beforehand, and the related agreements may be termed materials and data transfer agreements (MTDAs). For any sharing and use of protected health information, investigators need to comply with the Institutional Review Boards and the privacy standards of the respective countries, such as the Health Insurance Portability and Accountability Act in the United States and the European Union General Data Protection Regulation (US Department of Health and Human Services and National Institute of Health 2020; Staunton et al. 2019). When using de-identified data, researchers need to ensure that there is a negligible risk of “re-identification.” Finally, when collecting and sharing data, collaborators need to choose online platforms carefully to ensure fidelity and confidentiality of the data.

In recent years, research funding agencies, journal editors (Taichman et al. 2017), and the scientific community in general (Institute of Medicine 2015) have begun advocating for responsible sharing of data generated by scientific research, while recognizing some of the many challenges involved. Initial efforts have focused on data generated through clinical trials, but many of the same considerations pertain to survey data. In particular, encouraging open access to de-identified, individual-level data allows for greater transparency and reproducibility of research results, while facilitating greater use of the data for secondary analyses. In this way, more questions can be addressed with minimal additional financial investment, and greater benefits can be accrued from the efforts of the original data gatherers and research participants. Ideally, these data sets could be archived in a central repository, such as WHO's VMNIS, although additional resources would be required to maintain the infrastructure, curate individual-level data and manage access to the data sets.

6.6.3 Current frequency of national surveys, projected future frequency, and related laboratory support needs

According to the analyses described in **Section 4.5** and summarized **Figure 3**, approximately four national surveys that include MN biomarkers are currently being conducted each year. These surveys typically intend to collect an average of ~2000 clinical specimens from PSC and a similar number from WRA. Some surveys also include older children, adolescents and adult males. Surveys from larger countries sometimes collect considerably more clinical specimens. For example, the 2016-18 survey in India generated slightly more than 20,000 specimens each from preschool children and adolescents and the survey in Pakistan collected more than 60,000 specimens from PSC and WRA combined, so as to be able to disaggregate data to sub-national strata. To estimate the current demand for laboratory services to analyze clinical specimens from PSC and WRA globally, we can assume four surveys per year, with a total of 4000 blood or urine specimens (2000 each from PSC and WRA), resulting in a total of ~16,000 specimens annually. Each of these specimens might be analyzed for 10-12 biomarkers, depending on local interests, implying a total of 116,000-148,000 individual laboratory tests or twice that number if completed in duplicate.

To estimate the costs of completing these laboratory analyses, we compiled data from a total of 23 laboratories around the world, with the help of CDC IMMPACT and individual colleagues carrying out these analyses. As summarized in **Table 6**, these costs are extremely variable, depending on the particular biomarker, the individual laboratory, and the analytical methods used. Because it is not possible to predict which biomarkers any given country may decide to measure and what the actual costs may ultimately be in a dedicated resource laboratory, we assumed an average of $10 per test, which is slightly more than the average of each of the lowest reported analytical costs, regardless of analytical method, and somewhat less than the average costs, when all analytical methods are considered. Using this assumed average of $10 per test, ~$2.4-3.0 M would be required annually to support the foregoing number of laboratory analyses (completed in duplicate). Because, as noted, the actual cost per test may vary substantially from one laboratory to another, depending on local labor costs and many other factors, this is a fairly crude estimate, which is just meant to provide a rough idea of the likely total cost of these analyses.

Ideally, every country should complete a MN status survey every five years, both to track changes in status and to feed into national planning documents, which are typically revised every five years. This would indicate that, on average, ~28 of the total of 138 LMICs would need to conduct a survey each

year, which is approximately seven times the current number being completed. If just half of these countries follow a five year survey schedule, which seems to be a reasonable aspiration initially, then approximately three and a half times the current number of surveys would need to be completed annually, at a total cost of ~$8-10 M annually for laboratory analyses. This figure can be used as a basis to estimate the amount of funds that would have to be mobilized to cover these laboratory expenses each year, in addition to the costs of collecting and shipping the specimens, as described above in **Section 6.4.1 and Appendix 3**. Ideally, these expenses would be shared between individual countries and international donor agencies contributing to the MN data generation initiative, as described below.

6.6.4 Next steps for establishing resource laboratories

As a first step in establishing the proposed resource laboratories for analysis of MN biomarkers, a consensus building process will be required to determine the demand for these services and identify the umbrella organization(s) that will oversee these facilities, and to identify existing laboratories that could be enhanced and supported to provide these services. This could be accomplished by convening two regional workshops with members of the managing team, PPT, TAC, and representatives of possible umbrella organizations and hosting institutions. As noted above, possible umbrella organizations include WHO regional offices or collaborating centers, international health or agricultural research institutions, global professional societies, or individual academic institutions. In the case of the research institutions, laboratories within these facilities could be dedicated to serve as the MN biomarker resource labs.

It will be important to draft criteria for selecting the laboratory managing entity and the individual resource laboratories before the workshops are convened. The final selection process will likely require site visits to a short list of candidate laboratories by selected members of the management team, Project Partners Team (PPT) and/or Technical Advisory Committee (TAC). Some of the essential criteria that will need to be considered, in addition to national and institutional commitment, are the financial and political stability of the parent institution; the availability of critical infrastructure, like stable electricity, an ample supply of pure water, space for sample storage, and in-house information technology support services; the presence of qualified laboratory chemists and technicians; the local availability of critical supplies and reagents or ability to import these easily (and ideally import duty-free); the ability to import biological specimens from all countries; and the ability to host foreign nationals in the lab.

Once the central resource laboratories are identified and provided with any equipment not already available in the facility, the individual analytical methods described in **Appendix 4** will need to be implemented at these sites, possibly requiring external technical assistance, as well as access to external quality assessment systems. To support setting up the labs and the analytical methods, we propose that the MN data generation initiative should provide financial support for a full-time laboratory director, a full-time technician, core supplies and reagents, and equipment maintenance contracts for the first two years of operation. Thereafter, these recurring expenses and equipment maintenance and depreciation should be built into the fees charged for sample analysis, so that the laboratories can become self-sustaining.

6.7 Develop laboratory quality assurance procedures

To ensure the accuracy and reproducibility of laboratory analyses, quality assurance procedures need to be implemented as an integral component of laboratory operating procedures. Standardization of assays, even retrospectively (Durazo-Arvizu et al. 2017), provides confidence that data are accurate and

reliable and allows results to be compared from different studies and over time (Makris et al. 2020). Many references are available on best practices for internal laboratory quality control (WHO 2011b; Cooper 2008) , so this topic will not be reviewed herein. Issues of inter-laboratory standardization, certification, and reporting, as well as related global support systems, are described in **Appendix 5**. Several of these items have been addressed in a recent editorial, which highlighted some of the weaknesses in current laboratory practices related to nutrition biomarkers and reporting of results (O'Callaghan and Roth 2020).

6.8 Determine technical training needs and support professional training and refresher courses

Training needs for setting up recommended laboratory analysis methods in the regional resource laboratories are mentioned above. In cases where individual countries decide to analyze specimens in national laboratories, several possibilities for training exist. As noted in **Section 6.6.1**, the proposed resource laboratories can also serve as regional hubs for capacity building. This would mean that financial support would be required to help defray travel and subsistence expenses for laboratory scientists from national labs to spend time at the resource lab. Support would also be needed for the trainer(s) in the resource lab, as some of their time would be diverted from other responsibilities. We have built these expenses into the full budget for the future initiative.

Another option for national training would be to rely on existing training programs, such as the excellent program developed by the US CDC for training in serum and red blood cell folate analyses, using the microbiological assay (CDC 2018). However, we are not emphasizing this approach in the current strategy document, as we prefer to promote use of the regional resource facilities for the reasons described above. Nevertheless, for countries that want to develop national MN laboratories, it would be useful for the MN data generation initiative to compile an inventory of existing training curricula for different MN biomarkers, similar to the one described above for folate.

6.9 Develop and support data repositories, increase information dissemination, and enhance capacity for data interpretation and utilization

One of the stated goals of this initiative is not just to collect more high-quality information on population MN status, but to promote and support expanded use of this information for program design and evaluation. As discussed above, the initiative's Core Working Group strongly supports making data on MN status freely available in the public domain. This implies the need for a data repository where the information can be accessed following initial data quality review and standardized presentation of the information. The following sections describe current sources of information on population MN status and related surveys that produce the information, along with suggestions for promoting greater use of these resources.

6.9.1 Description of existing data repositories

As noted above, the primary source of information on MN status of populations is WHO's VMNIS. The Micronutrients Database was first developed as part of the VMNIS, formerly known as the Micronutrient Deficiency Information System, in 1991. This database is an interactive platform for summarizing data published in reports and manuscripts on the MN status of populations. Data are representative at national, regional (within country) and first administrative levels (e.g., canton, state, province). The database was originally designed to capture data on hemoglobin, serum retinol, xerophthalmia, urinary

iodine and goiter; and it has recently been greatly expanded to include information on most published indicators of MN status for over 40 indicators. Additional information on the type of biological sample, method of sample collection and analysis, relevant adjustments (e.g., for inflammation, smoking, altitude, etc.), and important survey characteristics are also recorded to aid in the interpretation and comparison of data. Most results published prior to 2020 have been entered into the VMNIS database, and verification of the data is ongoing. The database is continuously maintained, with data entry occurring as reports are received from Member States and partners. Formal search strategies are conducted annually to capture additional reports in the published literature

The Micronutrients Database has been expanded slowly over time due to the limited financial and human resources available for entering large amounts of data. Final validation of the backlog of data on new indicators currently requires 0.5 FTE of a staff member and 0.5 FTE of a consultant. Once this validation is completed, approximately 0.5 FTE of a staff member (at a cost of ~$105,000 per annum) will be required to maintain the database, perform continuous quality improvements and work with partners. Moving from the pilot data visualization platform of the Micronutrients Database to a platform that has improved performance, structure and capability within the WHO IT environment will require an investment of approximately $170,000.

WHO collaborates with other agencies, associations, networks and academic institutions to share data sources for variety of purposes. Data are used to generate global estimates of the burden of anemia and micronutrient malnutrition, to track progress towards the goal of eliminating the major micronutrient deficiencies and to improve decision-making related to micronutrient policies and actions. Starting this year, WHO has become a data custodian for the new SDG indicator 2.2.3 on anemia.

The Institute for Health Metrics and Evaluation (IHME), based at the University of Washington, produces the annual Global Burden of Disease Report (GBD), which estimates the causes of premature death and disability from more than 350 diseases and injuries in 195 countries, by age and sex, from 1990 to the present. The GBD report is used widely by global and national decision makers to identify priorities for action and to monitor progress (IHME 2019). The current IHME estimates of the global burden of MN deficiencies suffer from the lack of data on MN status, which obliges the modelers to use alternative, but flawed, methods. One example is the heavy reliance on data concerning per capita micronutrient availability in national food supplies, based on the FAO Food Balance Sheets, as a proxy for MN intake and status. As noted above, this approach does not account for the uneven distribution of food availability and dietary intakes throughout the population (including different geographies, age groups and socio-economic segments), nor the bioavailability of nutrients from these foods. Because this approach using food balance sheets does not correlate well with MN deficiency prevalence estimates based on MN status biomarkers, there remains a sizeable range of uncertainty of the prevalence estimates obtained in this way. For its future versions, IHME is interested in updating their MN projections by using and aligning a broader range of MN status data sources.

6.9.2 Clearinghouse of information on recent and upcoming nutrition surveys

IZiNCG maintains an inventory of recently completed and upcoming national surveys that have assessed or are planning to assess biomarkers of MN status. The original motivation for developing this inventory was to disseminate information on which countries have produced recent, nationally-representative data on plasma zinc concentration (PZC), and to advocate for the inclusion of PZC in upcoming surveys. However, it soon became apparent that these objectives were also relevant for many other “neglected”

MN biomarkers that are not often included in population-based surveys. Thus, the inventory was expanded to include biomarkers of folate, vitamin B12, and vitamin D status, in addition to the more commonly assessed MNs such as iron, vitamin A, and iodine.

Since its inception, this inventory has evolved into a tool that supports enhanced coordination and communication among various organizations and agencies involved in financing, planning, and otherwise assisting with or implementing MN status assessment surveys. An informal "Micronutrient Biomarker Working Group" has been formed, which includes representatives from UNICEF, WHO, the US CDC, USAID, GAIN, GroundWork, and Sight and Life, in addition to IZiNCG and the Micronutrient Forum. Information on upcoming surveys is solicited on a quarterly basis, including target populations to be assessed, anticipated sample sizes, MN biomarkers being considered for inclusion, and organizations providing technical or financial support. The inventory is updated continuously in an online spreadsheet accessible to all working group members, so that they can identify opportunities to advocate for more comprehensive MN status assessment in the upcoming surveys and identify any needs for additional technical support.

IZiNCG is well-positioned to continue to develop and manage this clearinghouse of surveys in the future. Opportunities also exist to expand the scope of the data base to include more information on issues such as survey implementation methods, laboratory methods used for biomarker analysis, sources of financial support, and the availability of individual-level data from survey participants. A more sophisticated, user-friendly data base could be posted to the IZiNCG and MN Forum websites and linked with the WHO VMNIS, which would likely capture the interest and engagement of a broader audience. Maintenance of this resource will require financial support for a part-time position to continue to update the data base and implement the proposed enhancements. Financing will also be required to provide technical support for the website development. The estimated amount of funding required is approximately $15,000 in start-up costs to develop the web pages and $17,000 annually to communicate quarterly with survey support agencies and government officials and to update the data base.

6.9.3 Periodic analyses of trends in MN deficiency prevalence

To help track the availability of relevant information and to estimate the global prevalence of individual MN deficiencies, global trends in population MN status and deficiency prevalence should be reassessed at least every five years. WHO has previously developed global estimates of vitamin A deficiency (Stevens et al. 2015) and anemia (Stevens et al. 2013). The anemia prevalence estimates have been updated at least every five years since 2007 for children (WHO 2017a) and women (WHO 2017b). There are plans to continue future updates every three to five years to monitor progress related to the Global Nutrition Target 2 (50% reduction in the prevalence of anemia in women of reproductive age by the year 2025) and the new SDG indicator 2.2.3 (prevalence of anemia in women 15-49 years of age, by pregnancy status). By contrast, there are presently no plans to update estimates of the prevalence of iodine and vitamin A deficiency, although WHO collaborates with the Iodine Global Network to monitor the prevalence of iodine deficiency through the Global Iodine Scorecard (Iodine Global Network 2020). WHO welcomes collaboration with additional networks, agencies and academic institutions to generate these estimates. USAID-Advancing Nutrition and its partners have recently decided to compile existing information on population MN status and to develop updated estimates of the prevalence of selected deficiencies and the number of individuals with one or more deficiencies, depending on data availability.

6.9.4 Information dissemination

As more data become available, additional attention should be directed to how best to disseminate this information more widely. In addition to the data repositories cited above, summary statistics on the global and regional prevalence of MN deficiencies should be reported actively and systematically. A potential platform for reporting this information is the Global Nutrition Report, which provides an annual, independent update on the state of global nutrition (Global Nutrition Report 2020). We propose, for example, that the GNR could include a section on global MN status, based on the periodic reviews of prevalence trends described above, along with information on MN program coverage. Another possible vehicle for dissemination of this information is the UNICEF report, "State of the World's Children."

6.9.5 Training in data analysis, interpretation and utilization

Another issue that was revealed during the IZiNCG interviews with key informants is the fact that in some cases where MN status data are available in particular countries, the information is not fully exploited for decision-making and program design. Although the survey results are typically published as individual reports for national dissemination, there is not always a clear link between the survey results and their implications for ongoing or future programmatic interventions. The reasons for this under-utilization of data are not fully understood, but likely relate to the lack of national professional capacity to analyze and interpret the data, poor communication between technical experts and policy makers, and the fact that other political and financial issues apart from the prevalence of MN deficiencies influence ultimate decision-making regarding intervention programs. To address this set of issues, training in data analysis, interpretation and dissemination would be useful, along with guidance on how best to package brief messages that summarize these results and their implications for program design. Additionally, the financial consequences of any program-related recommendations should be estimated and communicated at the same time that any such recommendations are issued.

The ultimate goal of the data generation process is to bring about improvements in health policy and practices. Such progress is only possible if the results of survey research are shared broadly through published reports, journal articles, conference presentations, and direct communication with key decision-makers, as well as mass media, social media, and other public formats as part of a broader communication plan. For survey reports, simple tabulations of the prevalence of deficiency or excessive MN status among selected population sub-groups, such as PSC and WRA, are usually of primary interest. Depending on the survey design, these results may be further disaggregated by geographic region, urban-rural site of residence, demographic and socioeconomic status, and other possible risk factors affecting MN status. The DHS web site provides useful examples of how data tabulations might be presented for different nutrition-related indicators (USAID 2020c). The results can be shown as data tables or bar graphs, including the confidence limits of the prevalence estimates. Ideally, the implications of the results for public health policy should be described. It is also desirable to circulate any related scientific articles published or in press, so that technical experts in the government or donor agencies have easy access to this material.

Survey results should be communicated directly to appropriate government authorities and funding sources so that the results can be considered for future policy formulation and priority setting. Reports should be formatted in a way that is accessible to individuals who are not necessarily experts in

nutrition, while remaining technically accurate. FAO has published useful guidance on how to prepare effective policy briefs (FAO 2008).

This proposed training in data analysis, interpretation, and dissemination could take place in the form of national or regional workshops, backed by on-line instruction materials. A formal training curriculum will need to be developed as one of the follow up activities after publication of this strategy document. One example of this type of training program is the Regional Initiative to Strengthen the Analytical Capacity and Evidence for Nutrition in West Africa, which has convened two workshops and provides open access supporting materials on the internet (Countdown to 2030 2020). The DHS Program also supports data analysis workshops and e-learning platforms related to data analysis and use (https://dhsprogram.com/What-We-Do/Capacity-Strengthening/Regional-Workshops.cfm).

7 Summary of recommended next steps

Many of the actions that are being recommended as part of this initiative have already been stated or implied within each of the foregoing sections that describe the steps required for promoting increased data generation and utilization (**Sections 6.1-6.9**). In the current section we briefly summarize the full set of recommendations and propose possible entities that could assume responsibility for implementing these actions. Where possible, we have also attempted to estimate the professional and financial support that will be needed to sustain these activities.

7.1 Establish a project management team

Once this strategy document is endorsed by a broad range of interested stakeholders, follow up activities should be directly managed or coordinated by a project management team that will have three primary functions: 1) advocacy and fund-raising on behalf of the initiative; 2) direct oversight of selected activities, including hiring consultants as needed; and 3) coordination with partner agencies. We propose that the management entity should be based within the MN Forum, building on the technical expertise and administrative support that backstopped the preparation of the current strategy document.

7.2 Prepare formal proposals to develop financial support

Many of the key components of this initiative will require leveraging new financial resources, as discussed below in **Section 8**. The project management team will need to prepare grant proposals and other supporting materials to justify and motivate investments in this initiative. This will require the project management team to work directly with potential donor agencies to identify specific components of the initiative that are most compatible with the individual agencies' existing portfolios and future priorities.

7.3 Establish a Project Partners Team, a Technical Advisory Committee, and a roster of technical experts

Three different groups of experts will need to be convened by the initiative's management team. First, a Project Partners Team (PPT) comprised of representatives of agencies that offer technical and financial support for field surveys and those supporting this global initiative, both financially and technically, should be convened quarterly by teleconference and annually in person (when possible) to help guide the initiative, review progress and share updates on their own organizations' activities. Second, the current Core Working Group should evolve into a standing Technical Advisory Committee (TAC), which

will meet semi-annually by teleconference (or at separately scheduled professional conferences) to provide technical guidance and critical feedback on individual project activities. Finally, the project management team should develop a roster of technical agencies and individual consultants who can be called upon to complete selected tasks prioritized by the management team, PPT, TAC, and individual donors on an ad hoc basis.

7.4 Create advocacy materials and implement related communication plan to motivate national decision makers, technical advisory groups and donor agencies to implement recommended data generation activities

An active advocacy and communication plan targeted to both key national decision-makers and champions of this initiative, as well as selected leaders and program officers of global and national technical assistance and donor organizations, should be prepared and implemented, with initial focus on high-risk countries that have not produced relevant information within the past five years. Following development of key messages for these target audiences and ancillary communication materials, the information should be delivered through global, regional, and national health and nutrition events, national media, and direct communication with key individuals, as indicated above. These activities should be overseen by the initiative's management team and implemented either by internal project staff and consultants or collaborating institutions or agencies.

7.5 Create and manage a central fund for MN status assessments

A key limiting factor in collecting information on population MN status is the lack of government funding in LMICs, both to collect clinical specimens and to complete the related laboratory analyses. To address this critical bottleneck in producing more high-quality information, we propose creating a multi-donor central fund for generating data on population MN status. This fund will be used for two primary purposes: 1) to establish and maintain one or more regional resource laboratories to complete the necessary biomarker analyses; and 2) to help defray costs of collecting samples in individual countries and analyzing the specimens that are obtained. The latter activity will necessitate specifying the conditions of these grants (as discussed in **Section 8.3**), developing criteria for selecting countries to receive these funds, and creating an appropriate governance structure. This fund will be administered by the initiative's managing team, with oversight provided by the PPT and independent auditing. The need for donor funds should reduce over time as economic conditions improve in LMICs and the value of the information becomes more widely appreciated.

7.6 Provide technical assistance to countries for the planning, implementation, and analysis of national surveys that include MN status assessment

One of the issues identified during interviews with key informants is the need for technical support to countries for survey design, selection of appropriate MN biomarkers, field implementation (including proper collection and handling of clinical specimens), and data analysis. Several technical support agencies exist, but they are unable to satisfy current demand, let alone the anticipated increased demand for these services. Therefore, these agencies will need to ramp up their capacity or new ones will need to be established, and funding will be required to support these services. Continued support for maintenance and periodic updating of the OpeN-Global web site is also recommended.

7.7 Compile list of recommended analytical methods, and support and expand external quality assessment systems and availability of certified reference material

To promote and support the accuracy of laboratory results and inter-laboratory comparability, we recommend using a commonly agreed upon set of analytical methods and reference biomarker materials. As a first step, the initiative should commission experts to reach consensus on a list of recommended analytical method(s) for each key biomarker and standard operating procedures for completing these analyses, building on the information provided in the present document. These methods should be promoted for use both in the regional resource laboratories and any national labs that continue to undertake these analyses in support of national surveys or for clinical care. To ensure the accuracy of the laboratory results, current external quality assessment programs should be expanded and new ones established to encompass the full range of recommended biomarkers. In cases where CRMs for these biomarkers are not yet available, the initiative should collaborate with existing authorities or commercial laboratories to produce these materials and make them available.

7.8 Establish two comprehensive, regional resource laboratories to analyze MN status biomarkers in clinical specimens collected in national surveys and to provide laboratory training

As explained in several sections of this document, we believe that the most cost-efficient, sustainable approach to ensuring high quality, carefully standardized laboratory analyses is to establish comprehensive, regional resource laboratories. This will require first confirming country-level demand for these services, identifying existing laboratories that can be further enhanced to provide the necessary services, and training and upgrading these facilities as needed to provide the full range of recommended analyses. Initially, we propose establishing two laboratories, one in sub-Saharan Africa and one in South or Southeast Asia under the administrative umbrella of an international or regional scientific agency or institution, although further discussion among stakeholders will be required to assess countries' commitment to this plan.

7.9 Prepare and publish an inventory of training curricula in laboratory analysis of MN status biomarkers

To support countries that want to develop national facilities for analyzing MN status biomarkers, a list of existing training curricula should be prepared, both to serve as a resource and to identify where additional curriculum development may be needed.

7.10 Support and expand existing data archives of MN status information, including publication of periodic updates on the global situation, and a clearinghouse of information on recent and upcoming surveys

The WHO VMNIS is the main source of curated information on population MN status, based on MN biomarkers. However, there is a backlog of survey data not yet posted on the VMNIS web site, several MNs of public health concern are not yet included in the VMNIS data base, and available data are reported only as aggregated results rather than individual-level data. Support should be provided to WHO to update and expand the VMNIS data base, prepare more user-friendly data visualization tools, and ultimately to curate appropriately anonymized, individual-level information. Support should also be provided to maintain the clearinghouse of MN status surveys developed by IZiNCG. Finally, global and regional information on the prevalence of various MN deficiencies and trends should be compiled

periodically so that updates can be widely disseminated, based on the data available in the VMNIS data base and other relevant and reliable data sources.

7.11 Develop and support training programs on the interpretation and application of information on population MN status for program design

To encourage more extensive application of survey results for policy formulation and program design, we recommend providing technical assistance in these areas of data interpretation and utilization and developing a related training curriculum, so that national scientists and program managers can further develop these skills.

7.12 Track progress of fund raising, data generation, and related activities proposed to support this initiative

As with any new initiative, it will be important to develop a set of indicators that will allow the management team and project partners to track progress. A set of indicators listed below can serve as a starting point to develop consensus on which of these indicators should be monitored systematically. These activities should be overseen by the initiative's management team and reported periodically to the PPT and TAC.

8 Generating financial support for recommended actions

To complete the proposed sets of activities – advocacy and information dissemination; field support for survey design and specimen collection; laboratory development, staff training, and quality assurance; subsidized laboratory analyses; technical assistance with data analysis, interpretation and dissemination; maintaining a curated data repository and clearinghouse of information on MN status surveys; and tracking progress – will require a substantial financial investment. This section of the document examines the approximate amount of funding that will be required, possible sources of funding, management of follow-up activities, and next steps to implement this agenda.

8.1 Roles of national governments, international agencies, donor groups

We envisage that a consortium of global donors will be needed to support the broad range of proposed activities, and we assume that different entities will take responsibility for implementing specific components of this initiative. National governments should remain the primary drivers of decisions concerning the design of individual surveys and related data collection and analysis activities. As such, the governments should bear the primary responsibility for funding the surveys or identifying donor support through country-specific relationships with entities like the World Bank, other international agencies and bilateral donors. On the other hand, shared facilities and services, such as the regional resource laboratories, participation in external quality assessment systems, data repositories, technical assistance, and central management of the initiative, should be considered a global common good to be supported by multi-lateral and bi-lateral agencies, private foundations, and interested commercial enterprises engaging in social responsibility actions. We believe that funding contributed by non-governmental sources for in-country activities should be conditional on the countries' willingness to adhere to certain common principles, as described below in **Section 8.3**.

8.2 Estimated costs of advocacy and information dissemination, laboratory development, national subsidies for specimen collection and laboratory analyses, technical assistance, information curation and utilization, training activities, and project management

At this stage of the process, we are not able to develop detailed budgets for individual components of this initiative. Nevertheless, we have attempted to estimate the general level of financial support that would likely be required to finance each of the major sub-projects, as summarized in **Table 7**.

To complete the proposed advocacy agenda, it will be necessary to develop specific messages and related written materials as well as a communication plan for disseminating these messages. Once these initial tasks have been completed, ongoing support will be required for a communication specialist with the necessary technical background to conduct or participate in the advocacy events and interact directly with focal points in high priority countries. We have estimated the costs of the start-up communication activities to be approximately $100,000 and the annual recurring costs for a half-time technical specialist, travel, and in-country support costs to be approximately $165,000 per year.

Development of the regional resource laboratories will require preliminary efforts to identify the umbrella organization to oversee these labs and to determine the specific facility sites. These steps can be accomplished by convening regional workshops and conducting site visits to a short list of potential lab facilities. The labs will then need to be equipped, and the staff members trained to implement the recommended analytical methods. Once the labs are fully operational, recurring costs will include partial support for the lab director and a laboratory technician for the first two years, initial purchase of essential reagents and supplies, initial equipment maintenance contracts, and participation in EQA programs. We assume that each of the two proposed laboratories will require up to $1,000,000 in start-up costs and approximately $160,000 in annual operating costs for the first two years. Individual assays should be performed on a fee-for-service basis.

From a national perspective, generating information on population MN status involves specimen collection (and related training of field staff) and shipping to a resource laboratory, as well as the fees for the laboratory analyses. Specimen collection costs include the additional salaries for survey field staff and transportation for the personnel who collect and process the specimens, which we have not attempted to estimate because of the substantial variability in these expenses across countries. We have estimated the supply needs at approximately $20 per survey participant (see **Section 6.4.1 and Appendix 3)** and the laboratory fees at $10 per assay (see **Section 6.6.3**). Thus, for a survey with 4000 participants (2000 PSC and 2000 WRA) approximately $80,000 would be required for supply costs, approximately $25,000 would be required for shipping specimens, and approximately $880,000 would be required to complete 4000 biomarker assays of 11 biomarkers in duplicate at a cost of $10 per assay (4000 X 2 X 11 X $10 = $880,000). To support 14 surveys per year, a total of nearly $14 million would be required (14 X (80,000 + 25,000 + 880,000) = 13,790,000). If half of this sum were provided from the initiative's central funds, and half generated by the countries themselves, the central funds would have to provide approximately $7 million per year to cover these expenses.

To provide technical assistance to countries, support is needed for technical specialists in survey design, field implementation and data processing and analysis. We estimate that each year three new countries will require part-time technical assistance over a period of two years each. This includes salaries for the technical experts plus international travel and per diem. These needs will escalate over the first few years of the project, reaching peak requirements of two full-time salaries and related travel cost, for a

total cost of $360,000 per year, when a total of six countries will be receiving assistance. An additional $40,000 per year is budgeted to support continued maintenance and updating of the OpeNGlobal web site.

The financial needs to improve and expand the VMNIS data repository and the IZiNCG clearinghouse on MN status assessment surveys are approximately $185,000 in initial costs and $122,000 in recurring costs, as described in **Sections 6.9.1 and 6.9.2**. Additionally, $125,000 per year is budgeted to complete data analyses to provide updates on the current situation and trends in the prevalence of deficiency of specific MNs.

For countries that want to complete analyses in national laboratories or develop the capacity to complete future analyses in national clinical laboratories, we recommend offering training opportunities through the initiative's central resource laboratories. This would require travel support and per diems for the national personnel participating in the training activities and for the trainers based in the central laboratories. This has been budgeted at $128,000 per year to cover a total of 16 trainees and four trainers.

To provide training in data analysis, interpretation and utilization for policy formulation and program planning and evaluation, a training curriculum needs to be developed and delivered regionally or nationally. Additional materials for on-line training would also be useful. Support for the curriculum development is estimated at $50,000 and is shown under consultant fees. Data analysis training could be accomplished through regional or national workshops of one week duration at a cost of approximately $25,000 per workshop to cover support for a workshop facilitator, local co-organizer, meeting space and travel and per diem for up to 10 participants. If two workshops are scheduled per year, the total cost would be $50,000 annually.

Finally, funds are budgeted to hire short-term consultants to complete specific tasks, including development of laboratory procedures manuals, conducting a review of existing laboratory training curricula, development of a data analysis workshop curriculum, and others.

8.3 Central funding mechanism to support increased data generation and utilization

To expand the generation and utilization of data on population MN status and to encourage countries to include MN status assessment in their national surveys, we propose establishing a central fund that countries could draw on to help defray the costs of specimen collection and laboratory analysis of MN status biomarkers. This fund would be administered by the initiative's management team, which is described below (**Section 8.4**), with oversight by members of the PPT and external auditing. The funds could be used to support some proportion of the cost of the specimen collection and laboratory analyses, with the remainder to be provided by the national government (either directly from government sources or government-leveraged commitments from bilateral donors or bank loans), as a sign of national commitment that the data generated will be used for program planning and management. For the purposes of budgeting, we estimated a 50% cost-sharing by the central fund and national governments, or a total expenditure of $7 million annually from the central fund. If, instead, the central fund were to provide 80% of the estimated total costs, this would increase the annual outlay from the central fund by approximately $4.2 million.

We further propose several conditions that countries should accept to be able to utilize these funds. First, the countries should agree to complete all laboratory analyses in the central resource laboratories

identified and supported by the project or in a national laboratory that is pre-certified by the project's designated quality assurance systems. This condition will guarantee the reliability of any laboratory analyses supported by the central fund. Secondly, the countries should commit to a policy of open access to data, whereby the final results of the analyses become publicly available after an appropriate period of time required for data analysis, interpretation and publication by national authorities. Other conditions may be developed by the PPT following further discussion with stakeholders.

At the early stages of the initiative, a governance plan for the central funds will need to be developed. We propose that representatives of the PPT and the project management team should draft the governance plan, which should be ratified by the full PPT.

8.4 Project management

The core project management team should consist of a full-time project manager and a part-time administrative assistant initially, both under the supervision of the Executive Director of the MN Forum and supported by the MN Forum's administrative systems. Individual sub-projects could be implemented through partner agencies, depending on the willingness and capacity of those agencies to assume responsibility for specific components of the initiative, or by existing or newly hired personnel within the MN Forum and the project management team or external consultants, as needed. The proposed lines of authority for the management team and related activities are shown in **Figure 5**. The primary responsibilities of the project management team are listed above (**Section 7.1**). The project director should be a senior public health or nutrition specialist with at least 10 years of international professional experience in survey design and implementation, laboratory science, or both, in addition to experience with project management.

9 Tracking progress

The project management team should take responsibility for tracking progress of each of the components of the initiative, including creation and convening of the PPT and TAC, grant proposal preparation, advocacy and communication, MN status survey design and implementation, sample collection and laboratory analyses, data archiving and analysis, and utilization of information for program design and utilization. Specific indicators will be developed in each domain for systematic tracking. Examples of such indicators are displayed in **Table 8**.

Of course, the most important results of this effort will be the development of new policies and new or modified intervention programs based on the data generated. These outcomes will be tracked by conducting interviews with key decision makers and representatives of development partners in countries that have conducted recent population assessment surveys.

References

Allen, L. H., J. W. Miller, L. de Groot, I. H. Rosenberg, A. D. Smith, H. Refsum, and D. J. Raiten. 2018. 'Biomarkers of Nutrition for Development (BOND): Vitamin B-12 Review', *Journal of Nutrition*, 148: 1995S-2027S.

Arroyave, G., and L. A. Mejia. 2010. 'Five decades of vitamin A studies in the region of Central America and Panama', *Food and Nutrition Bulletin*, 31: 118-29.

Arsenault, J. E., R. J. Hijmans, and K. H. Brown. 2015. 'Improving nutrition security through agriculture: an analytical framework based on national food balance sheets to estimate nutritional adequacy of food supplies', *Food Security*, 7: 693-707.

ATCC. 2020. 'Certified reference maternials (CRMS)', ATCC, Accessed Aug 2. https://www.lgcstandards-atcc.org/Standards/Standards_Programs/Certified_Reference_Materials_CRMs.aspx.

Bailey, L. B., and D. B. Hausman. 2018. 'Folate status in women of reproductive age as basis of neural tube defect risk assessment', *Annals of the New York Academy of Sciences*, 1414: 82-95.

Bailey, L. B., P. J. Stover, H. McNulty, M. F. Fenech, J. F. Gregory, J. L. Mills, C. M. Pfeiffer, Z. Fazili, M. Zhang, P. M. Ueland, A. M. Molloy, M. A. Caudill, B. Shane, R. J. Berry, R. L. Bailey, D. B. Hausman, R. Raghavan, and D. J. Raiten. 2015. 'Biomarkers of Nutrition for Development-Folate Review', *Journal of Nutrition*, 145: 1636-80.

Bailey, R. L., R. A. Durazo-Arvizu, R. Carmel, R. Green, C. M. Pfeiffer, C. T. Sempos, A. Carriquiry, and E. A. Yetley. 2013. 'Modeling a methylmalonic acid-derived change point for serum vitamin B-12 for adults in NHANES', *American Journal of Clinical Nutrition*, 98: 460-7.

Barennes, H., K. Sengkhamyong, J. P. Rene, and M. Phimmasane. 2015. 'Beriberi (thiamine deficiency) and high infant mortality in northern Laos', *PLoS Negl Trop Dis*, 9: e0003581.

Bath, Sarah C. 2019. 'The effect of iodine deficiency during pregnancy on child development', *Proceedings of the Nutrition Society*, 78: 150-60.

Binkley, N., and C. T. Sempos. 2014. 'Standardizing vitamin D assays: the way forward', *Journal of Bone and Mineral Research*, 29: 1709-14.

Black, M. M. 2008. 'Effects of vitamin B12 and folate deficiency on brain development in children', *Food and Nutrition Bulletin*, 29: S126-31.

Black, R. E., C. G. Victora, S. P. Walker, Z. A. Bhutta, P. Christian, M. de Onis, M. Ezzati, S. Grantham-McGregor, J. Katz, R. Martorell, R. Uauy, Maternal, and Group Child Nutrition Study. 2013. 'Maternal and child undernutrition and overweight in low-income and middle-income countries', *Lancet*, 382: 427-51.

Blencowe, H., V. Kancherla, S. Moorthie, M. W. Darlison, and B. Modell. 2018. 'Estimates of global and regional prevalence of neural tube defects for 2015: a systematic analysis', *Annals of the New York Academy of Sciences*, 1414: 31-46.

BRINDA. 2017. 'Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia', Accessed Aug 26.

Brindle, E., L. Lillis, R. Barney, S. Y. Hess, K. R. Wessells, C. T. Ouedraogo, S. Stinca, M. Kalnoky, R. Peck, A. Tyler, C. Lyman, and D. S. Boyle. 2017. 'Simultaneous assessment of iodine, iron, vitamin A, malarial antigenemia, and inflammation status biomarkers via a multiplex immunoassay method on a population of pregnant women from Niger', *PLoS One*, 12: e0185868.

Brindle, E., D. Stevens, C. Crudder, C. E. Levin, D. Garrett, C. Lyman, and D. S. Boyle. 2014. 'A multiplex immunoassay method for simultaneous quantification of iron, vitamin A and inflammation status markers', *PLoS One*, 9: e115164.

Brown, K. H., R. Engle-Stone, J. Kagin, E. Rettig, and S. A. Vosti. 2015. 'Use of Optimization Modeling for Selecting National Micronutrient Intervention Strategies: An Example Based on Potential

Programs for Control of Vitamin A Deficiency in Cameroon', *Food and Nutrition Bulletin*, 36: S141-8.
Brown, K. H., J. M. Peerson, S. K. Baker, and S. Y. Hess. 2009. 'Preventive zinc supplementation among infants, preschoolers, and older prepubertal children', *Food and Nutrition Bulletin*, 30: S12-40.
Brown, K. H., J. A. Rivera, Z. Bhutta, R. S. Gibson, J. C. King, B. Lönnerdal, M. T. Ruel, B. Sandström, E. Wasantwisut, and C. Hotz. 2004. 'International Zinc Nutrition Consultative Group (IZiNCG) technical document #1. Assessment of the risk of zinc deficiency in populations and options for its control', *Food and Nutrition Bulletin*, 25: S99-203.
Bubela, T., J. Guebert, and A. Mishra. 2015. 'Use and misuse of material transfer agreements: lessons in proportionality from research, repositories, and litigation', *PLoS Biol*, 13: e1002060.
Cashman, K. D., K. G. Dowling, Z. Škrabáková, M. Gonzalez-Gross, J. Valtueña, S. De Henauw, L. Moreno, C. T. Damsgaard, K. F. Michaelsen, C. Mølgaard, R. Jorde, G. Grimnes, G. Moschonis, C. Mavrogianni, Y. Manios, M. Thamm, G. B. Mensink, M. Rabenberg, M. A. Busch, L. Cox, S. Meadows, G. Goldberg, A. Prentice, J. M. Dekker, G. Nijpels, S. Pilz, K. M. Swart, N. M. van Schoor, P. Lips, G. Eiriksdottir, V. Gudnason, M. F. Cotch, S. Koskinen, C. Lamberg-Allardt, R. A. Durazo-Arvizu, C. T. Sempos, and M. Kiely. 2016. 'Vitamin D deficiency in Europe: pandemic?', *American Journal of Clinical Nutrition*, 103: 1033-44.
CDC Foundation. 2020. 'Capacity building for folate measurement', Accessed Aug 26.
Centers for Disease Control and Prevention. 1987. "Recommendations for prevention of HIV transmission in health-care settings." In *MMWR*, 36 (2S).
———. 2017. 'VDSCP: Vitamin D standardization - certification program', Centers for Disease Control and Prevention, Accessed Aug 2. https://www.cdc.gov/labstandards/vdscp.html.
———. 2018. "Folate microbiologic assay. Training manual." In. Atlanta: Department of Health & Human Services USA, Centers for Disease Control and Prevention.
———. 2019. 'Performance verification program for serum micronutrients', Accessed Aug 26. https://www.cdc.gov/nceh/dls/nbb_micronutrient_performance.html.
———. 2020. 'EQUIP: Ensuring the quality of urinary iodine procedures', Accessed Oct 9. https://www.cdc.gov/labstandards/equip.html.
Centers for Disease Control and Prevention, World Health Organization, and UNICEF. 2020. "Micronutrient survey manual." In. Geneva: World Health Organization.
Cervo, S., P. De Paoli, E. Mestroni, T. Perin, L. Escoffier, V. Canzonieri, and A. Steffan. 2016. 'Drafting biological material transfer agreement: a ready-to-sign model for biobanks and biorepositories', *International Journal of Biological Markers*, 31: e211-7.
Clinical and Laboratory Standards Institute (CLSI). 1998. "Determination of serum iron, total iron-binding capacity, and percent transferrin saturation; Approved standard." In. Wayne, PA: Clinical and Laboratory Standards Institute.
Cooper, G. 2008. "Basic lessons in laboratory quality control." In.: Bio-Rad Laboratories, Inc.
Countdown to 2030. 2020. 'Regional Initiative to strengthen the analytical capacity and evidence for nutrition in West Africa. Advanced analytical workshop', Accessed Aug 26. https://www.countdown2030.org/west-and-central-africa/countdown-to-2030-regional-initiative-to-strengthen-the-analytical-capacity.
Daru, J., J. Zamora, B. M. Fernandez-Felix, J. Vogel, O. T. Oladapo, N. Morisaki, O. Tuncalp, M. R. Torloni, S. Mittal, K. Jayaratne, P. Lumbiganon, G. Togoobaatar, S. Thangaratinam, and K. S. Khan. 2018. 'Risk of maternal mortality in women with severe anaemia during pregnancy and post partum: a multilevel analysis', *Lancet Glob Health*, 6: e548-e54.
de Benoist, B. 2008. 'Conclusions of a WHO Technical Consultation on folate and vitamin B12 deficiencies', *Food and Nutrition Bulletin*, 29: S238-44.

Durazo-Arvizu, R. A., L. Tian, S. P. J. Brooks, K. Sarafin, K. D. Cashman, M. Kiely, J. Merkel, G. L. Myers, P. M. Coates, and C. T. Sempos. 2017. 'The Vitamin D Standardization Program (VDSP) Manual for Retrospective Laboratory Standardization of Serum 25-Hydroxyvitamin D Data', *Journal of AOAC International*, 100: 1234-43.

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA), D Turck, JL Bresson, B Burlingame, T Dean, S J Fairweather-Tait, M Heinonen, KI Hirsch-Ernst, I Mangelsdorf, H. J. McArdle, A Naska, G Nowicka, K Pentieva, Y Sanz, A Siani, A Sjödin, M Stern, D Tomé, H Van Loveren, M Vinceti, P Willatts, C Lamberg-Allardt, H Przyrembel, I Tetens, JG Rakic, S Ioannidou, A de Sesmaisons-Lecarré, Forss AC, and M Neuhäuser-Berthold. 2016. 'Dietary reference values for thiamin', *EFSA Journal*, 14: 4653.

Figueiredo, ACMG, I. S. Gomes-Filho, R. B. Silva, P. P. S. Pereira, Fafd Mata, A. O. Lyrio, E. S. Souza, S. S. Cruz, and M. G. Pereira. 2018. 'Maternal Anemia and Low Birth Weight: A Systematic Review and Meta-Analysis', *Nutrients*, 10.

Finch, C. A., V. Bellotti, S. Stray, D. A. Lipschitz, J. D. Cook, M. J. Pippard, and H. A. Huebers. 1986. 'Plasma ferritin determination as a diagnostic tool', *Western Journal of Medicine*, 145: 657-63.

Food and Agriculture Organization of the United Nations. 2008. 'What are policy briefs? Writing effective reports', Food and Agriculture Organization of the United Nations. http://www.fao.org/3/i2195e/i2195e03.pdf.

Gibson, R. S., and E.L. Ferguson. 2008. "An interactive 24-hour recall for assessing the adequacy of iron and zinc intakes in developing countries." In *HarvestPlus Technical Mongraph 8*. Washington, DC and Cali: International Food and Policy Research Institute (IFPRI) and International Center for Tropical Agriculture.

Global Nutrition Report. 2020. 'About the Global Nutrition Report'. https://globalnutritionreport.org/about/.

Green, R. 2011. 'Indicators for assessing folate and vitamin B-12 status and for monitoring the efficacy of intervention strategies', *The American Journal of Clinical Nutrition*, 94: 666S-72S.

Greig, A. J., A. J. Patterson, C. E. Collins, and K. A. Chalmers. 2013. 'Iron deficiency, cognition, mental health and fatigue in women of childbearing age: a systematic review', *J Nutr Sci*, 2: e14.

Hall, A. G., J. C. King, and C. M. Mc Donald. submitted. 'Comparison of serum, plasma, and liver zinc measures by Atomic Absorbance Spectrometry (AAS), Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES), and ICP Mass Spectrometry (ICP-MS) in diverse laboratory settings'.

Haskell, M. J., J. D. Ribaya-Mercado, and the Vitamin A Tracker Task Force. 2005. "Handbook on Vitamin A Tracer Dilution Methods to Assess Status and Evaluate Intervention Programs." In *HarvestPlus Technical Monograph 5*. Washington DC and Cali: International Food Policy Research Institute (IFPRI) and International Center for Tropical Agriculture (CIAT).

Hess, S. Y., J. M. Peerson, J. C. King, and K. H. Brown. 2007. 'Use of serum zinc concentration as an indicator of population zinc status', *Food and Nutrition Bulletin*, 28: S403-29.

Hotz, C., J. M. Peerson, and K. H. Brown. 2003. 'Suggested lower cutoffs of serum zinc concentrations for assessing zinc status: reanalysis of the second National Health and Nutrition Examination Survey data (1976-1980)', *American Journal of Clinical Nutrition*, 78: 756-64.

Imdad, A., E. Mayo-Wilson, K. Herzer, and Z. A. Bhutta. 2017. 'Vitamin A supplementation for preventing morbidity and mortality in children from six months to five years of age', *Cochrane Database Syst Rev*, 3: CD008524.

Institute of Health Metrics and Evaluation. 2019. 'The Global Burden of Disease', Institute of Health Metrics and Evaluation, Accessed 18 October. http://www.healthdata.org/gbd.

Institute of Medicine. 2006. *Dietary Reference Intakes: The essential guide to nutrient requirements* (The National Academic Press: Washington, DC).

———. 2015. *Sharing clinical trial data: Maximizing benefits, minimizing risk* (The National Academies Press: Washington, DC).

Institute of Medicine Committee to Review Dietary Reference Intakes for Vitamin D and Calcium. 2011. 'The National Academies Collection: Reports funded by National Institutes of Health.' in A. C. Ross, C. L. Taylor, A. L. Yaktine and H. B. Del Valle (eds.), *Dietary Reference Intakes for Calcium and Vitamin D* (National Academies Press (US): Washington (DC)).

Institute of Medicine Standing Committee on the Scientific Evaluation of Dietary Reference Intakes and its Panel on Folate Other B. Vitamins and Choline. 1998. 'The National Academies Collection: Reports funded by National Institutes of Health.' in, *Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B(6), Folate, Vitamin B(12), Pantothenic Acid, Biotin, and Choline* (National Academies Press (US): Washington (DC)).

International Atomic Energy Agency. 2020. "How the retinol isotope dilution test can help assess vitamin A status in public health programmes. IAEA Factsheets." In. Vienna: International Atomic Energy Agency.

International Zinc Nutrition Consultative Group. 2020. 'Assessment of zinc status'. www.izincg.org.

International Zinc Nutrition Consultative Group (IZiNCG), KH Brown, JA Rivera, Z Bhutta, RS Gibson, JC King, B Lönnerdal, MT Ruel, B Sandtröm, E Wasantwisut, and C Hotz. 2004. 'International Zinc Nutrition Consultative Group (IZiNCG) technical document #1. Assessment of the risk of zinc deficiency in populations and options for its control', *Food and Nutrition Bulletin*, 25(1 Suppl 2): S99-203.

Iodine Global Network. 2020. 'Global iodine nutrition scorecard and map', Iodine Global Network, Accessed Aug 2. https://www.ign.org/scorecard.htm.

Karakochuk, C. D., A. M. Henderson, K. L. I. Samson, A. M. Aljaadi, A. M. Devlin, E. Becquey, J. P. Wirth, and F. Rohner. 2018. 'Comparison of a New Multiplex Immunoassay for Measurement of Ferritin, Soluble Transferrin Receptor, Retinol-Binding Protein, C-Reactive Protein and α^1-Acid-glycoprotein Concentrations against a Widely-Used s-ELISA Method', *Diagnostics (Basel)*, 8.

Karakochuk, C. D., S. Y. Hess, D. Moorthy, S. Namaste, M. E. Parker, A. I. Rappaport, R. Wegmüller, and O. Dary. 2019. 'Measurement and interpretation of hemoglobin concentration in clinical and field settings: a narrative review', *Annals of the New York Academy of Sciences*, 1450: 126-46.

Kavishe, F , I Ndombi, M Irie, MC Desilets, and B Rakotomalala. 2019. "Iodine deficiency in Madagascar: time for action." In *IDD Newsletter*.

King, J. C., K. H. Brown, R. S. Gibson, N. F. Krebs, N. M. Lowe, J. H. Siekmann, and D. J. Raiten. 2016. 'Biomarkers of Nutrition for Development (BOND)-Zinc Review', *Journal of Nutrition*.

König, F., M. Andersson, K. Hotz, I. Aeberli, and M.B. Zimmermann. 2011. 'Ten Repeat Collections for Urinary Iodine from Spot Samples or 24-Hour Samples Are Needed to Reliably Estimate Individual Iodine Status in Women', *The Journal of Nutrition*, 141: 2049-54.

Labuschaigne, M., A. Dhai, S. Mahomed, K. Behrens, A. Nienaber, K. Moodley, P. Cleaton-Jones, A. Olckers, N. Maepa, and C. Penny. 2019. 'Protecting participants in health research: The South African Material Transfer Agreement', *S Afr Med J*, 109: 353-56.

Larson, L. M., S. M. Namaste, A. M. Williams, R. Engle-Stone, O. Y. Addo, P. S. Suchdev, J. P. Wirth, V. Temple, M. Serdula, and C. A. Northrop-Clewes. 2017. 'Adjusting retinol-binding protein concentrations for inflammation: Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) project', *American Journal of Clinical Nutrition*.

Lynch, S., C. M. Pfeiffer, M. K. Georgieff, G. Brittenham, S. Fairweather-Tait, R. F. Hurrell, H. J. McArdle, and D. J. Raiten. 2018. 'Biomarkers of Nutrition for Development (BOND)-Iron Review', *Journal of Nutrition*, 148: 1001S-67S.

Makris, K., C. Sempos, and E. Cavalier. 2020. 'The measurement of vitamin D metabolites: part I-metabolism of vitamin D and the measurement of 25-hydroxyvitamin D', *Hormones (Athens)*, 19: 81-96.

Manger, M. S., and C. M. McDonald. 2020. 'Barriers and enablers of including micronutrient biomarkers in national surveys and surveillance systems. Unpublished', Accessed Aug 27. https://www.izincg.org/technical-documents

McDonald, C. M., P. S. Suchdev, N. F. Krebs, S. Y. Hess, K. R. Wessells, S. Ismaily, S. Rahman, F. T. Wieringa, A. M. Williams, K. H. Brown, and J. C. King. 2020. 'Adjusting plasma or serum zinc concentrations for inflammation: Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) project', *American Journal of Clinical Nutrition*, 111: 927-37.

Mei, Z., S. M. Namaste, M. Serdula, P. S. Suchdev, F. Rohner, R. Flores-Ayala, O. Y. Addo, and D. J. Raiten. 2017. 'Adjusting total body iron for inflammation: Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) project', *American Journal of Clinical Nutrition*.

Miller, W. G., H. Schimmel, R. Rej, N. Greenberg, F. Ceriotti, C. Burns, J. R. Budd, C. Weykamp, V. Delatour, G. Nilsson, F. MacKenzie, M. Panteghini, T. Keller, J. E. Camara, I. Zegers, and H. W. Vesper. 2018. 'IFCC Working Group Recommendations for Assessing Commutability Part 1: General Experimental Design', *Clinical Chemistry*, 64: 447-54.

Mimouni-Bloch, A., H. Goldberg-Stern, R. Strausberg, A. Brezner, E. Heyman, D. Inbar, S. Kivity, A. Zvulunov, I. Sztarkier, R. Fogelman, and A. Fattal-Valevski. 2014. 'Thiamine deficiency in infancy: long-term follow-up', *Pediatric Neurology*, 51: 311-6.

Molloy, A. M., F. Pangilinan, J. L. Mills, B. Shane, M. B. O'Neill, D. M. McGaughey, A. Velkova, H. O. Abaan, P. M. Ueland, H. McNulty, M. Ward, J. J. Strain, C. Cunningham, M. Casey, C. D. Cropp, Y. Kim, J. E. Bailey-Wilson, A. F. Wilson, and L. C. Brody. 2016. 'A Common Polymorphism in HIBCH Influences Methylmalonic Acid Concentrations in Blood Independently of Cobalamin', *American Journal of Human Genetics*, 98: 869-82.

Namaste, S. M., G. J. Aaron, R. Varadhan, J. M. Peerson, P. S. Suchdev, and Brinda Working Group. 2017. 'Methodologic approach for the Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) project', *American Journal of Clinical Nutrition*.

Namaste, S. M., F. Rohner, J. Huang, N. L. Bhushan, R. Flores-Ayala, R. Kupka, Z. Mei, R. Rawat, A. M. Williams, D. J. Raiten, C. A. Northrop-Clewes, and P. S. Suchdev. 2017. 'Adjusting ferritin concentrations for inflammation: Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) project', *American Journal of Clinical Nutrition*, 106(Suppl 1): 359S-71S.

National Institutes of Health. 2018. 'Vitamin D standardization program (VDSP)', Accessed Aug 26. https://ods.od.nih.gov/Research/vdsp.aspx.

Neufeld, L. M., L. M. Larson, A. Kurpad, S. Mburu, R. Martorell, and K. H. Brown. 2019. 'Hemoglobin concentration and anemia diagnosis in venous and capillary blood: biological basis and policy implications', *Annals of the New York Academy of Sciences*, 1450: 172-89.

NIST. 2010. 'SRM definitions', National Institute of Standards and Technology, Accessed Aug 2. https://www.nist.gov/srm/srm-definitions.

Nutrition International. 2020. 'Nutrition Folate Task Team', Accessed Aug 26. https://www.nutritionintl.org/what-we-do/nteam/folate-task-team/.

O'Callaghan, K.M., and D.E. Roth. 2020. 'Standardization of laboratory practices and reporting of biomarker data in clinical nutrition research', *The American Journal of Clinical Nutrition*, 112: 453S-57S.

OpeN-Global. 2019. 'OpeN-Global', Accessed Aug 27. https://open-global.kcl.ac.uk/#.

Paudyal, N., S. Chitekwe, S. Rijal, K. Parajuli, C. Pandav, M. Maharjan, R. Houston, and J. Gorstein. 2020. 'The evolution, progress, and future direction of Nepal's universal salt iodization program', *Maternal & Child Nutrition*, n/a: e12945.

Pena-Rosas, J. P., L. M. De-Regil, M. N. Garcia-Casal, and T. Dowswell. 2015. 'Daily oral iron supplementation during pregnancy', *Cochrane Database Syst Rev*: CD004736.

Qureshi, U. A., A. Sami, U. Altaf, K. Ahmad, J. Iqbal, N. A. Wani, Z. Mir, and I. Ali. 2016. 'Thiamine responsive acute life threatening metabolic acidosis in exclusively breast-fed infants', *Nutrition*, 32: 213-6.

Raiten, D. J., S. Namaste, B. Brabin, G. Combs, Jr., M. R. L'Abbe, E. Wasantwisut, and I. Darnton-Hill. 2011. 'Executive summary--Biomarkers of Nutrition for Development: Building a Consensus', *American Journal of Clinical Nutrition*, 94: 633S-50S.

Raiten, D. J., F. A. Sakr Ashour, A. C. Ross, S. N. Meydani, H. D. Dawson, C. B. Stephensen, B. J. Brabin, P. S. Suchdev, B. van Ommen, and Inspire Consultative Group. 2015. 'Inflammation and Nutritional Science for Programs/Policies and Interpretation of Research Evidence (INSPIRE)', *Journal of Nutrition*, 145: 1039S-108S.

Rappaport, A I , C D Karakochuk, S Y Hess, S M L Namaste, O Dary, L M Neufeld, M E Parker, S Newton, M K Serdula, R Wegmuller, Jr R D Whitehead, and D Moorthy. 2020. 'Variability in hemoglobin concentration by measurement tool and blood source: an analysis from seven countries', *Journal of Clinical Pathology*: in press.

Richards, P., and Colleagues. 2020. *Iodophor: Tamed iodine: The status of iodine in Tasmania* (Clifford Craig Foundation: Launceston, Tasmania).

Rohner, F., M. Zimmermann, P. Jooste, C. Pandav, K. Caldwell, R. Raghavan, and D. J. Raiten. 2014. 'Biomarkers of nutrition for development--iodine review', *Journal of Nutrition*, 144: 1322S-42S.

Roth, D. E., S. A. Abrams, J. Aloia, G. Bergeron, M. W. Bourassa, K. H. Brown, M. S. Calvo, K. D. Cashman, G. Combs, L. M. De-Regil, M. E. Jefferds, K. S. Jones, H. Kapner, A. R. Martineau, L. M. Neufeld, R. L. Schleicher, T. D. Thacher, and S. J. Whiting. 2018. 'Global prevalence and disease burden of vitamin D deficiency: a roadmap for action in low- and middle-income countries', *Annals of the New York Academy of Sciences*, 1430: 44-79.

Selhub, J., P. F. Jacques, G. Dallal, S. Choumenkovitch, and G. Rogers. 2008. 'The use of blood concentrations of vitamins and their respective functional indicators to define folate and vitamin B12 status', *Food and Nutrition Bulletin*, 29: S67-73.

Staunton, C., S. Slokenberga, and D. Mascalzoni. 2019. 'The GDPR and the research exemption: considerations on the necessary safeguards for research biobanks', *European Journal of Human Genetics*, 27: 1159-67.

Stevens, G. A., J. E. Bennett, Q. Hennocq, Y. Lu, L. M. De-Regil, L. Rogers, G. Danaei, G. Li, R. A. White, S. R. Flaxman, S. P. Oehrle, M. M. Finucane, R. Guerrero, Z. A. Bhutta, A. Then-Paulino, W. Fawzi, R. E. Black, and M. Ezzati. 2015. 'Trends and mortality effects of vitamin A deficiency in children in 138 low-income and middle-income countries between 1991 and 2013: a pooled analysis of population-based surveys', *Lancet Glob Health*, 3: e528-36.

Stevens, G. A., M. M. Finucane, L. M. De-Regil, C. J. Paciorek, S. R. Flaxman, F. Branca, J. P. Pena-Rosas, Z. A. Bhutta, M. Ezzati, and Group Nutrition Impact Model Study. 2013. 'Global, regional, and national trends in haemoglobin concentration and prevalence of total and severe anaemia in children and pregnant and non-pregnant women for 1995-2011: a systematic analysis of population-representative data', *Lancet Glob Health*, 1: e16-25.

Suchdev, P. S., S. M. Namaste, G. J. Aaron, D. J. Raiten, K. H. Brown, R. Flores-Ayala, and Brinda Working Group. 2016. 'Overview of the Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) Project', *Adv Nutr*, 7: 349-56.

Taichman, D. B., P. Sahni, A. Pinborg, L. Peiperl, C. Laine, A. James, S. T. Hong, A. Haileamlak, L. Gollogly, F. Godlee, F. A. Frizelle, F. Florenzano, J. M. Drazen, H. Bauchner, C. Baethge, and J. Backus. 2017. 'Data Sharing Statements for Clinical Trials - A Requirement of the International Committee of Medical Journal Editors', *New England Journal of Medicine*, 376: 2277-79.

Tanumihardjo, S. A., R. M. Russell, C. B. Stephensen, B. M. Gannon, N. E. Craft, M. J. Haskell, G. Lietz, K. Schulze, and D. J. Raiten. 2016. 'Biomarkers of Nutrition for Development (BOND)-Vitamin A Review', *Journal of Nutrition*, 146: 1816S-48S.

The Republic of the Union of Myanmar Ministry of Health, and United Nations Children's Fund. 2014. "Study on cause of under-five mortality." In.

Tinker, Sarah C., Heather C. Hamner, Yan Ping Qi, and Krista S. Crider. 2015. 'U.S. women of childbearing age who are at possible increased risk of a neural tube defect-affected pregnancy due to suboptimal red blood cell folate concentrations, National Health and Nutrition Examination Survey 2007 to 2012', *Birth Defects Research Part A: Clinical and Molecular Teratology*, 103: 517-26.

UNICEF. 2020. 'Multiple Indicator Cluster Surveys (MICS)', Accessed Aug 26. http://mics.unicef.org/.

US Agency for International Development. 2020a. 'DHS model questionnaires', The DHS Program Accessed Aug 26. https://dhsprogram.com/What-We-Do/Survey-Types/DHS-Questionnaires.cfm.

———. 2020b. 'DHS Survey design: Micronutrient status', Accessed Oct 9. https://dhsprogram.com/pubs/pdf/DHSM20/DHSM20.pdf.

———. 2020c. 'Publication summary. Tabulation plan for DHS final report', The DHS Program Accessed Aug 26. https://www.dhsprogram.com/publications/publication-dhsm6-dhs-questionnaires-and-manuals.cfm.

———. 2020d. 'What we do', The DHS Program, Accessed Oct 9. https://dhsprogram.com/what-we-do/survey-search.cfm.

US Department of Health and Human Services, and National Institute of Health. 2020. 'How Can Covered Entities Use and Disclose Protected Health Information for Research and Comply with the Privacy Rule?', national Institute of Health, Accessed April 30. https://privacyruleandresearch.nih.gov/pr_08.asp.

Vosti, S. A., J. Kagin, R. Engle-Stone, and K. H. Brown. 2015. 'An Economic Optimization Model for Improving the Efficiency of Vitamin A Interventions: An Application to Young Children in Cameroon', *Food and Nutrition Bulletin*, 36: S193-207.

Vosti, S. A., J. Kagin, R. Engle-Stone, H. Luo, A. Tarini, A. Clermont, J. G. Assiene, M. Nankap, and K. H. Brown. 2020. 'Strategies to achieve adequate vitamin A intake for young children: options for Cameroon', *Annals of the New York Academy of Sciences*, 1465: 161-80.

Walters, G. O., F. M. Miller, and M. Worwood. 1973. 'Serum ferritin concentration and iron stores in normal subjects', *Journal of Clinical Pathology*, 26: 770-2.

Wessells, K. R., and K. H. Brown. 2012. 'Estimating the global prevalence of zinc deficiency: results based on zinc availability in national food supplies and the prevalence of stunting', *PLoS One*, 7: e50568. doi:10.1371/journal.pone.0050568.

Wessells, K. R., J. C. King, and K. H. Brown. 2014. 'Development of a plasma zinc concentration cutoff to identify individuals with severe zinc deficiency based on results from adults undergoing experimental severe dietary zinc restriction and individuals with acrodermatitis enteropathica', *Journal of Nutrition*.

Whitfield, K. C., M. W. Bourassa, B. Adamolekun, G. Bergeron, L. Bettendorff, K. H. Brown, L. Cox, A. Fattal-Valevski, P. R. Fischer, E. L. Frank, L. Hiffler, L. M. Hlaing, M. E. Jefferds, H. Kapner, S. Kounnavong, M. P. S. Mousavi, D. E. Roth, M. N. Tsaloglou, F. Wieringa, and G. F. Combs, Jr.

2018. 'Thiamine deficiency disorders: diagnosis, prevalence, and a roadmap for global control programs', *Annals of the New York Academy of Sciences*, 1430: 3-43.
Whitton, T., J. Nielsen, and D. Nicol. 2019. 'Terms of Engagement: Transfer of Biological Materials for Research in Australia', *J Law Med*, 27: 338-54.
Willis, G. B., and K. Miller. 2011. 'Cross-Cultural Cognitive Interviewing: Seeking Comparability and Enhancing Understanding', *Field Methods*, 23: 331-41.
Wirth, J. P., N. Petry, S. A. Tanumihardjo, L. M. Rogers, E. McLean, A. Greig, G. S. Garrett, R. D. Klemm, and F. Rohner. 2017. 'Vitamin A Supplementation Programs and Country-Level Evidence of Vitamin A Deficiency', *Nutrients*, 9.
World Health Organization. 1996. "Indicators for assessing vitamin A deficiency and their application in monitoring and evaluating intervention programmes." In. WHO, Geneva; WHO/NUT/96.10.
———. 2011a. "Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity." In *Vitamin and Mineral Nutrition Information System*. Geneva: World Health Organization.
———. 2011b. "Laboratory quality management system: handbook." In. Geneva: World Health Organization.
———. 2011c. "Serum ferritin concentrations for the assessment of iron status and iron deficiency in populations." In *Vitamin and Mineral Nutrition Information System*, WHO/NMH/NHD/MNM/11.2. Geneva: World Health Organization.
———. 2011d. "Serum retinol concentrations for determining the prevalence of vitamin A deficiency in populations." In *Vitamin and Mineral Nutrition Information System*. Geneva: World Health Organization.
———. 2013. "Urinary iodine concentrations for determining iodine status in populations." In, edited by Vitamin and Mineral Nutrition Information System, WHO/NMH/NHD/EPG/13.1. Geneva: World Health Organization.
———. 2014a. "C-reactive protein concentration as a marker of inflammation or infection for interpreting biomarkers of micronutrient status." In, edited by Vitamin and Mineral Nutrition Information System, WHO/NMH/NHD/EPG/14.7. Geneva: World Health Organization.
———. 2014b. "Serum transferrin receptor levels for the assessment of iron status and iron deficiency in populations." In *Vitamin and Mineral Nutrition Information System*, WHO/NMH/NHD/EPG/14.6. Geneva: World Health Organization.
———. 2015. "Serum and red blood cell folate concentrations for assessing folate status in populations." In *Vitamin and Mineral Nutrition Information System*, WHO/NMH/NHD/EPG/15.01. Geneva: World Health Organization.
———. 2017a. 'Anaemia in children <5 yrears. Estimates by WHO region', World Health Organization, Accessed Aug 26. https://apps.who.int/gho/data/view.main.ANEMIACHILDRENREGv.
———. 2017b. 'Prevalence of aneamie in women', Switzerland, Accessed Aug 26. https://apps.who.int/gho/data/node.main.ANAEMIAWOMEN?lang=en.
———. 2020. "WHO guideline on use of ferritin concentrations to assess iron status in individuals and populations." In. Geneva: World Health Organization.
World Health Organization, and United States Centers for Disease Control and Prevention. 2014. "Methodological approaches to estimating global and regional prevalence of vitamin and mineral deficiencies. Report on the joint WHO/USCDC technical consultation. Atlanta, USA 7-9 December 2010." In. Geneva: World Health Organization.
Yetley, E. A., C. M. Pfeiffer, K. W. Phinney, R. L. Bailey, S. Blackmore, J. L. Bock, L. C. Brody, R. Carmel, L. R. Curtin, R. A. Durazo-Arvizu, J. H. Eckfeldt, R. Green, J. F. Gregory, 3rd, A. N. Hoofnagle, D. W. Jacobsen, P. F. Jacques, D. A. Lacher, A. M. Molloy, J. Massaro, J. L. Mills, E. Nexo, J. I. Rader, J. Selhub, C. Sempos, B. Shane, S. Stabler, P. Stover, T. Tamura, A. Tedstone, S. J. Thorpe, P. M.

Coates, C. L. Johnson, and M. F. Picciano. 2011. 'Biomarkers of vitamin B-12 status in NHANES: a roundtable summary', *American Journal of Clinical Nutrition*, 94: 313S-21S.

Young, Ian S. 2018. 'The Enduring Importance and Challenge of Commutability', *Clinical Chemistry*, 64: 421-23.

Zhang, M., M. R. Sternberg, and C. M. Pfeiffer. 2018. 'Harmonizing the Calibrator and Microorganism Used in the Folate Microbiological Assay Increases the Comparability of Serum and Whole-Blood Folate Results in a CDC Round-Robin Study', *Journal of Nutrition*, 148: 807-17.

Zimmermann, M. B., I. Aeberli, T. Torresani, and H. Bürgi. 2005. 'Increasing the iodine concentration in the Swiss iodized salt program markedly improved iodine status in pregnant women and children: a 5-y prospective national study', *American Journal of Clinical Nutrition*, 82: 388-92.

Zimmermann, M. B., and M. Andersson. 2012. 'Assessment of iodine nutrition in populations: past, present, and future', *Nutrition Reviews*, 70: 553-70.

Zimmermann, M. B., and K. Boelaert. 2015. 'Iodine deficiency and thyroid disorders', *Lancet Diabetes Endocrinol*, 3: 286-95.

Zou, Y., X. Lou, G. Ding, Z. Mo, W. Zhu, and G. Mao. 2014. 'Iodine nutritional status after the implementation of the new iodized salt concentration standard in Zhejiang Province, China', *BMC Public Health*, 14: 836.

Tables

Table 1 – Estimated average requirements[1], metabolic functions and deficiency signs of selected micronutrients considered to have likely or possible public health importance

Nutrient	Estimated average requirement (EAR), NPNL adult female[1]	Metabolic functions	Deficiency signs and symptoms	Safe upper intake level (UL)	Ratio of UL/RI
Vitamins					
Retinol (vitamin A)	500 µg	Constituent of retinal pigments required for night vision; epithelial cell maturation; erythrocyte production	Xerophthalmia, blindness; increased incidence & severity of diarrhea/pneumonia (death); anemia	3000 µg	4.3
Thiamine (vitamin B1)	0.9 mg	Coenzyme for carbohydrate metabolism	Infantile beriberi; (death) encephalopathy	None	-
Riboflavin (vitamin B2)	0.9 mg	Coenzyme for carbohydrate, fat, protein metabolism; involved in iron metabolism	Oral, lingual lesions; anemia	None	-
Niacin (vitamin B3)	11 mg niacin equivalents (also synthesized from tryptophan)	Coenzyme in oxidation/reduction reactions	Pellagra (dermatitis, diarrhea, dementia, death)	35 mg	2.5
Pyridoxine (vitamin B6)	1.1 mg	Involved in hemoglobin synthesis and coenzyme for macronutrient metabolism	Deficiency is uncommon; convulsions in newborns	100 mg	76.9
Folate (vitamin B9)	320 µg	Erythrocyte production; DNA synthesis	Fetal neural tube defects (death); megaloblastic anemia	1000 µg	2.5
Cobalamin (vitamin B12)	2.0 µg	Erythrocyte production; nerve myelination; folate metabolism	Megaloblastic anemia, neuropathy	None	-
Ascorbate (vitamin C)	60 mg	Collagen synthesis, bone formation; facilitated iron absorption; antioxidant	Scurvy; connective tissue disorders and bleeding	2000 mg	26.7
Cholecalciferol & ergocalciferol (vitamin D)	10 µg	Involved in calcium & phosphorus absorption and metabolism	Rickets, osteomalacia; increased risk of asthma and respiratory infections (death?)	50 µg	10
Tocopherols (vitamin E)	12 mg	Antioxidant	Hemolytic anemia in newborn	1000 mg	66.7

Nutrient	Estimated average requirement (EAR), NPNL adult female[1]	Metabolic functions	Deficiency signs and symptoms	Safe upper intake level (UL)	Ratio of UL/RI
Minerals					
Calcium[2]	800 mg	Structural component of bones/teeth; involved in nerve conduction, muscle function and coagulation	Rickets, osteomalacia; hypocalcemic seizures, death	2500 mg	2.1
Copper	700 µg	Enzyme cofactor; involved in cellular electron transfer (energy production) and hemoglobin synthesis	Anemia; impaired activity of antioxidant enzymes	10 mg	11.1
Iodine	95 µg	Component of thyroid hormone	Hypothyroidism, goiter; impaired neuro-cognitive development.	1100 µg	7.3
Iron	8.1 mg	Component of hemoglobin, myoglobin and multiple enzymes. Involved in cellular respiration.	Anemia, impaired neuro-cognitive development	45 mg	2.5
Selenium	45 µg	Component of antioxidant glutathione peroxidase	Abnormalities of cardiac function (hypothesized role in pre-term birth)	400 µg	7.3
Zinc	6.8 mg	Cofactor for multiple enzymes. Involved in immune function, wound healing, protein synthesis.	Increased incidence of diarrhea, pneumonia (death), impaired linear growth	40 mg	5

[1]Estimated average requirements are based on the US Institute of Medicine's values for non-pregnant, non-lactating women 19-50 years of age

[2]Calcium is sometimes considered a "macro-mineral" because of its relatively high daily requirement

Table 2 - Characteristics of six nationally representative surveys that analyzed MN biomarkers and were included in IZiNCG key informant interviews

	Cambodia	Pakistan	Malawi	Ghana	Uganda	Uzbekistan
Survey year	2014	2019	2015-16	2017	2018	2018
In-country lead	UNICEF	Ministry of Health; Aga Khan Univ	Ministry of Health; NSO	University of Ghana	Ministry of Health; NBOS	UNICEF
External technical support agency[1]	IRD	UNICEF; WFP	CDC; Emory Univ	GrndWork	CDC	GrndWork
External funding sources	UNICEF; World Vision; IRD; WFP; ILSI	DFID; USAID; Australian Aid	Irish Aid; World Bank; UNICEF; USAID	UNICEF; Global Affairs Canada	USAID; UNICEF	UNICEF
Survey platform	"Follow-on" from DHS	Broader nutrition survey	"Follow-on" from DHS	Broader nutrition survey	National Panel Survey	Broader nutrition survey
Level of representativeness	National; Urban-rural	National; Province; District	National; Urban-rural; Regional (North, Central, South)	National; Regional (North, Central, South)	National	National; Regional (14 regions)
Site of laboratory analyses	Internatl; Regional; Domestic	Domestic	Internatl; Domestic	Internatl	Internatl	Domestic

IRD, Institut de Recherche pour le Développement; WFP, World Food Programme; ILSI, International Life Sciences Institute; DHS, Demographic and Health Survey; NSO, National Statistics Office (Malawi); CDC, US Centers for Disease Control; DFID, Department for International Development, United Kingdom; NBOS, National Bureau of Statistics (Uganda); Grndwork, GroundWork.

[1] ICF provided technical support for the over-arching DHS in Cambodia and Malawi.

Table 3 – **Key biomarkers recommended for assessing selected MNs by BOND, NYAS, WHO and other expert groups**

Nutrient	Primary recommended biomarkers	Expert groups
Vitamin A	s/p retinol, retinol binding protein	BOND, WHO
	total body stores (retinol isotope dilution method)	IAEA
Thiamine	RBC or whole blood thiamine diphosphate, RBC transketolase activity	NYAS
Folate	RBC/s/p folate, homocysteine	BOND, WHO, MNF, NI
Vitamin B12	s/p cobalamin, transcobalamin-2, methyl malonic acid, homocysteine	BOND
Vitamin D	s/p 25-OH vitamin D	Multiple (see Roth, 2018)
Iodine	u iodine, s/p thyroglobulin	BOND, IGN
Iron	s/p ferritin, soluble transferrin receptor, transferrin saturation, RBC zinc protoporphyrin	BOND, WHO
Zinc	s/p zinc	BOND, IZiNCG

BOND, Biomarkers of Nutrition for Development; IAEA, International Atomic Energy Agency; IGN, Iodine Global Network; IZiNCG, International Zinc Nutrition Consultative Group; NYAS, New York Academy of Sciences. s/p means serum or plasma can be used for analyses.

Table 4– Recommended cutoffs of MN status biomarkers used to define deficiency and insufficiency

Nutrient and Biomarker	Population sub-group	Minimum value	Basis for cutoff	Reference
Vitamin A Serum retinol		0.7 µmol/L	Whole body deficiency, based on severe depletion of liver vitamin A stores.	(WHO 1996)
Thiamine ETK activity coefficient ThDP[1]		Adequacy <1.15; Insufficiency 1.15-1.25; Deficiency >1.25 NA[2]	Based on experimental thiamine deficiency studies (EFSA 2016)	(IOM 1998)
Folate Serum folate RBC folate		Deficiency <10nmol/L Deficiency <340 nmol/L Insufficiency <906 nmol/L	For deficiency, based on a metabolic indicator (increased plasma total homocysteine (Selhub et al. 2008)) For insufficiency, based on elevated risk of NTDs (Bailey and Hausman 2018)	(de Benoist 2008)
Vitamin B12 Plasma B12		Deficiency < 150 pg/mL	Based on 98%-ile of a group having elevated MMA (>376nmol/L) (Bailey et al. 2013)	(de Benoist 2008)
Vitamin D Serum 25(OH)D		Deficiency < 25-30nmol/L	Levels indicative of increased risk of musculoskeletal disorders	(IOM 2011; Roth et al. 2018)
Iodine Urinary iodine concentration[2]	Schoolchildren	Median UIC < 100 µg/L	Indicative of low dietary iodine intake, with intake requirements based on thyroid iodine accumulation and turnover (IOM 2006).	(WHO 2013)
	Pregnant women	Median UIC < 150 µg/L		

[1] ThDP No established cutoffs (Whitfield et al. 2018). Data should be reported with reference range used by the reporting laboratory.

[2] Population based cut-offs

Note: cutoffs may depend on assay method.

	Lactating women	Median UIC < 100 µg/L		
Iron Ferritin	Healthy infants and children < 5 years	Deficiency < 12 µg/L	Levels indicative of low body iron stores in all age groups.	(WHO 2020)
	Infants and children < 5 years with infection or inflammation	Deficiency < 30 µg/L		
	Healthy individuals 5 years or older	Deficiency < 15 µg/L		
	Individuals 5 years or older with infection or inflammation	Deficiency < 70 µg/L		
	Pregnant women (first trimester)	Deficiency < 15 µg/L		
Zinc Serum zinc	Children <10 y, morning	Deficiency <65 ug/dL	All cutoffs based on 2.5%-ile of presumably healthy population (NHANES-2)	(IZiNCG 2004)
	Children <10 y, afternoon	Deficiency <57 ug/dL		
	Females ≥10 y, fasting	Deficiency <70 ug/dL		
	Females ≥10 y, morning, non-fasting	Deficiency <66 ug/dL		
	Females ≥10 y, afternoon, non-fasting	Deficiency <59 ug/dL		
	Males ≥10 y, fasting	Deficiency <74 ug/dL		
	Males ≥10 y, morning, non-fasting	Deficiency <70 ug/dL		
	Males ≥10 y, afternoon, non-fasting	Deficiency <61 ug/dL		

	Pregnant females, (trimester 2-3)	Deficiency <50 ug/dL		

Notes: For folate, WHO has also established a RBC cutoff for maternal folate insufficiency (<906 mmol/L), based on the level at which the risk of fetal neural tube defects begins to increase (WHO 2015). For vitamin D, different expert groups have proposed distinct cutoffs for serum 25OHD (Roth et al. 2018). For vitamin A, iron, and zinc different approaches have been proposed for adjusting for the effects of inflammation.

Table 5 – Equipment needs for central resource laboratory using recommended methods for MN biomarker analyses, and approximate costs

Equipment	Nutrients & biomarkers or analytical methods														Cost (US$)
	Vit A	Thiamine		Folate		Vit B12			Vit D	Iodine	Iron			Zinc	
	Serum retinol	ThDP (HPLC)	ETK	Microbiology	LC-MS/MS	Serum B12	Holo TC	MMA	Serum 25(OH)D	Urinary I	Hematology	Clin-chem[3]	ELISA		
Incubator			●	●											2,500
Multiskan plate reader (Thermo)			●												9,000
Pipetting robot[1]				Optional[1]											60,000
Spectrophotometer	●	●		●	●				●	●					6,000
LC-MS/MS					●				●						390,000
Positive pressure / vacuum manifold					●				●						5,000
Multi-tube vortex mixer	●	●			●				●						6,500
96-well plate shaker					●				●	●					650
HPLC with UV detector	●	●													125,000
Refrigerated centrifuge	●	●	●						●						7,500
Vacuum evaporator/ sample concentrator	●							●	●						2,500
Plate reader / washer[2]			●	●									●		13,000
ICPMS										●				●	260,000
Plate washer							●						●		6,500

Clinical chemistry analyzer						●						●			75,000
Hematology analyzer[4]											●				65,000
General lab equip															
-80°C freezer															13,000
-20°C freezer															1,300
Fridge															750
pH meter															650
Roller mixer															1,000
Vortex mixer (single tube)															250
Single- and multi-channel pipettes															1,250
Balance															2,000
Magnetic stirrer															500
Grand total															1,054,850

[1] Pipetting robot is optional and only required for very high throughput laboratories

[2] Fully automated system with separate plate washer

[3] Includes iron, ferritin, TIBC, soluble transferrin receptor, transferrin (and CRP and AGP)

[4] Hematology analyses may be done at field site rather than central laboratory

Table 6 – Laboratory analysis costs reported by different laboratories, by MN biomarker and analysis method

Nutrient	Biomarker	Analytical method	N labs	Cost range ($)	Mean cost ($)	Lowest cost ($)
Vitamin A	Serum retinol	HPLC	4	12-50	23.62	12
	Retinol binding protein	ELISA	1	1	1	1
		All	**5**	**1-50**	**19.10**	**1**
Thiamine	ETKAC	**Colorimetric enzyme assay**	**1**	**16**	**16**	**16**
	ThDP	**HPLC**	**1**	**25**	**25**	**25**
Folate	RBC folate	Microbiological assay	6	20-47	26.67	20
		Chemiluminescence	1	10	10	10
		All	**7**	**10-47**	**24.29**	**10**
Vitamin B12	Serum cobalamin	**Chemiluminescence**	**3**	**10-45**	**24.33**	**10**
Vitamin D	Serum 25(OH)D	HPLC	3	15-65	33.33	15
		Immunoassay	1	28	28	28
		LC/MS-MS	2	20-40	30	20
		All	**6**	**15-65**	**31.33**	**15**
Iodine	Urinary iodide	Spectrophotometric assay	6	6-18	9.20	6
		ICP-MS	1	16	16	16
		All	**7**	**6-18**	**10.29**	**6**
Iron	Serum ferritin	ELISA	1	1	1	1
	Serum soluble transferrin receptor	ELISA	1	1	1	1
		All	**1**	**1**	**1**	**1**
Zinc	Serum zinc	AAS	1	18	18	18
		ICP-AES	1	10	10	10
		ICP-OES	1	11	11	11
		ICP-MS	2	16-39	27.50	16
		All	**5**	**10-39**	**19.05**	**11.80**
Mean, based on any method for each MN					18.00	8.62

Table 7 – Estimated direct costs for centrally funded components of the initiative*

Category of expense	Items	Year 1	Year 2	Year 3	Years 1-3
Project management	Full-time project director, part-time admin asst, fraction of MNF Exec Dir	267,500	267,500	267,500	802,500
Advocacy & communication	Message development & communication plan design	100,000	-	-	100,000
	Implementation (part-time technical specialist, travel, in-country support)	82,500	165,000	165,000	412,500
Lab development and operation	Identification of labs (workshops, site visits)	134,000	-	-	134,000
	Establishment of labs (Equip purchase & installation - 2 labs)	2,000,000	-	-	2,000,000
	Operating expenses (lab director, technician, core supplies – 2 labs)	-	300,000	300,000	600,000
	Participation in EQA schemes (2 labs, 10 biomarkers each)	-	20,000	20,000	40,000
Financial support for natl surveys	50% subsidy for specimen collection supplies, shipping, & lab analyses, 14 surveys	-	7,000,000	7,000,000	14,000,000
Technical assistance for natl surveys	Salaries for two full-time technical specialists (year 3), travel and per diem, and maintenance of OpeN-Global web site	130,000	220,000	400,000	750,000
Data repositories & analysis	VMNIS updates & upgrades	170,000	-	-	170,000
	VMNIS management	105,000	105,000	105,000	315,000
	Survey clearinghouse upgrades	15,000	-	-	15,000
	Survey clearinghouse management	17,000	17,000	17,000	51,000
	Data analysis (prevalence estimates)	-	125,000	125,000	250,000
Laboratory training	Travel & per diem for national scientists, partial salary support for trainers	-	128,000	128,000	256,000
Data analysis training	Workshops	-	50,000	50,000	100,000

Consultants	Lab manuals, lab training curriculum inventory, data analysis curriculum development, etc.	100,000	50,000	50,000	200,000
Total		3,121,000	8,447,500	8,627,500	20,196,000

*All estimates shown in US dollars

Table 8 – Examples of indicators for tracking progress of the MN data generation initiative

Category of information	Indicator	Source of data	Frequency of updating
Financial resource generation	Number of grant proposals prepared by management team	Management team records	Semi-annually
	Total value of grant proposals	Management team records	Semi-annually
	Number (%) of grant proposals funded	Management team records	Semi-annually
	Total funds leveraged	Management team records	Semi-annually
Advocacy materials prepared and distributed	Number of messages prepared, briefs distributed, by target audience	Advocacy team records	Quarterly
	Number of countries & individuals contacted to promote data generation	Advocacy team records	Quarterly
Surveys completed	Number of countries conducting surveys	Survey clearinghouse	Annually
	Number and type of biomarkers measured, by population sub-group	Survey clearinghouse	Annually
Laboratory analyses completed	Number (%) of analyses completed at central resource labs	Central resource labs	Annually
	Number (%) of laboratories participating in external quality assessment, by assay type	Survey of labs supporting all surveys	Annually
Data archives	Number (%) of completed surveys providing data for central archive and individual-level open access data	Survey clearinghouse and data repository staff	Annually
	Timelines of updating of archives in relation to receipt of data	Data repository staff	Semi-annually
Training in data interpretation & utilization	Number of workshops convened and individuals trained	Management team records	Annually
Policy formulation and program planning & evaluation	Number of new policies & new or modified programs based on data from recent surveys	Interviews with country officials and partners	Annually

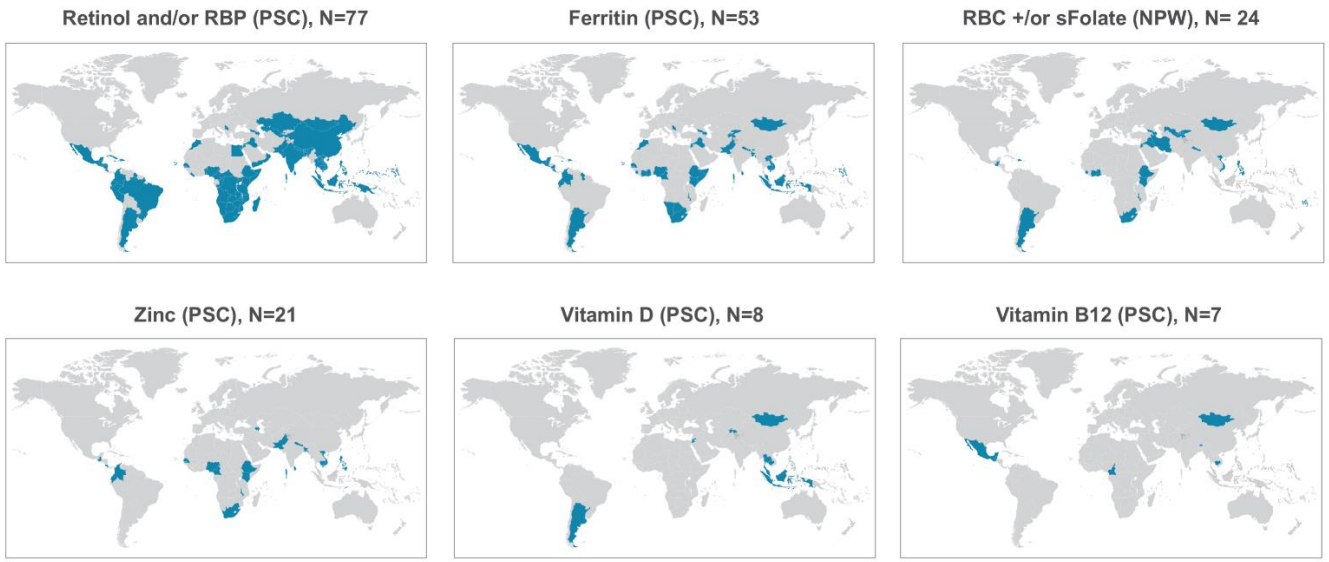
Figures

Figure 1 – Low- and middle-income countries with representative data on anemia prevalence among preschool-age children that were obtained since 1980 and included in the World Health Organization's Vitamin and Mineral Nutrition Information System (N = 107 LMICs) [1]

Data from:
https://extranet.who.int/sree/Reports?op=vs&path=/WHO_HQ_Reports/G9/PROD/EXT/vmnis
(February 27, 2020).

[1]Data from high-income countries not included in tally.

Figure 2 – Number of low- and middle-income countries with specified data on MN status of selected population sub-groups since 1980 according to the World Health Organization's Vitamin and Mineral Nutrition Information System[1]



PSC, pre-school-age children; NPW, non-pregnant women of child-bearing age

[1] Data from: https://www.who.int/vmnis/database/en/ (February 28, 2020).
[2] Data from high-income countries not included in tallies.

Figure 3 – MN and inflammation biomarkers included in national surveys completed from 2015-2019 or currently being planned

	Africa									S/SE Asia			W Pacific			E Mediterranean			Eur	C Am
	Burkina Faso, N=6750	Ethiopia, N =2768	Gambia, N=3094	Ghana, N=2450	Malawi, N=2280	Nigeria, N=12,600	Rwanda, N=5500	Uganda, N=3900	Zambia, N=919	Bangladesh, N=2000	India, N=20,350	Nepal, N=4060	Kiribati, N=665	Mongolia, N=4250	Vietnam, N=13,645	Jordan, N=1100	Pakistan, N=63,656	Somalia, N=2300	Uzbekistan, N=4797	Guatemala, N=950-2290, depending on biomarker
Hemoglobin	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Ferritin	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Transferrin receptor	x	x	x	x	x	x		x					x	x						x
Retinol/RBP, MRDR	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Zinc	x	x			x				x	x	x	x	x		x	x	x	x		x
Folate	x	x		x	x	x	x	x	x	x	x	x	x		x	x	x	x	x	x
Vitamin B12	x	x		x	x	x	x	x	x	x	x		x		x	x	x	x	x	x
Vitamin D	x									x	x			x	x	x	x			x
Thiamine diphosphate						x							x							
Selenium					x								x							
CRP and/or AGP	x	x	x	x	x	x	x	x	x		x	x	x	x		x	x	x	x	x
Urinary Iodine	x	x	x		x	x	x	x		x	x	x		x	x	x	x	x	x	x

N = total number of specimens intended for analysis for WRA and PSC combined. (Urinary iodine generally analyzed in school-age children.)
Guatemala data are from individual rounds of national surveillance system, reflecting period from 2015-2019.

Figure 4 – Theory of change for generating reliable data on population MN status globally

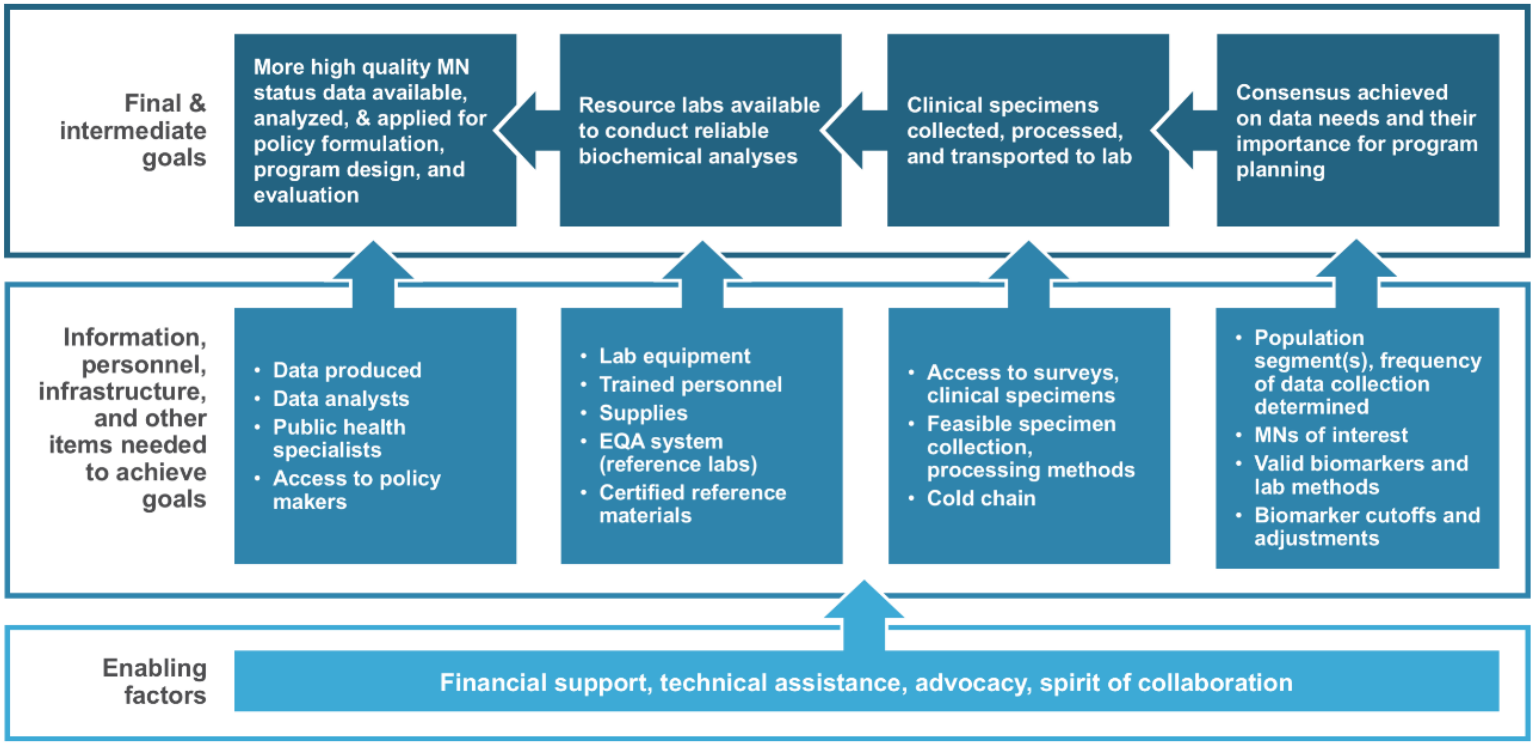
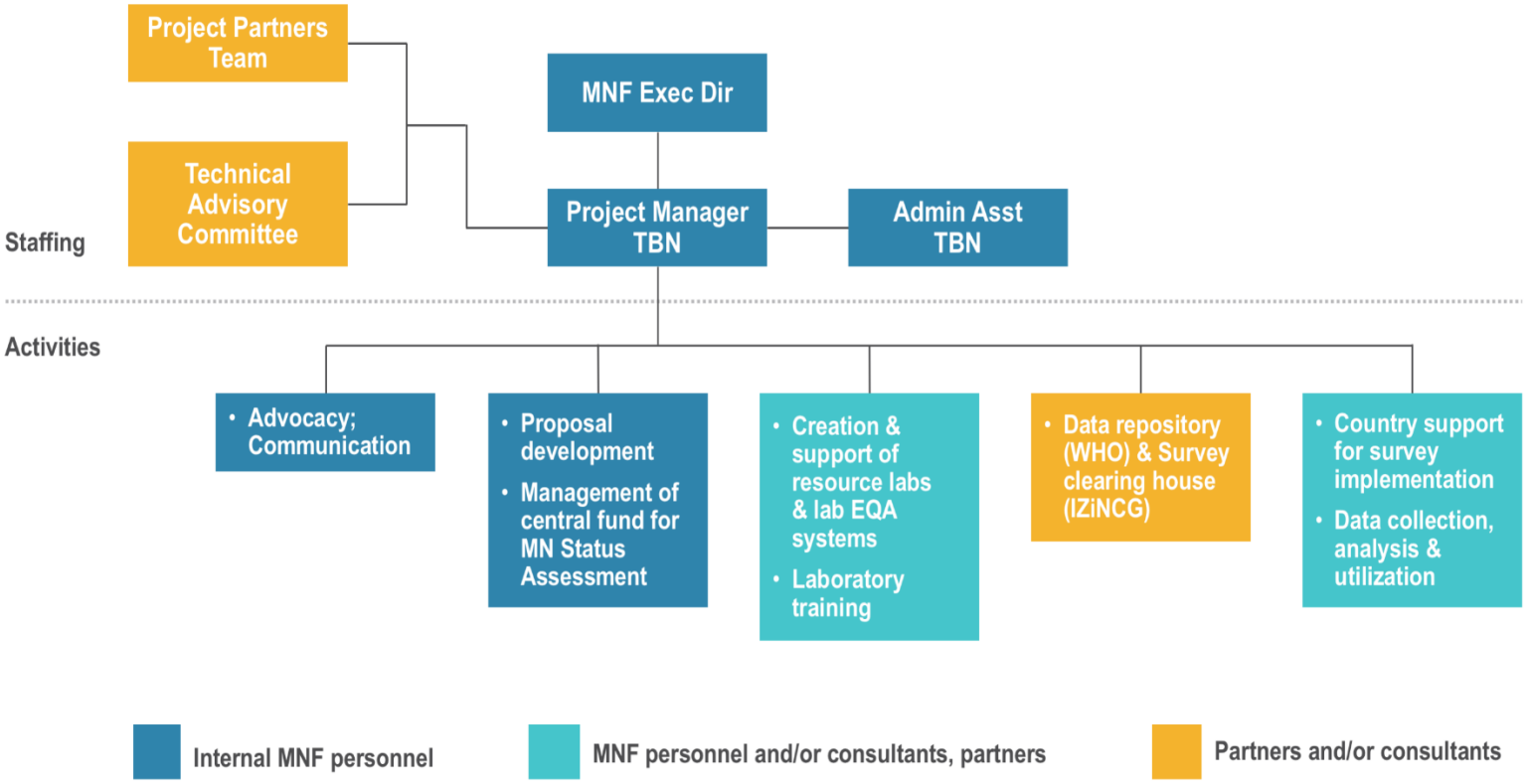


Figure 5 – Proposed organizational structure for MN Data Initiative



Abbreviations and Acronyms

AAS – Atomic absorption spectrophotometer

BMGF – Bill & Melinda Gates Foundation

BOND – Biomarkers of Nutrition for Development

CLSI – Clinical and Laboratory Standards Institute

CRM – Certified reference material

DataDENT – Data for Decisions to Expand Nutrition Transformation

DBS – Dried blood spot

DHS – Demographic and Health Surveys

EAR – Estimated average requirement

eLENA – e-Library of Evidence for Nutrition Action (WHO)

EQA – External quality assessment

FAO – Food and Agriculture Organization

GAIN – Global Alliance for Improved Nutrition

GBD – Global burden of disease

GDD – Global Dietary Data Base

GIFT – Global Individual Food Consumption Data Tool

GINA – Global Data Base on the Implementation of Nutrition Action (WHO)

Hb – Hemoglobin

Hct - Hematocrit

HCES – Household consumption and expenditure survey

Hcy – Homocysteine

HoloTC – Holo-transcobalamin

HPLC – High performance liquid chromatograph

IAEA – International Atomic Energy Agency

ICP-MS – Inductively coupled plasma-mass spectrometer

IGN – Iodine Global Network

IHME – Institute for Health Metrics and Evaluation (University of Washington)

IMMPaCt – International Micronutrient Malnutrition Prevention and Control Program (US Centers for Disease Control)

IRD – French Institute for Development Research

IZiNCG – International Zinc Nutrition Consultative Group

LC-MS/MS – Liquid chromatograph tandem mass spectrometer

LMIC – Low- and middle-income countries

MCH – Mean corpuscular hemoglobin

MCV – Mean corpuscular volume

MICS – Multi-indicator Cluster Survey

MMA – Methyl-malonic acid

MN – Micronutrient

MTA – Materials transfer agreement

N4G – Nutrition for Growth

NIST – National Institute of Standards and Technology

NGO – Non-governmental organization

NPNL – Non-pregnant, non-lactating

NPW – Non-pregnant women

NTD – Neural tube defect

NYAS – New York Academy of Sciences

OpeN-Global - online resource to support assessment of nutritional biomarkers

POC – Point of care (or point of contact)

POSHAN – Partnership and Opportunities to Strengthen and Harmonize Actions for Nutrition

PPT – Project Partners Team

PSC – Pre-school children

RBC – Red blood cell

RDW – Red cell distribution width

RID – Retinol isotope dilution test

RMP – Reference method procedure

SDG – Sustainable Development Goal

STfN – Standing Together for Nutrition

sTfR – Soluble transferrin receptor

SUN – Scaling Up Nutrition

TAC – Technical Advisory Committee

Tg – Thyroglobulin

TIBC – Total iron binding capacity

TSAT – Transferrin saturation

UIC – Urinary iodine concentration

USAID-AN – United States Agency for International Development-Advancing Nutrition

VAS – Vitamin A supplementation

VMNIS – WHO Vitamin and Mineral Nutrition Information System

WHO – World Health Organization

WRA – Women of reproductive age

ZPP – Zinc protoporphyrin

25(OH)D – 25-hydroxy vitamin D

Appendices

Appendix 1 – Working group members and other co-authors

Core Working Group

Sylvia Alayon	USAID Advancing Nutrition
Kenneth H Brown	University of California, Davis; Micronutrient Forum
Sonja Y Hess	University of California, Davis
Maria Elena Jefferds	US Centers for Disease Control and Prevention
Christine M McDonald	University of California, San Francisco; International Zinc Nutrition Consultative Group
Sophie E Moore	Kings College London; OpeN-Global
Saskia Osendarp	Micronutrient Forum

Additional Co-Authors

Jennifer Coates	Tufts University
Kerry Jones	NIHR BRC Nutritional Biomarker Laboratory, MRC Epidemiology Unit, University of Cambridge
Mari Manger	IZiNCG
Sarah Meadows	NIHR BRC Nutritional Biomarker Laboratory, MRC Epidemiology Unit, University of Cambridge

Multi-Stakeholder Advisory Group

Seth Adu-Afarwuah	University of Ghana, Legon
Maria Andersson	Iodine Global Network; University Children's Hospital, Zurich
Mandana Arabi	Nutrition International
Sufia Askari	Children's Investment Fund Foundation
Shawn Baker	USAID
Gilles Bergeron	Nutrition Institute, New York Academy of Sciences
Eleanor Brindle	University of Washington
Kevin Cashman	University College, Cork
Jennifer Coates	Tufts University
Nichola Connell	Eleanor Crook Foundation
Omar Dary	USAID
Jonathan Gorstein	Iodine Global Network
Ralph Green	University of California, Davis
Sarah Hanck	Rockefeller Foundation
Andreas Hasman	UNICEF
Rebecca Heidkamp	Johns Hopkins Bloomberg School of Public Health
Rolf Klemm	Helen Keller International; Johns Hopkins Bloomberg School of Public Health
Germana Leyrna	Tanzania Food and Nutrition Center
Cornelia U Loechl	International Atomic Energy Agency

Renata Micha	Global Nutrition Report
Sorrel Namaste	The DHS Program, ICF
Lynnette Neufeld	Global Alliance for Improved Nutrition
Abigail Perry	UK Foreign, Commonwealth, and Development Office
Christine Pfeiffer	US Centers for Disease Control
Felix Phiri	Malawi MOH
Rahul Rawat	Bill & Melinda Gates Foundation
Lisa Rogers	World Health Organization
Fabian Rohner	GroundWork Switzerland
Kyoko Shibata	World Bank
Ben Siddle	Irish Aid
Parminder Suchdev	Dept of Pediatrics and Emory Global Health Institute, Emory University
Masresha Tessama	Ethiopian Public Health Institute

Appendix 2 – Expert group recommendations concerning MN status biomarkers

Multiple expert groups have been convened over the past two decades to review available scientific literature and develop consensus on the best available biomarkers of MN status that can be applied for population assessment. These groups are described briefly in the following paragraphs in relation to the MNs addressed by each group; publications resulting from their deliberations are cited.

The Biomarkers of Nutrition for Development (BOND) project was initiated in 2009 under the leadership of the US National Institutes of Health, with support from the Bill & Melinda Gates Foundation. The goals of the BOND project were to: 1) identify, develop, and apply valid and reliable biomarkers of MN exposure and status, and 2) harmonize the global health and nutrition communities' decision-making process for determining which biomarkers are most useful under defined conditions and settings (Raiten et al. 2011). The project convened multiple expert groups that reviewed the scientific literature and formulated recommendations on key biomarkers of vitamin A, iron, zinc, iodine, folate and vitamin B12 status. The results of the working group deliberations have been published (Green 2011; Rohner et al. 2014; King et al. 2016; Bailey et al. 2015; Tanumihardjo et al. 2016; Allen et al. 2018; Lynch et al. 2018); the primary recommended biomarkers are summarized in **Table 3** of this report. The BOND initiative also convened separate working groups to assess the effects of inflammation on these biomarkers (Raiten et al. 2015), and how best to adjust for these effects (Suchdev et al. 2016; Mei et al. 2017).

Building on the BOND experience, the Nutrition Program of the New York Academy of Sciences convened two working groups to focus on the assessment and control of thiamine and vitamin D deficiencies. These groups were also tasked with identifying appropriate biomarkers for the respective MNs (Whitfield et al. 2018; Roth et al. 2018), and their recommendations are also listed in **Table 3**. Several other groups have also published recommendations on assessing vitamin D status, as described previously (Roth et al. 2018). The MN Forum and Nutrition International assembled a task force on folate deficiency, which reexamined and endorsed the BOND recommendations regarding folate biomarkers (Bailey and Hausman 2018). Individual MN interest groups have published independent recommendations for specific nutrients, and these are generally aligned with the aforementioned conclusions. For example, IZiNCG has published recommendations for assessing population zinc status (IZiNCG 2004; Hess et al. 2007; IZiNCG 2020). The International Atomic Energy Agency (IAEA) has recently published a recommendation for using the retinol isotope dilution method to assessing total body vitamin A stores across the spectrum from deficiency to hypervitaminosis, especially in situations where concerns have been raised about possible excessive intakes (International Atomic Energy Agency 2020).

WHO also issues guidance on how best to assess individual and population nutritional status for selected nutrients. These are described in a series of publications on serum and red blood cell folate for assessing folate status (WHO 2015), serum ferritin (WHO 2011c, 2020) and transferrin receptor for assessing iron status (WHO 2014b), serum retinol for determining the prevalence of vitamin A deficiency (WHO 2011d), urinary iodine for assessing the adequacy of iodine intake (WHO 2013), hemoglobin concentrations for the diagnosis of anemia (WHO 2011a), C-reactive protein concentration as a marker of inflammation (WHO 2014a), and general methodological approaches for estimating the prevalence of vitamin and mineral deficiencies (WHO/CDC 2014).

OpeN-Global is an open access source of information on nutritional biomarkers that was launched in 2019 (OpeN-Global 2019). Developed by King's College London, but supported by a large network of global collaborators, the OpeN-Global web site provides information on biomarker assessment, laboratory methods, and quality control and accreditation procedures. OpeN-Global covers more than 20 nutritional biomarkers, including those of particular public health importance, as noted above. The primary objective of OpeN-Global is to support the assessment of nutritional biomarkers by bringing together into a single website all the required details, together with sign-posting to collaborators for technical support. OpeN-Global utilizes other resources (e.g. BOND) and organizations (e.g. IZiNCG) to ensure the best technical support and advice is available.

Reference ranges for MN status biomarkers have been developed for specific age, sex and physiological categories (**Table 4**). Ideally, these ranges should be based on selected clinical or functional outcomes that occur when the biomarker is less than or greater than some cutoff. For example, cutoffs for folate deficiency and insufficiency have been set based on the respective levels of RBC folate concentrations at which the risk of megaloblastic anemia or the incidence of neural tube defects begins to increase. In some cases, when the cutoffs have not been linked to clinical or functional outcomes, these ranges are defined statistically, based on the distribution of values in a presumably healthy population. For example, IZiNCG originally proposed cutoffs for serum zinc concentration based on the distribution of values observed in healthy Americans (Hotz et al. 2003). In this latter case, it is conceivable that values less than the lower cutoff could be compatible with health, but the cutoff is assumed to represent a suitably conservative criterion for motivating programmatic action. Subsequent research linking serum zinc concentration to specific clinical signs of zinc deficiency supports the use of these statistically defined cutoffs (Wessells et al. 2014).

Appendix 3 – Specimen collection and processing, and related data collection instruments and costs

There are many issues that need to be considered to ensure that biological specimens obtained for analysis of MN status biomarkers are of adequate quality and representative of the population groups of interest. Many of these issues have been reviewed in the Micronutrient Survey Manual that was recently published by CDC, WHO and UNICEF (CDC et al. 2020). We summarize herein some of the key items related to specimen collection and processing and related matters of critical supplies and information needs.

For plasma specimens, blood is collected into tubes containing an anticoagulant, such as EDTA or heparin; and for serum, blood is collected into tubes containing a serum separator. If trace metals, such as serum zinc or selenium, are being measured, special tubes certified as being free of trace metals must be used. For some biomarkers, the specimens should also be protected from direct light to prevent photo-degradation. Whole blood from all collection tubes must be refrigerated in a cold box and maintained at ~4˚C immediately after collection and until processed. Whole blood from the EDTA or heparin tube can be used for field tests, including hemoglobin and malaria rapid diagnostic tests, and for preparation of dried blood spots (DBS).

Urine is collected in specimen cups for biomarkers like iodine or selenium. Breast milk is usually collected by manual expression for casual samples, or by manual or automated pump for full breast expression. Breast milk (~10 mL) can be collected in specimen cups or polypropylene tubes to assess certain MN biomarkers, such as vitamin A, B vitamins or vitamin D. The breast milk needs to be refrigerated at 4˚C immediately, and protected from light for selected MN biomarkers, as noted above for blood-derived specimens. The breast milk must either be analyzed within 24 hours of collection or stored frozen at -80˚C and analyzed within one year of collection. Before freezing, precise aliquots of milk that will be used for measuring nutrient content should be prepared, as thawed samples can be difficult to homogenize.

Universal precautions, defined by CDC as “a set of precautions designed to prevent transmission of human immunodeficiency virus (HIV), Hepatitis B virus (HBV), Hepatitis C virus (HCV), and other blood-borne pathogens” must be followed when specimens are being collected from human subjects (CDC 1987). This requires the use of gloves during specimen collection and processing, proper waste disposal, and access to hand-washing facilities or sanitizers.

Proper and accurate labeling of all specimens is one of the most important aspects of the survey. The label identifies the participant with a unique sequence of numbers and/or letters, which matches with the barcode on the label and provides key survey information. Participants are assigned a sheet of labels that follows them through the entire process of specimen collection and processing so that all forms, tubes, and storage vials are labelled with the same unique identification. Barcode scanners can be used to prepare an inventory of specimens for storage. Tablets used in the field can also be used to scan the barcode to store this information for quality control and tracking during specimen collection in order to link with paper forms and electronic databases.

To prevent any deterioration of biomarkers, the specimens should be processed as soon as possible (and always within 24-48 hours, depending on the biomarker). Specimens are processed using portable

centrifuges that are powered using electricity, car batteries, or portable power sources. Specimens used to analyze trace metals should be processed using a fume hood or locally fabricated plastic container to avoid contamination with dust. Specimens are then aliquoted into cryovials and labelled, and the vials are stored in cryovial boxes, and placed into the portable -20°C freezers. Ideally, processed specimens are separated according to specimen type (e.g., serum vial 1 for retinol, serum vial 2 for inflammation markers, plasma vial 1 for trace elements, etc.) to facilitate storage logistics, shipping, and tracking the inventory; but they can also be sorted later.

Cold boxes (i.e., coolers) and portable freezers are used to store specimens in the field prior to and following initial processing. For specimens that require freezing, portable -20°C freezers are available. Alternatively, liquid nitrogen stored in cylinders at -196°C can be used in remote locations when electricity is not available or temperatures <-20°C are needed. Because portable freezers and liquid nitrogen tanks take up space in the vehicle, this must be considered when planning the number of vehicles required per field team.

It is usually necessary to transfer specimens from the field daily or every few days, as the portable freezers used in the field have limited storage capacity. Ultimately, the specimens are stored frozen in a -80°C freezer until they are analyzed in-country or shipped to international laboratories for analysis. As a safeguard, temperature monitoring devices, back-up generators and extra fuel for the generators are needed to ensure that the freezers remain functional for the duration of the survey and the period of time that the specimens are stored prior to analysis.

One example of the costs of supplies and equipment required for specimen collection, processing and cold chain maintenance for a national MN survey is presented in the table below. In this example, based on a field survey conducted in sub-Saharan Africa, temporary field laboratories were set up to process all specimens at the end of the day, and specimens were stored in regional laboratories until final transport to the central laboratory in the capital.

A data collection questionnaire and related survey tools need to be prepared to allow relevant information to be linked with the biological specimens. The data collection tools should be as concise as possible, focusing on information that meets the survey objectives. The figure below provides an example of the steps involved in developing the main survey instrument, which requires field testing and possible revisions prior to implementation. The survey questionnaire should be designed to elicit information for monitoring current programs and planning future strategies. It needs to be clear how the results of the questionnaire will be used and whether the inclusion of a particular indicator can potentially affect overall program 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, if not identical, in the two surveys. Similarly, it is best to use the same laboratory methods and equipment models across surveys when possible to reduce variability.

The questionnaire is usually designed to collect certain types of information, for example:

- Information about biological specimens or food samples that align with the survey objectives
  - Confirmation that the biological specimens or food samples were collected from respective individuals or households and dates of collection

  - Results of any field-based testing (for example, the hemoglobin concentration or the result of a rapid diagnostic test for malaria)
- Information on participants that can facilitate cross tabulations.
  - Demographic information
  - Socioeconomic information
  - Information reflecting respondent knowledge, attitudes, and practices (KAP), usually in relation to MN interventions
  - Participation in nutrition interventions
- Information that helps interpret measurements of micronutrient biomarkers, 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
  - Presence in household or consumption patterns of fortified/fortifiable foods
  - Altitude of the cluster location
  - Whether the respondent is a smoker and, if so, the average number of cigarettes smoked per day. It may be relevant to ask about pipes or electronic cigarettes as well, but there is less data and guidance available for these products.
- Information to assess the impact of certain nationally-relevant practices on MN status, such as
  - The frequency of using salt substitutes (such as bouillon), to estimate their iodine content and potential contribution to iodine intake, as well as other MN-enriched condiments, foods, and beverages
  - Behaviors related to infant feeding practices, to assess the impact of a targeted behavior-change communication campaign
  - The use of cooking ash (e.g., sodium bicarbonate), which affects food's acidity and in turn affects the bioavailability of micronutrients in multiple MN powders and foods
  - Consumption of teas and other beverages and foods that may reduce mineral bioavailability

When questionnaire modules are similar to those included in the DHS and the UNICEF MICS surveys, 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 and with surveys conducted in other countries. The DHS model questionnaires are available in English and French (USAID 2020a); and the UNICEF MICS questionnaires are available in English, Spanish, French, and Russian (UNICEF 2020). Standard methods for analyzing the data to fill suggested table shells are provided in the DHS and MICS documentation, available on the respective websites. Suggested wording for questions used in micronutrient surveys among different population groups can be found online in the questionnaire examples. New questions that have not been part of and tested in previous surveys need to be developed systematically, preferably following the cognitive interviewing process (Willis and Miller 2011).

The overall format of the questionnaires will vary by survey depending on the objectives, the number and types of MNs being assessed, and the 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 are the recommended method for collecting this information.

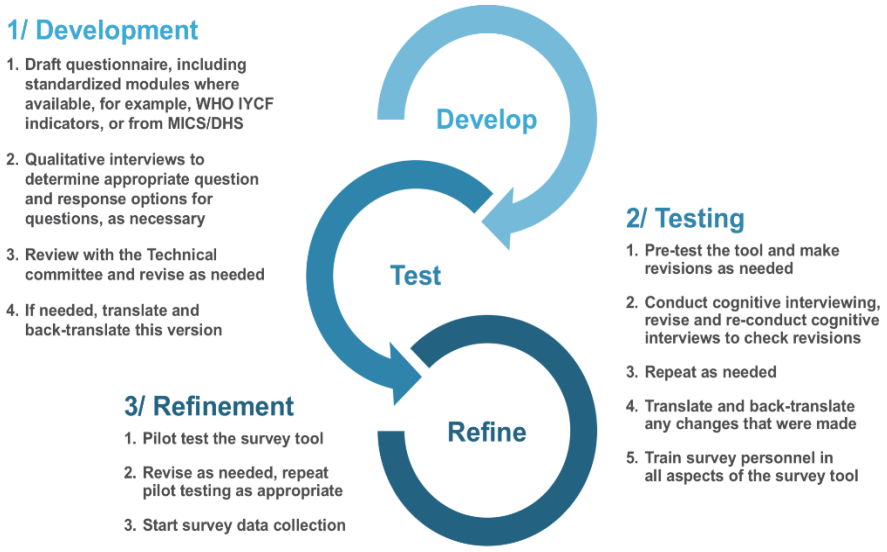
Designing an electronic data collection program, with all the required logic and error checks, can take several months. Developing these electronic data entry systems ideally takes place after the paper-based versions of these tools have been pretested with appropriate target populations.

Table - Estimated costs of supplies required for MN status survey, by category of supplies*

Category of supplies	Brief description	Cost
Blood drawing supplies	Alcohol swabs, needles, band-aids, syringes, collection tubes, storage vials, storage boxes, other	$21,543
Urine collection supplies	Specimen containers, storage tubes, storage boxes, other	$10,683
General laboratory supplies	Portable centrifuge and freezers, balances, pipettes, plastic bags and wrap, biohazard disposal, other	$47,171
Hemocue devices	9 devices, 8140 cuvettes, control solutions, batteries	$21,662
Malaria test kits	Test kits and 10 digital timers	$5,539
Anthropometry equipment	Length/height boards, digital balances, circumference tapes, blood pressure monitors (10 each)	$13,877
Shipping of supplies	Estimated	$8,000
Total		$128,475

*Survey of 1688 children 6-59 months of age and 4824 women 15-49 years of age (WRA) in 3260 households (total sample size 6512 individuals + 25% overage for field specimen collection and laboratory supplies). Blood specimens obtained for hemoglobin; serum retinol; ELISA testing (serum ferritin, soluble transferrin receptor, retinol binding protein, C-reactive protein, alpha-1 acid glycoprotein); malaria test; and (in subsample) modified relative dose response test. In addition, whole blood obtained from WRA for RBC and serum folate, serum vitamin B12, hemoglobin A1c and other markers of non-communicable diseases. Urine specimens obtained for urinary iodine. Total cost of $128,475 = ~$20 per individual for all supplies.

Figure - Example of steps involved in developing a survey questionnaire



Appendix 4 – Analytical methods for recommended biomarkers and related laboratory equipment needs and quality assessment schemes

Dependable data on population MN status requires reliable laboratory analyses in addition to careful specimen collection and processing. Information on the physiological roles and metabolism of selected MNs of public health concern and a review of the laboratory methods available for analyzing their respective biomarkers are provided in this section of the report.

Vitamin A (retinol)

Vitamin A is a fat-soluble vitamin required for normal vision, growth and development, immune function, and hematopoiesis. Although vitamin A deficiency is the primary public health focus, vitamin A excess is also a potential concern under certain circumstances. Serum retinol is the most commonly used biomarker to assess vitamin A status. Serum retinol is homeostatically controlled across a broad range of total body vitamin A stores, but the concentration decreases when liver reserves are depleted. Thus, serum retinol concentration is a useful indicator of vitamin A deficiency. Serum retinol may be depressed in the presence of inflammation associated with infection or obesity and its comorbidities, especially in PSC, so it is recommended that acute phase proteins are also measured to adjust for the effects of inflammation. Other biochemical markers that can be used to assess vitamin A status are plasma retinol binding protein, the relative dose response (RDR) test, and breast milk retinol, among others. The advantages and disadvantages of each method are discussed in the BOND review (Tanumihardjo et al. 2016).

Serum retinol is less useful for detecting excess vitamin A status before the appearance of clinical signs of toxicity, so IAEA has recently recommended the use of the retinol isotope dilution (RID) technique for population measurements in situations where high intakes may occur (Haskell et al. 2005). However, the RID technique is more costly, imposes additional field logistics challenges, and requires greater expertise to measure than serum retinol, which must be considered when deciding whether and when to use the RID method.

Sample Type

Serum /plasma

Laboratory methods for measuring vitamin A status biomarkers and their respective advantages and disadvantages

Method	Advantages	Disadvantages
LC-MS/MS	Sensitive Specific Matched internal standards	Sample preparation required Expensive equipment Experienced analyst
HPLC – reference method	Less expensive than MS	Fairly expensive equipment Sample preparation required Experienced analyst Lower sensitivity than LC-MS/MS Non-specific internal standards

GC-MS for Retinol Isotope Dilution technique (deuterium tracer)	Measures total body stores Sensitive	Sample preparation required Expensive equipment Experienced analyst Multiple samples required Larger isotope dose required than ^{13}C label
GC-C-IRMS for Retinol Isotope Dilution technique or LC-MS/MS (^{13}C tracer)	Measures total body stores Very sensitive	Sample preparation required Expensive equipment Experienced analyst Multiple samples required More expensive than deuterium
Immunoassay (RBP)	High throughput Automated Low cost Kits are available	Proxy for serum retinol Less sensitive Retinol should still be measured in a sub-sample of the population to determine relationship between RBP and retinol in the study population

Equipment required for retinol assay using HPLC or LC-MS/MS

HPLC with UV detector *or* LC-MS/MS

Centrifuge

Vacuum evaporator *or* sample concentrator

Multi-tube vortex mixer

Spectrophotometer

Notes

Solvent supply, storage and disposal need to be considered

Vitamin A is light sensitive

US National Institute of Standards and Technology (NIST) Standard Reference Material is available

External Quality Assessment (EQA) Schemes

UK NEQAS Vitamins, CDC VITAL EQA, and CDC Performance Verification Program for Serum Micronutrients (CDC 2019)

Additional Resources

BOND Vitamin A report (Tanumihardjo et al. 2016)

Thiamine (vitamin B1)

Thiamine is a water-soluble vitamin that plays an important role in energy metabolism. The clinical manifestations of thiamine deficiency are variable, and may include respiratory distress, other signs of cardiac failure, and/or neurological signs. This variability of clinical presentation along with the lack of a readily accessible and widely agreed biomarker of thiamine status complicate efforts to diagnose deficiency. Populations with a dietary reliance on low thiamine-containing staple foods, such as rice and cassava, may be at particular risk of deficiency (Whitfield et al. 2018).

There are two ways to assess thiamine status: 1) measuring the activity of a thiamine-dependent enzyme, erythrocyte transketolase (ETK), and 2) quantifying thiamine metabolites. The ETK assay provides a functional marker of thiamine status. The activity of the enzyme is measured in the absence and presence of exogenous thiamine diphosphate (ThDP) to calculate the activity coefficient. This assay is not readily available and requires washed red blood cells, a time consuming and cumbersome procedure. There is no universal agreement on criteria to define deficiency, although an increase of >25% in the ETK activity coefficient following addition of ThDP has been proposed as indicating functional thiamine deficiency and an increase >15% and <25% has been proposed as indicating marginal thiamine status (Whitfield et al. 2018).

Free (unphosphorylated) thiamine, thiamine monophosphate, and ThDP can be measured by HPLC, and of these ThDP measured in whole blood or erythrocytes is considered the best indicator of thiamine status. Whole blood ThDP has been shown to correlate with erythrocyte ThDP, making sample preparation simpler and quicker as this obviates the need to prepare washed red blood cells. There has been no standardization of thiamine assays and there remains high variability between laboratories and methods. Thiamine is photosensitive and samples for any assay need to be treated correctly to avoid loss of the analytes; samples should be kept in the dark and stored and transported at or below -20°C to prevent degradation of the analytes and to lyse the red blood cells prior to analysis.

Sample Type

Whole blood / red blood cells (erythrocytes)

Laboratory methods for measuring thiamine status biomarkers and their respective advantages and disadvantages

Method	Advantages	Disadvantages
ETK	Functional assay of status Equipment is less expensive Small sample volume required No calibration required	Not readily available Constant temperature over the whole plate is required Needs an experienced analyst Inter-assay precision can be poor
HPLC	Direct measure of thiamine status	Fairly expensive equipment No suitable internal standards Chromatographic separation required Larger sample volume (>300 µL) required
LC-MS/MS	Indicates thiamine status	Very expensive equipment

	Isotopically labelled internal standards available Chromatographic separation not required	

Equipment required for ETK assay

Microplate incubator and photometer

Equipment required for assay HPLC

HPLC with UV detector *or* LC-S/MS

Centrifuge

Multi-tube vortex mixer

Vacuum manifold

Spectrophotometer

Notes

Thiamine is light sensitive, so sample preparation for HPLC should be carried out under gold light

Freezing samples will lyse the erythrocytes, so samples for the ETK assay using red blood cells must be washed prior to freezing.

EQA Schemes

Royal College of Pathologists of Australasia (RCPA) for thiamine metabolites.

None for ETK assay.

Folate (vitamin B9)

Folate plays a key role in the development, growth and maintenance of cells. Folate nutrition is of public health concern because folate insufficiency during pregnancy leads to birth defects, specifically neural tube defects, and severe folate deficiency is a cause of nutritional anemia. Serum and red blood cell folate are measured to assess folate status. Serum folate is an acute indicator of recent intake or changes in folate metabolism, and red blood cell folate is a marker of longer-term status that correlates with tissue stores.

There are three main types of assays to measure folate. The BOND review (Bailey et al. 2015) and WHO (2015) recommend the use of protein binding assays in the clinical setting where high throughput is essential, the microbiological or LC-MS/MS assay in public health settings, where assay stability is required, and LC-MS/MS in research work where differentiation of the various forms of folate is needed. Choosing between the microbiological and the LC-MS/MS method will depend on the availability of resources, sample type, sample volume and whether total folate or individual folate species are of interest. Red cells contain polyglutamate folates that must be converted to monoglutamates before

analysis. There remain difficulties with this conversion for analysis by LC-MS/MS, so this method is currently only recommended for serum analysis, where the folates are already in monoglutamate form. Improvements in the microbiological assay (Zhang et al. 2018) have enhanced its robustness; and the introduction of a chloramphenicol resistant bacterium means that sterilization and aseptic addition are no longer required, making this assay particularly well suited for low income settings. Both the microbiological assay and LC-MS/MS require skilled laboratory staff.

Sample Type

Serum /plasma / whole blood / dried blood spot (DBS, for microbiological method only)

Laboratory methods for measuring folate and their respective advantages and disadvantages

Method	Advantages	Disadvantages
Protein binding assays	Very high throughput Direct sample assay Minimum operator input	Total folate only Limited dynamic range Underestimation of some folate species
Microbiological assays – preferred method	High sensitivity Inexpensive Low technology	Total folate only Sensitive to antibiotics and antifolates Less precise than other methods 2-day assay Experienced analyst
Chromatography	Individual species measured Chromatographic separation Large dynamic range	Experienced analyst required Sample preparation required Non-specific internal standards Larger sample volume (>300 µL) required
LC-MS/MS	Individual species measured High sensitivity High Specificity Matched Internal standards Large dynamic range	Experienced analyst Expensive equipment Sample preparation required Chromatographic separation limited

Equipment required for microbiological assay

Incubator

Plate reader

Pipetting robot (optional, depending on required throughput)

Spectrophotometer

Reagents – The Centers for Disease Control and Prevention (CDC), Atlanta, US provide a training program and kit with all the reagents including the bacterium ready for use (CDC Foundation 2020)

Equipment required for LC-MS/MS assay

LC-MS/MS

Positive pressure manifold

Multi-tube vortex mixer

96-well plate shaker

Spectrophotometer

Stable isotope-labelled internal standards >$10,000 (this will last a long time but require the initial outlay)

Notes

Folate is sensitive to temperature and UV light, so samples should be stored at ≤-70°C and analyzed under gold light

National Institute of Standards and Technology (NIST) Standard Reference Material and WHO standards from the National Institute for Biological Standards and Controls (NIBSC) are available

Available quality assurance schemes

UK National External Quality Assessment Services (NEQAS) haematinics, CDC Vitamin A Laboratory – External Quality Assurance (VITAL EQA), and CDC Performance Verification Program for Serum Micronutrients (CDC 2019)

Additional resources

BOND folate report (Bailey et al. 2015)

Nutrition International folate task team (Nutrition International 2020)

Vitamin B12 (cyanocobalamin)

Vitamin B12 (cyanocobalamin, also called cobalamin) was first recognized historically as the cause of pernicious anemia. It is now known that there is also a high prevalence of dietary deficiency in many populations. There are four available biomarkers for vitamin B12 status: 1) serum vitamin B12, 2) serum holo-transcobalamin (HoloTC), 3) plasma homocysteine (Hcy), and 4) serum or urine methylmalonic acid (MMA). Serum vitamin B12 gives information on long term status; the assays are widely available and are unaffected by age or infection. It gives an indication of vitamin B12 bound to transcobalamin, which is metabolically active, and to haptocorrin, which may not be available for cellular uptake. Serum HoloTC provides information on serum B12 available to cells; it is most sensitive to recent intake and is also unaffected by infection. Hcy and MMA are both functional markers of vitamin B12; increased serum MMA reflects inadequacy of B12 for metabolic function and is the most sensitive marker of vitamin B12

status, although MMA also increases with renal dysfunction and with bacterial overgrowth. MMA also tends to be higher in low income populations independent of vitamin B12 status, and with a specific genetic polymorphism (Molloy et al. 2016). Increased Hcy also reflects inadequacy of vitamin B12 for metabolic function and responds rapidly to improvement in vitamin B12 status of deficient individuals. Hcy is not specific for B12, as it also increases with deficiencies of folate, riboflavin and vitamin B6 as well as with renal insufficiency and hypothyroidism.

No single biomarker provides sufficient sensitivity and specificity for diagnosing vitamin B12 deficiency. In recent years there has been a move towards multi-analyte testing to address this problem, and most people advocate for measuring a direct marker of vitamin B12 status (i.e., serum vitamin B12 or HoloTC) along with a functional biomarker (MMA or Hcy). In 2012 an NHANES expert committee decided that active B12 (HoloTC) was a better direct biomarker than serum B12, and MMA was a preferable functional marker rather than Hcy due to the lack of specificity of Hcy. Thus, we focus primarily on HoloTC and MMA when describing the required laboratory equipment. Nevertheless, in situations where it is not possible to measure these two biomarkers, another option is to assess serum vitamin B12 and Hcy.

Sample Type

Plasma / Serum for each of the four biomarkers

Urine (for MMA only)

Laboratory methods for measuring vitamin B12 and their respective advantages and disadvantages

Method	Advantages	Disadvantages
Immunoassay (vitamin B12)	Automated High throughput Rapid turn around time Inexpensive reagents	Expensive equipment Samples and calibrators should be protected from light Equipment needs regular maintenance Interference from anti-intrinsic factor antibodies, so cannot be used in patients with pernicious anemia Variation between platforms
Microbiological assay (vitamin B12)	Inexpensive	Can be affected by antibiotics Experienced analyst required Sample preparation required
Competitive protein binding radio-assay	Inexpensive High throughput	Radioactivity Gamma counter required High variability
Automated antibody assay (HoloTC)	Automated High throughput Rapid turn around time Inexpensive reagents Harmonization of methods	Expensive equipment Equipment needs regular maintenance

ELISA (HoloTC)	Rapid turn around time Inexpensive reagents Can be automated	Only a few manufacturers of kits
LC-MS/MS or GC-MS/MS (MMA and Hcy)	Small sample volumes Precise Specific High throughput	Expensive equipment Sample preparation required Experienced and skilled analyst required Equipment requires expert maintenance
HPLC with fluorescence detection (Hcy)	Small sample volumes Precise Specific High throughput Instrumentation commonly available	Fairly expensive equipment Sample preparation required Experienced analyst required Equipment requires expert maintenance
Immunoassay (Hcy)	High throughput Quick turn around time Commercial kits available Good precision Small sample volume	Limited range Relatively high reagent cost Expensive equipment Equipment needs regular maintenances Requires patented antibody
Enzymatic assay (Hcy)	Very small sample volume Can be automated	Sample preparation required Background determination of D amino acids needed
Capillary electrophoresis with laser induced fluorescence	No solvent use Can be automated Short analysis time	Requires specialized expensive equipment Experienced analyst required Complex sample extraction with derivatization

<u>Equipment required for ELISA assay for HoloTC</u>

Plate reader

Plate washer

<u>Equipment required for LC-MS/MS assay for MMA</u>

GC *or* LC-MS/MS

Multi-tube vortex mixer

96-well plate shaker

Centrifuge

Sample concentrator with heating abilities

<u>Notes</u>

For serum vitamin B12 measurement, samples should be protected from light during collection and handling.

There are standard reference materials available from NIST for serum B12 and homocysteine

EQA Schemes

UK NEQAS haematinics, RIQAS immunoassay, WEQAS haematinics, and CDC Performance Verification Program for Serum Micronutrients (CDC 2019)

Additional resources

BOND Vitamin B12 reports (Green 2011; Allen et al. 2018)

Biomarkers of vitamin B12 status in NHANES: a roundtable summary (Yetley et al. 2011)

Vitamin D

Vitamin D is a fat-soluble vitamin essential for maintaining calcium homeostasis and bone health, including prevention of rickets. Vitamin D deficiency has also been implicated in a wide range of other adverse health outcomes, such as hypertension, diabetes, and a range of autoimmune and infectious diseases. Several reviews have highlighted the fact that there are very few nationally representative studies of population vitamin D status. However, available information from smaller-scale studies suggests that vitamin D deficiency may be widespread, even in peri-equatorial countries where sunlight exposure is limited because of cultural practices related to clothing and outdoor activities. (Roth et al. 2018).

The recommended biomarker of vitamin D status is serum/plasma 25 hydroxy-vitamin D (25(OH)D). Laboratory measurements of 25(OH)D have shown significant inter-assay and inter-laboratory differences, which has motivated efforts to standardize procedures and increase the use of LC-MS/MS as the reference measurement technique. Immunoassays have shown varying levels of imprecision partly due to cross reactivity between 25 hydroxyvitamin D_2 and 25 hydroxyvitamin D_3 as well as interference from other vitamin D metabolites, such as 24,25-dihydroxyvitamin D.

LC-MS/MS is able to distinguish all vitamin D metabolites, although this method requires cinematographic separation of the C3-epimer of 25(OH)D from 25(OH)D. The high specificity and accuracy of LC-MS/MS have established it as the reference method, although fully trained staff are essential.

Sample Type

Serum /plasma

Laboratory methods for measuring vitamin D and their respective advantages and disadvantages

Method	Advantages	Disadvantages
LC-MS/MS – preferred method	Sensitive Specific Matched internal standards	Sample preparation required Expensive equipment Experienced analyst required

	Distinguishes between individual vitamin D metabolites	Many columns cannot separate the C3 epimer
HPLC with UV detection	Less expensive than MS Distinguishes individual vitamin D metabolites	Fairly expensive equipment Sample preparation required Experienced analyst required Many columns cannot separate the C3 epimer Low sensitivity Non -specific internal standards
Immunoassay	No cross reactivity with epimers Can be automated No pre-assay sample preparation required High throughput	Cross reactivity with other vitamin D metabolites Unequal binding to the antibody of $25OHD_2$ and $25OHD_3$ Automated systems are expensive

Equipment for LC-MS/MS assay

LC-MS/MS

Centrifuge

Vacuum evaporator *or* sample concentrator

Multi-tube vortex mixer

96-well plate shaker

Spectrophotometer

Notes

Solvent supply, storage and disposal need to be considered

NIST Standard Reference Material is available

EQA Schemes

UK NEQAS vitamin D, DEQAS, Vital EQA and CDC Performance Verification Program for Serum Micronutrients (CDC 2019)

Additional resources

Vitamin D Standardization Program (NIH 2018)

Iodine

Iodine is a mineral required for the synthesis of thyroid hormone, hence its importance for energy metabolism and physical growth and development. Iodine deficiency impairs thyroid hormone production and causes multiple adverse effects throughout the life cycle (Zimmermann and Boelaert 2015). In particular, severe iodine deficiency causes thyroid enlargement (goiter) and hypothyroidism. Mild iodine deficiency and excessive iodine intake can both impair thyroid function, and iodine deficiency early in life may impair neuromuscular and cognitive function.

Urinary iodine concentration (UIC) measured in spot urine samples is the recommended biomarker of population iodine intake (WHO, 2013). UIC is a good marker of recent intake, as more than 90% of ingested iodine is excreted within 24–48 h (Zimmermann and Andersson 2012). However, dietary iodine intake varies considerably from day-to-day, so UIC is not a reliable biomarker of individual status (König et al. 2011). WHO defines adequate iodine intake in populations based on the overall median UIC (WHO 2013)(**Table 4**).

Thyroglobulin (Tg) measured in serum or whole blood on dried blood spots has proved to be a sensitive biomarker of iodine status. Neonatal thyroid stimulation hormone (TSH) may be used to assess iodine sufficiency in the population. Measurement of serum TSH and one or both thyroid hormones is routine in clinical settings to diagnose thyroid disorders, but thyroid function markers are usually within normal ranges in mild iodine deficiency and not recommended for monitoring iodine nutrition in populations (Rohner et al. 2014).

Many analytical methods for measuring UIC are based on spectrophotometric measurements of the Sandell-Kolthoff reduction reaction catalyzed by iodine and using varying oxidizing reagents. These have been modified to include semi-quantitative methods, microplate methods and automated methods. Inductively coupled plasma mass spectroscopy (ICP-MS) is considered the most accurate method of analysis of urinary iodine and is often used as the reference technique, Although WHO recommends the Sandell-Kolthoff-method for epidemiological studies.

Sample Type

Urine

Laboratory methods for measuring iodine status biomarkers and their respective advantages and disadvantages

Method	Advantages	Disadvantages
ICP-MS	Reference method Most accurate measurement Good precision Minimal sample preparation High sample throughput	Expensive equipment Experienced analyst required
Spectrophotometric (Sandell-Kolthoff)	Inexpensive equipment Can be semi-automated Can be semi-quantitative If automated, sample throughput can be increased	Sample preparation required Toxic waste generated
Autoanalyzer	High throughput	Expensive equipment

Equipment needed for ICP-MS assay

ICP-MS

Notes

Iodine contamination from external sources is common, so meticulous attention is required during sample collection and analysis to avoid this.

EQA Schemes

CDC EQUIP, Centre de Toxicologie du Quebec PCI and QMEAS

Additional Resources

BOND Iodine report (Rohner et al. 2014)

Iron

Iron is used in the body for energy production, synthesis of hemoglobin and myoglobin for oxygen transport and utilization, cell proliferation, and immune function. Unlike other minerals, iron has no regulated excretion pathway, so absorbed iron is completely utilized as a component of functional proteins or is bound to storage proteins.

The absence of stainable iron in bone marrow is generally regarded as the gold standard for diagnosing iron deficiency, but the invasive nature of this procedure makes it an unsuitable tool for epidemiological studies. Iron status biomarkers can be categorized into biochemical or hematological indicators, and most analytical methods that measure these biomarkers are well established and widely used in clinical evaluation and research settings. Serum ferritin, serum iron and transferrin saturation (TSAT), serum transferrin receptors, and red blood cell protoporphyrin can be used to assess iron status and determine the severity of iron deficiency.

Serum ferritin is an important biomarker of iron status. Even though serum ferritin only represents a tiny fraction of the total ferritin, most of which is stored intracellularly, it is well correlated with body iron stores (Walters et al. 1973; Finch et al. 1986). Analysis of serum ferritin is widely available and well standardized using both automated analyzers and ELISA, and it is thought to be the only measure of iron status that can reflect both a reduction in storage iron as well as iron overload. Serum ferritin has the disadvantage that it is positively affected by acute and chronic inflammation and is therefore much more difficult to interpret in areas where infectious diseases are common. However, methods have been developed to adjust for the effects of inflammation on serum ferritin concentration (Namaste, Rohner, et al. 2017), and this remains the primary indicator of iron deficiency recommended by WHO (WHO 2011c, 2020).

Serum iron is bound to transferrin and exhibits a circadian rhythm with a morning peak and evening nadir. When absorption and release from stores is insufficient, serum iron levels fall and transferrin concentration increases (customarily expressed as total iron binding capacity (TIBC)). Transferrin saturation is a commonly employed biomarker in clinical nutrition studies as a marker of iron deficiency. Serum iron and TIBC are customarily reported together, but it must be recognized that the utility of

these markers, as with many markers of iron status, can be limited by circadian rhythm, infectious diseases and many other clinical disorders. In 1998, the Clinical and Laboratory Standards Institute (CLSI) approved standards for the measurement of serum iron, TIBC and TSAT (CLSI 1998). Rigorous elimination of iron contamination is a critical concern for manual colorimetric methods, but this has not been a problem with automated analyzers. Most automated analyzers measure unsaturated iron binding capacity (UIBC) and then calculate TIBC, as the measurement of UIBC is more easily automated. Comparisons between manual and automated methods have shown no significant differences and good precision. Serum transferrin can also be measured directly using an immunologic assay.

Transferrin receptors (TfR) are specific cell surface receptors that bind the iron-transferrin complexes that transport iron in plasma. The number of cell surface TfR molecules reflects the cellular iron requirement, and reduced iron supply results in up-regulation of TfR synthesis. The soluble transferrin receptor (sTfR) present in plasma is a truncated monomer of the transmembrane receptor. Recent studies have indicated that malaria infections and inflammatory disease are associated with slightly greater circulating levels of sTfR, more so in PSC than WRA (Rohner, 2017). Therefore, as with ferritin, the clinical interpretation of sTfR measurements is influenced by ongoing acute phase responses, albeit in the opposite direction. Moreover, there is poor agreement between commercially available sTfR assays, likely due to the use of standards derived from different tissues, different antibodies and different reporting units. An international reference material has been available since 2010, but use has been limited due to poor commutability. Lack of commutability and high reagent costs are reasons that this biomarker has not been widely adopted in clinical practice.

During normal heme synthesis, ferrous iron is incorporated into a protoporphyrin molecule. Zinc is an alternative metal substrate, which is normally found in just trace amounts. However, when iron levels are deficient, zinc protoporphyrin (ZPP) rises progressively, thereby providing an index of the severity of functional iron deficiency. ZPP is incorporated into the red blood cells while they are maturing and then remains there for their lifespan, so ZPP provides a measure of the iron available to the erythrocyte marrow in the preceding 3-4 months. Along with many other measures of iron status this can also be affected by other diseases. Nevertheless, values >150µmol/mol heme are highly suggestive of iron deficiency. Measurement of erythrocyte protoporphyrin by chemical extraction is no longer widely used due to the lengthy sample preparation using a hazardous chemical, so the hematofluorimeter is now being employed more often. This can be used in low resource settings, but has two limiting factors: 1) it needs to be regularly calibrated to ensure alignment of the mirrors and filters if transported under unfavorable conditions, and this calibration needs to be carried out by the manufacturer, and 2) there are concerns about interferences from non-porphyrin fluorescent compounds.

Multiplex assays to measure multiple biomarkers of iron status in a single ELISA assay are currently under development; but more work needs to be done to improve the precision, inter assay variability, assay ranges, standardization, and correlation with established methods before they can be used widely (Brindle et al. 2014; Brindle et al. 2017; Karakochuk et al. 2018).

Hemoglobin measurements are used to assess anemia at the individual or population level. The accepted reference method is the photometric determination method (Karakochuk et al. 2019); however, the reagents are light sensitive and toxic. Portable POC analyzers, like the Hemocue device, can also be used to complete Hb determinations in the field. The HemoCue analyzer is widely used and is calibrated against the reference method from the International Council for Standardization in

Haematology (ICSH). The largest source of error with this method is the use of improperly collected capillary samples, although the specific Hemocue model used may also affect the results (Neufeld et al. 2019; Rappaport et al. 2020). Hematocrit does not offer any further information in addition to Hb, so unless this is needed to normalize other blood-based measurements, it is not often measured in the field. Other red blood cell parameters, including MCV, MCH and RDW, are part of a full blood count profile on auto-analyzers, but these indices and reticulocyte counts are not used commonly in field surveys at present. Where relevant, Hb and other hematology indices can be measured in routine clinical laboratories, using automated devices without the need for transportation to a central laboratory.

Sample Type

Plasma / Serum for ferritin, TIBC, TfR; washed RBCs for ZPP; whole blood for Hb, RBC indices and reticulocyte counts.

Laboratory methods for measuring iron and their respective advantages and disadvantages

Method	Advantages	Disadvantages
ELISA (SF and soluble transferrin receptor)	Equipment relatively inexpensive Commercial kits available	Extensive sample preparation required Moderate precision Relatively high reagent costs Experienced analyst required
Automated immunoassay analyzers (SF, soluble transferrin receptor and transferrin	High sample throughput Quick turn around time Commercial kits available Minimum operator involvement Good precision	Moderately expensive equipment Equipment requires regular maintenance Lot-to-lot variability
Colorimetric assay (serum iron / TIBC)	Inexpensive photometric instrument Inexpensive reagents	Sample preparation required High sample volume Moderate precision
Clinical chemistry analyzer (serum iron / TIBC)	High sample throughput Quick turn around time Commercial kits available Minimum operator involvement Good precision Low cost reagent	Fairly expensive equipment Instrumentation requires regular maintenance Lot-to-lot variability
Automated flow cytometry (Hb, PCV and Hct)	Fully automated Good precision Multi blood cell analysis	Fresh blood specimens required Instrumentation requires regular maintenance
HemoCue (Hb)	Battery-operated, hand-held instrument Very small sample volume	Fresh blood specimens required

	Inexpensive Suited to low resource settings	Blood sampling technique needs to be standardized to avoid considerable variability
Microcentrifuge (Hct)	Inexpensive Little sample preparation Portable instrument Suitable for low resource settings	Fresh blood specimens required Needs power supply Poor reproducibility Capillary tubes need proper sealing and careful use
Hematofluorimeter (erythrocyte protoporphyrin ZPP)	Simple instrument Portable instrument Small sample volume required Inexpensive	Needs stable power supply Fresh blood specimens required
Chemical extraction with conventional fluorimetry (free erythrocyte protoporphyrin)	Stored blood samples can be used Suitable for DBS and washed red cells Small sample volume Inexpensive equipment	Extensive sample preparation required Moderate precision External fluorescent contamination possible Subdued lighting required Requires Hct to correct for packed cells

Equipment needed for colorimetric assay for serum iron / TIBC

Plate reader

Plate washer

Notes

The above equipment can be utilized for other ELISA or plate based assays with purchase of the reagents / kits to measure additional iron status biomarkers.

EQA Schemes

UK NEQAS general hematology, hematinics, specific proteins and clinical chemistry, RIQAS hematology, immunoassay and clinical chemistry and WEQAS hematinics and serum chemistry, and CDC Performance Verification Program for Serum Micronutrients (CDC 2019)

Additional resources

BOND iron report (Lynch et al. 2018)

Biomarkers Reflecting Inflammation and Nutrition Determinants of Anemia (BRINDA 2017)

Zinc

Zinc is a necessary component of multiple proteins involved in DNA synthesis, catalytic reactions and membrane structure. Zinc deficiency increases the risk and severity of common infections, like diarrhea and pneumonia, and impairs child growth. Zinc status is measured using plasma/serum zinc concentration; and this biomarker has been endorsed by several expert committees, especially for population assessment (Brown et al. 2004; King et al. 2016). Plasma/serum zinc concentrations respond to severe dietary zinc restrictions and supplementation, and they are related to clinical signs of zinc deficiency (Wessells et al. 2014). However, there are limitations with plasma zinc concentration measurement. For example, concentrations respond less to additional food containing zinc than to supplements; there is considerable inter-individual variation with changes in dietary zinc; and it is influenced by recent food consumption, time of day, inflammation and certain drugs or hormones (Hotz, Peerson, and Brown 2003). Several different methods can be used to measure plasma / serum zinc, depending on instrument availability and other laboratory infrastructure and support systems; there is no particular instrument that is preferred (Hall et al. submitted). In all cases, care must be taken during blood collection, processing, and analysis to avoid contamination with zinc in the environment.

Sample Type

Plasma / Serum

Laboratory methods for measuring zinc and their respective advantages and disadvantages

Method	Advantages	Disadvantages
ICP-MS	High sensitivity Ability for multi-element analysis Good precision Minimal sample preparation High sample throughput Small sample volume	Expensive equipment Experienced analyst required Matrix effects Higher operating costs
ICP-atomic emission spectroscopy	High sensitivity Good precision Minimal sample preparation High sample throughput Small sample volume	Expensive equipment Higher operating costs
ICP-optical emission spectroscopy	High sensitivity Good precision Minimal sample preparation High sample throughput Small sample volume	Expensive equipment Higher operating costs
AAS	Less expensive operating costs Minimal sample preparation Relatively sensitive	Relatively expensive equipment Interference from atmosphere or other compounds in sample
Neutron activation analysis	Non-destructive Multi-element analysis Very sensitive High accuracy High precision	Nuclear reactor required Expensive equipment required Radioactive element produced Very slow analysis time

	Little sample preparation	
Proton induced x-ray emission	Non-destructive Multi-element analysis	Very expensive equipment Experienced analyst required

Equipment (depending on assay method)

ICP-MS

Flame atomic absorption spectrometer

Notes

Due to low concentrations of plasma / serum zinc, laboratory facilities must be designed to prevent zinc contamination.

Gases are required; storage and use needs to be considered.

EQA Schemes

UKNEQAS trace elements and Centre de Toxicologie du Quebec PCI

Additional resources

BOND zinc report (King et al. 2016)

International Zinc Nutrition Consultative Group (IZINCG 2020)

Markers of inflammation

As noted previously, markers of inflammation are needed to help interpret some of the biomarkers of MN status, including iron status (ferritin), zinc and vitamin A (Raiten et al. 2015). CRP and AGP are the most commonly measured markers of systemic inflammation. CRP increases within 6 to 8 hours of an infectious or inflammatory disease state and concentrations peak between 24 and 48 hours. AGP (also known as orosomucoid) has a more delayed appearance profile and therefore provides a marker of infection 4 to 5 days after the initial immunological challenge. Ideally, both CRP and AGP should be measured to provide a more complete picture of the full course of an episode of inflammation.

Several approaches have been proposed for how best to use inflammation markers to adjust specific MN biomarkers for the presence of inflammation. One approach is to exclude observations with CRP > 5 mg/L and/or AGP > 1 mg/L. However, this method can result in large decreases in sample size and associated precision and introduce bias. Regression correction approaches developed by the BRINDA consortium provide a more nuanced approach that adjusts MN biomarkers across the concentration range (Namaste et al. 2017).

CRP and AGP can be measured by ELISA, with clinical chemistry analysers using immunoturbidimetry, or with antibody-based nephelometric methods using a nephelometer. For both CRP and AGP, immunotubidometric assays have a number of advantages: they are easier to run, provide greater throughput and do not require sample pre-dilutions. Earlier clinical chemistry assays for CRP were

designed to identify the absence or presence of acute inflammation (typically CRP > 5 or 10 mg/L) to support clinical decisions. These assays typically measured to ~3 mg/L. Modern clinical chemistry based systems typically offer 'high sensitivity' (hsCRP) or 'extended range sensitivity' assays. hsCRP is used to identify small, chronic elevations in CRP as a cardiovascular risk marker; these assays typically measure to 0.5 mg/L or lower with an upper range in the tens of mg/L. Extended range assays tend to have a higher limit of quantitation (~1 mg/L) and greater range into 100s of mg/L and therefore may be more suitable for interpretation of MN status. hsCRP tests are not recommended during acute illness. Extended range assays are also generally more economical than hsCRP assays. While the work of the BRINDA consortium has shown that relationships between inflammation markers and MN status are linear, CRP cut-offs for the effect and relevance of the lowest concentrations of CRP for interpretation of MN status concentrations are unconfirmed.

There are no reference methods for either CRP or AGP, so it is not possible to recommend one method over another; the choice of assay may depend on cost, availability and throughput, and users are recommended to consult the literature for up to date performance of particular assays. The absence of reference methods also results in a lack of concordance between assays that can complicate comparison between studies.

Because of the broad range of CRP assays available, particular attention should be paid to the detection limit of the chosen assay. Consideration should be given to how values less than the limit of detection or quantification will be reported, as such values may influence the interpretation of the relationship between CRP and MN status (O'Callaghan and Roth 2020).

Sample type

Serum or lithium heparin plasma

Equipment

Clinical chemistry analyser; nephelometer; or (for ELISA) plate reader, plate washer

EQA Schemes

CRP – RIQAS Specific Protein EQA, NEQAS CRP and ultra-sensitive CRP schemes (immunochemistry EQA scheme), CAP CRP and high-sensitivity CRP schemes, WEQAS (CRP including hsCRP), RCPAQAP, hsCRP, CDC Performance Verification Program for Serum Micronutrients (CDC 2019)

AGP – RIQAS Specific Protein EQA, CDC Performance Verification Program for Serum Micronutrients (CDC 2019)

Appendix 5 – Laboratory quality assurance

To confirm the reliability of laboratory results and support inter-laboratory standardization of data, internal and external quality assurance programs will be essential core activities of laboratories participating in the MN data generation initiative. As noted in **Section 6.7** of this report, internal quality control measures are carried out routinely in clinical laboratories. External quality assessment and inter-laboratory standardization are somewhat more challenging and are described briefly in the following paragraphs.

Laboratory standardization and accreditation

External quality assessment (EQA) or proficiency testing allows a laboratory to assess how its own assay performs compares to other laboratories; and, more broadly, the compiled results can be used to assess how all laboratories and assay types are performing for that assay. This inter-laboratory standardization is necessary to allow for comparison of results across laboratories and application of a single set of cut-offs or reference ranges across laboratories and populations.

While the details of individual EQA programs vary, there are a number of common elements: 1) clinical specimens are provided by the reference laboratory as whole blood, serum, urine or lyophilized samples on a routine basis; 2) samples are analyzed in the participating laboratories, and results are submitted to a central repository; 3) the results are analyzed and compiled; and 4) reports are produced describing individual laboratory and method performance against a benchmark result. Some schemes may provide the participating laboratory with certification for the assay.

Most EQA schemes benchmark laboratory results against the trimmed mean of all submitted results or as method mean. For some schemes, results may also be compared to target values produced by a high-level reference method or reference measurement procedure (RMP). For example, RMPs are used to assign target values to materials distributed by DEQAS and CAP for 25(OH)D measurement. For some analytes, particularly those measured routinely in clinical settings, such as serum vitamin B12, there are multiple EQA schemes available, with hundreds of participating laboratories and well-characterized assays. For other specialized assays, like vitamin B1, there may be only a handful of participating laboratories.

For larger or sufficiently resourced smaller laboratories, formal laboratory accreditation may be an option. As an example, accreditation of vitamin D assays is provided by CDC's Vitamin D Standardization Certification Program, which evaluates the accuracy and reliability of vitamin D assays, certifying those that meet designated standards for a routine laboratory (± 5% mean bias compared to reference measurement procedure result and overall imprecision <10%) (CDC 2017). Standards for vitamin D analysis is stricter in reference laboratories, where bias should be ≤1.7% and imprecision ≤5%. The process of standardization as applied to the field of 25(OH)D analysis is well-documented and demonstrates what can be achieved with concerted effort and cooperation among researchers, manufacturers and other stakeholders (Binkley and Sempos 2014; Makris et al. 2020; Durazo-Arvizu et al. 2017; Cashman et al. 2016). CDC's EQUIP program for urinary iodine is another example of an excellent EQA system (Centers for Disease Control and Prevention 2020). However, CDC does not reveal

the identity of underperforming labs. This can be considered a limitation of the EQUIP program, as participation does not guarantee acceptable performance. CDC currently assists more than 126 iodine laboratories in more than 60 countries under this program.

In addition to specific assay accreditation, other schemes may cover wider aspects of the laboratory work flow and staff training and provide complete quality management systems. For example the College of American Pathologists Accreditation programs and the International Standards Organization (ISO) standards for medical laboratories (ISO 15189) are examples of such programs.

Certified reference materials

Certified Reference Materials (CRMs) are materials that are "homogenous and stable with respect to one or more specified properties and for which traceability and values of uncertainty at a stated level of confidence are established" (ATCC 2020). CRMs from the US National Institute of Standards and Technology (NIST) are referred to as Standard Reference Materials (SRM). Characterization of each batch of CRM requires considerable time and effort; consequently CRMs are typically fairly expensive and in short supply. As such, they are not designed for routine use, but are used to perform instrument calibration, to verify the accuracy of methods and to support the development of new assays and methods. NIST CRMs either have 'certified values', for which NIST has the highest confidence in the accuracy and where values have been determined from either reference methods or RMPs, or 'reference values' which are best estimates of the true value (NIST 2010). Reference values for WHO International Standards from the National Institute for Biological Standards and Control, UK (NIBSC) are consensus values derived from a number of laboratories and assays. Issues of commutability of the reference materials, that is, their applicability to different measurement systems and procedures also must be considered (Young 2018; Miller et al. 2018), but a full discussion of this issue is beyond the scope of the present document,

CRMs exist for most, but not all, of the main MN status biomarkers highlighted in this document (**Table 7**). In cases where CRMs do not yet exist in the appropriate matrix, for example for the thiamine erythrocyte transketolase assay and methyl malonic acid for vitamin B12 assessment, investments will be needed to produce these materials. Ideally, the MN data initiative's management team will be able to work with the government agencies that are charged with producing these materials to explain why CRMs for MN biomarkers are critically important.

Table – Certified Reference Materials currently available for MN status biomarkers

Analyte	CRM	Sample type	Cost*
Retinol (fat-soluble vitamins)	NIST 968f	Frozen human sera	$836
Retinol and carotenoids	NIST 1950	Frozen human plasma	$1117
Folate vitamers	NIST 3949	Frozen human serum	$894

Folate, WHO International Standard	NIBSC 95/528	Whole blood hemolysate; lyophilized.	$163
MTHF and folic acid	NIST 1950[a]		
Serum vitamin B12	NIST 3951	Frozen human serum	In development, not yet available
Vitamin B12, serum folate, holoTC, WHO International Standard	NIBSC 03/178	Serum; lyophilized.	$163
25OHD	NIST 972a	Frozen human sera	$819
25OHD (calibration solutions)	NIST 2972a	Calibration solutions	$730
Urinary iodine	NIST 3668	Frozen human urine	$1,133
Ferritin; WHO International Standard	NIBSC 94/572	Human recombinant	$164
Zinc, 25OHD	NIST 1949	Frozen human prenatal serum	$1,140
C-reactive protein	NIST 2924	Solution	$727
C-reactive protein	NIBSC 85/506	CRP in pooled human serum (freeze-dried)	$164
C-reactive protein	IRMM, product ERM-DA474/IFCC	Frozen human serum	$95

* As of July, 2020 from www-s.nist.gov or www.nibsc.org

[a] NIST 1950 (Metabolites in human serum) also includes amongst others homocysteine, lutein, carotenes, lycopene, retinol, tocopherol, 25OHD2, 25OHD3, vitamin D binding protein