TOWARDS AN ACCESSIBLE, NONINVASIVE MICRONUTRIENT STATUS ASSESSMENT METHOD: A COMPREHENSIVE REVIEW OF EXISTING TECHNIQUES

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August 21, 2024

ABSTRACT

Nutrients are critical to the functioning of the human body and their imbalance can result in detrimental health concerns. The majority of nutritional literature focuses on macronutrients, often ignoring the more critical nuances of micronutrient balance, which require more precise regulation. Currently, micronutrient status is routinely assessed via complex methods that are arduous for both the patient and the clinician. To address the global burden of micronutrient malnutrition, innovations in assessment must be accessible and noninvasive. In support of this task, this article synthesizes useful background information on micronutrients themselves, reviews the state of biofluid and physiological analyses for their assessment, and presents actionable opportunities to push the field forward. By taking a unique, clinical perspective that is absent from technological research on the topic, we find that the state of the art suffers from limited clinical relevance, a lack of overlap between biofluid and physiological approaches, and highly invasive and inaccessible solutions. Future work has the opportunity to maximize the impact of a novel assessment method by incorporating clinical relevance, the holistic nature of micronutrition, and prioritizing accessible and noninvasive systems.

***K*eywords** health sensing · mobile health · precision nutrition · micronutrients · nutrition assessment · malnutrition · point-of-care devices · accessibility

1 Introduction

Balanced nutrition is important for the development and functioning of the human body and can have many downstream health effects. The World Health Organization (WHO) reports that individuals with proper nutrition have increased lifespans, are more likely to break cycles of poverty, and have lower risks of disease, which impacts productivity and mortality [125]. Malnutrition is the most critical issue related to nutritional intake, with undernutrition associated with about 45% of deaths in children younger than five years old. Anemia is a major resulting disease, affecting 37% of pregnant women and 40% of children under 5. The WHO defines malnutrition as nutrient imbalance, which consists of deficiencies or excesses in essential nutrients [185]. It is a condition that includes several interconnected factors: 1) being overweight, 2) obesity, 3) diet-related noncommunicable diseases like anemia, heart disease, and diabetes, and 4) undernutrition, which encompasses being underweight, wasting, and stunting, as well as a lack of essential micronutrients.

According to Hummell and Cummings [68], malnutrition can be caused by insufficient intake, malabsorption, acute and chronic diseases, increased nutrient need, and weight loss surgery. The impact of acute or chronic diseases and weight loss surgery is self-explanatory, whereas other factors are less visible. Insufficient intake in particular can be influenced by a wide range of factors, making it a challenging problem. These factors include income, access to nutritious foods, culture, and upbringing (malnutrition patterns can be passed down to generations). Insufficient intake is a particularly high risk for low-income families. Furthermore, there is often a lack of awareness about insufficient intake, making

behavior change more difficult. Malabsorption can also be a cause, as the absorption of nutrients found in food and supplements is highly individual and can be affected by diseases, genetic issues, and one's stage of life. In addition, individual nutrient requirements change over time, and several factors can increase nutrient demand, such as growth from infancy to adulthood, pregnancy, lactation, and recovery from illness or other trauma.

Nutrients can be classified into two main categories: macronutrients and micronutrients. Both are essential in precise balance to prevent malnutrition, also referred to as nutritional imbalance. Macronutrients include carbohydrates, fats, and proteins, which are major energy sources and are required in larger amounts than micronutrients [64]. Although required in smaller amounts, optimal intake of micronutrients is crucial. Micronutrients include vitamins and minerals, and they play a critical role in chemical reactions that produce energy from macronutrients acquired from food, as well as other essential functions [50]. Micronutrient intake through diet or supplements is crucial because, unlike macronutrients, our bodies cannot synthesize micronutrients, nor can they be substituted for one another [20]. A precise intake amount is required and slight deviations can result in either deficiency or excess, both with significant health impacts [64]. For these reasons, micronutrient imbalance is a problem that is unique and deserving of attention.

Micronutrient Deficiency The estimated number of people with micronutrient deficiency is 2 billion worldwide [177]. It is also estimated that micronutrient deficiencies are the cause of between 425,000 and 745,000 deaths in children under five years old [21]. The population of the United States (US) has significant risk of micronutrient deficiency due to the prevalence of a high-energy, low-nutrient diet [50, 42]. The US' National Health and Nutrition Examination Survey (NHANES) estimates that 31% of the US population is at risk for micronutrient deficiency, with calcium, potassium, iron, and vitamins A, D, C, and E being of particular concern [50, 42]. Since NHANES is a survey of self-reported dietary intake with little biochemical testing, it is likely that this number is actually underestimating the true burden in the US. Deficiencies can cause developmental issues, metabolic disorders, impaired immune system, altered endocrine and cognitive functioning, chronic disease, and more. For example, low magnesium intake is associated with a greater risk of chronic diseases such as cardiovascular disease (CVD) [50]. Those most at risk for micronutrient deficiencies include children less than five years old, pregnant women, victims of chronic disease, and those living in developing nations [42, 23]. Risks of inadequate intake are exacerbated since different micronutrients are needed in different amounts at different stages in life [20]. In the first 1000 days of life, iron, iodine, folate, and vitamin D have high dietary requirements, and a failure to meet them could result in poor physical and cognitive development. During adolescence, iron, calcium, folate, and vitamin D intake is critical, especially for those who menstruate. Pregnancy sees an increase in iron, folate, vitamin B12, and vitamin D requirements. The elderly are more at risk for vitamin D, B12, and B6 deficiency. Medications that influence micronutrient status or absorption must also be more heavily considered during this stage. Micronutrient deficiencies rarely manifest alone, and it is common to see multiple deficiencies arise simultaneously [12].

Micronutrient Excess Like deficiency, micronutrient excess can also lead to health detriments, with the risk increasing as intake levels surpass an individual's upper limit [56]. Toxicity from micronutrient excess can occur in any person who exceeds this limit. However, there is an exceptional risk for vulnerable populations, namely infants, young children, and pregnant women [70, 135, 56, 46]. For example, an excess of iron increases the risk of diarrhea, sepsis, meningitis, and gut inflammation for infants and young children, with a lethal dose of 150 mg/kg [135]. Similarly, surplus iron can lead to an increased risk of gestational and type 2 diabetes for pregnant women [135]. Excess of vitamins A and D, calcium, and iodine additionally pose elevated health risks for these populations [135, 46]. Individuals in these groups could be affected by micronutrient toxicity because they are more likely to be taking supplements to address an initial deficit [22, 56]. When coupled with population-level nutrition interventions, such as food fortification, one's micronutrient intake increases, potentially surpassing the recommended value. Research suggests that ingesting an excess of micronutrients through diet alone is unlikely and may only occur for those who take supplements [46, 22, 135]. There is minimal research on micronutrient excess because it is usually limited to the above population groups. As a result, the work covered in this review largely focuses on micronutrient deficiency. For more information on excessive micronutrient intake and its specific effects for each micronutrient, we recommend referring to an up-to-date nutrition overview (e.g. Espinosa-Salas and Gonzalez-Arias [46]).

Micronutrition Assessment When a micronutrient imbalance is identified, it is addressed through intervention. This involves modifying diet, providing micronutrient supplements, or on a population scale, fortifying food products such as iodized salt and fortified flour [12]. Interventions need to be carefully planned and monitored to avoid providing too little or too much of a particular micronutrient. There is a strong need for tools that can help guide intervention programs to effectively reach at-risk populations while minimizing the impact on those who have sufficient nutrient levels. This requires employing "different risk assessment methods to make the monitoring process more efficient, reliable, and cost-effective" [12]. Nutritional status assessment is a way to evaluate an individual's overall nutritional health and is necessary to identify and address any imbalances. This review focuses on micronutrient status assessment

methods in particular because we find that existing methods are insufficient and new techniques are under-researched. The visibility of micronutrient status suffers as a result of this lack of attention, further perpetuating associated health issues. An ideal assessment method is *accessible* and *noninvasive*. An *accessible* method is one that is available to more individuals, without compromising clinical effectiveness. This can be achieved through improvements in cost, efficiency, or mobility. Such a method should ultimately alleviate the need for laboratory tests, decrease reliance on high-effort surveys (such as dietary intake logging), and increase efficiency and accuracy for clinicians. A *noninvasive* method would either eliminate the need for a biofluid sample or obtain it in an unobtrusive manner. An ideal method could obtain micronutrient status regularly, without much effort for the patient. In our review, we aim to consider both clinical and technical aspects of micronutrition assessment and explore the potential for novel, accessible, and noninvasive methods. We argue that such an interdisciplinary approach is key to finding optimal solutions.

1.1 Paper Scope

Few existing papers that review novel micronutrient assessment methods are comprehensive and clinically relevant [172, 156, 121, 78, 67, 25]. Campuzano et al. [25] and Kalita et al. [78] focus particularly on electrochemical sensors, with the latter considering few vitamins and mostly out of body status. Shi et al. [156] concentrate specifically on wearable sensors, and Nimbkar et al. [121] focus on microfluidic assessment. The novelty of our paper lies in its comprehensive review of accessible and noninvasive micronutrient assessment methods, uniquely emphasizing clinical relevance—an aspect often overlooked in prior work. It includes a background on micronutrients, tailored for a non-clinical perspective. We also explore a wide range of techniques for assessing physiology and biofluids, including assay-based technologies, electrochemistry-based methods, spectroscopy-based approaches, and analytic methods utilizing artificial intelligence (AI) and machine learning (ML). This review contributes several relevant tables throughout the paper and in the Appendix providing a comprehensive reference about micronutrients and their assessment. Ultimately, our paper calls for actionable potential opportunities to advance these methods for clinical use.

With the aim of pushing innovative research towards an accessible, noninvasive method of assessing micronutrient status, this review consists mostly of methods that are both accessible and noninvasive, or at least one of these. We additionally include few methods that are neither accessible nor noninvasive, yet are interesting and valuable for future work within this scope. This review does not include methods for assessing micronutrient levels in entities outside of humans, such as food or pharmaceuticals. Our focus is on vitamins and trace minerals (micronutrients), thus we do not discuss nutrients such as proteins, fats, and carbohydrates (macronutrients) or major minerals (e.g. potassium, calcium, etc.). These are sometimes considered separate from micronutrients because of their higher intake requirements and larger quantities in the body.

This review is structured as follows. We first present background information in Section 2. Next, we discuss micronutrient status assessment methods based on biofluid analysis in Section 3, and physiology-based methods in Section 4. We present suggestions for future work in Section 5. Finally, we end with a conclusion in Section 6, followed by reference tables in Section A.

2 Background

In this section we describe micronutrient characteristics, their presence in biofluids, and related physiological effects. We begin with a summary of micronutrients, their characteristics, their presence in biofluids, and their impacts on our physiological functioning. Such a background is necessary to contextualize solutions to micronutrient status assessment because of the variety of possible approaches.

2.1 Micronutrient Characteristics

Micronutrients are divided into three categories: water-soluble vitamins, fat-soluble vitamins, and minerals [24]. Water-soluble vitamins, such as vitamin C and the B vitamins, are absorbed directly into the bloodstream and are quickly excreted in urine. They are not stored for long periods in the body, so regular intake is necessary to prevent deficiencies, and there is less concern about toxicity from excess intake. On the other hand, fat-soluble vitamins like A, D, E, and K are absorbed into lymph vessels along with dietary fats. Fat-soluble vitamins are stored in larger quantities in fatty tissues and the liver, so deficiencies take longer to develop, and daily intake is less critical. However, due to their efficient storage and the lack of a rapid excretion mechanism, toxicity is more of a concern.

Minerals can be categorized as major or trace minerals based on the daily requirement. Major minerals, such as sodium, potassium, chloride, phosphorus, and magnesium, are required in amounts greater than 100 mg per day, while trace minerals like iron, copper, zinc, selenium, iodine, chromium, fluoride, and manganese are needed in amounts of 100 mg or less per day. Minerals are water-soluble and are absorbed directly into the bloodstream, sometimes with the help of

transport proteins. It is important to note that minerals have an electric charge, and their function and storage can be influenced by various factors. A comprehensive summary of the characteristics of micronutrients is presented in the Appendix in Tables 11, 12, and 13.

Dietary guidelines for intake vary across organizations and countries and are based on factors such as age and sex. These guidelines are not standardized due to the individualized nature of micronutrient metabolism, the diversity of micronutrients, their interactions, and the specific values at which they are needed. In the US, dietary reference intakes are used, mostly utilizing the Recommended Dietary Allowance (RDA). The RDA represents the average daily level of intake sufficient to meet the nutrient requirements of 97-98% of healthy individuals. The National Institutes of Health also defines terms to describe intake levels, such as an Upper Limit (UL), highlighting the potential for excess intake [120]. In cases where there is insufficient evidence to establish these guidelines, there exists the more general term, Adequate Intake, further emphasizing the uncertainty surrounding the appropriate levels of micronutrients in diet.

Micronutrient status assessment usually relies on the combination of the analysis of micronutrient biomarkers in biofluids as well as the physiological symptoms presented by an imbalance. Micronutrient imbalances have well-documented physical symptoms, which provides opportunities to apply noninvasive methods to determine micronutrient status [68, 109, 45, 39, 140]. These symptoms provide noninvasive insights into internal processes and help identify appropriate biomarkers for further testing. Micronutrient biomarkers are still debated, but can generally serve as a reliable gold standard for assessment methods [43].

2.2 Micronutrients in Biofluids

Biofluid analysis for micronutrient status remains challenging since it is often unclear how other biofluids reflect the gold-standard matrix for that micronutrient. Some biofluids include blood, saliva, sweat, tears, and urine. Besides blood, each of these can be collected and analyzed noninvasively. Blood and urine are clinically relevant for representing in-body micronutrient status, as will be demonstrated in Section 3.1. However, evidence is less clear for saliva, sweat, and tears [69].

In the clinical literature, the equivalence of saliva, sweat, and tears to blood and urine in micronutrient status assessment remains ambiguous. Some evidence for correlation of micronutrient levels in saliva with blood levels was found on a by-micronutrient basis [69]. For example, serum and saliva levels of vitamin D were found to have a correlation of 0.56 [11]. Additionally, some correlation of iron levels in saliva and serum (a blood derivative) have been found. However, validity remains inconclusive as some sources have found a high positive correlation between salivary and serum level [26, 60], while others report a high negative correlation [49, 10, 53]. Despite this, research still exists that aims to quantify micronutrient status with saliva as well as tears [145, 150]. Sempionatto et al. [150] in particular argue that tears are a good biofluid for analysis since they are noninvasive, less complex than blood yet still contain a "variety of biomarkers", and they "reflect concurrent blood levels" because of passive leakage of compounds from blood plasma. It is important to note, however, that neither of these works provide comparison to the in-body status of their target micronutrients as measured by gold-standard, urine or blood-based assays.

The relevance of sweat for micronutrition seems to have been the subject of more research. Indeed, sweat receives significant attention in emerging micronutrient detection methods, especially in wearables. When we discuss sweat, we refer to eccrine sweat. Eccrine sweat is mostly sodium and chloride, with smaller amounts of micronutrients and metabolites (at the micro and nanomolar scale), similar to or smaller than their concentrations in blood plasma [14]. Micronutrients found in sweat include potassium, calcium, magnesium, iron, copper, zinc, vitamin C, and vitamin B1. Out of the compounds in sweat, mostly sodium and chlorine ions are well-studied. Some research has explored water-soluble vitamins in sweat, such as vitamin C and vitamin B1, but there is no such attention on fat-soluble vitamins. Many confounding factors can impact sweat composition, such as whether the sweating is passive or active. Active sweating usually produces higher concentrations of sodium, chloride, and metabolites. Contamination of sweat by skin-derived substances, notably iron, can affect composition as well. This is combated by pre-rinsed skin, removal of initial sweat (concentrations stabilize after 20 to 30 minutes of sweating), and the analysis of cell-free sweat. The region and method of collection can also have an impact, as many micronutrient concentrations can vary two to four times depending on the region. Finally, sweat can be reabsorbed into the body. Skin temperature and the flow rate of sweat both impact the rate of this reabsorption.

Unfortunately, the clinical literature notes a general "lack of association between dietary micronutrient intake and corresponding sweat micronutrient concentrations" [14]. This lack of an association exists in comparison to blood as well. A review by Baker and Wolfe [14] finds that there is no established correlation between sweat and blood composition, and there is "little support for using sweat as a surrogate for blood". Concentrations of minerals in sweat are much more varied than in plasma, likely because minerals bind to carrier proteins in blood. The review also reports little to no correlation between sweat and blood concentrations of vitamin C and iron status. This finding for iron is

echoed in another paper that found both iron and calcium have no correlation between sweat and blood concentrations [13]. However, it was reported in the same paper that iron concentrations in sweat have been observed to be lower in anemic patients and higher in patients undergoing iron therapy.

2.3 Micronutrient Effects on Physiological Processes

Most of the physiological effects of micronutrients are related to the autonomic functions of the body. The literature documents the importance of micronutrients to the proper functioning of the nervous system [1, 54, 32]. For example, a study of vitamin B12 deficiency compared responses to a 60-degree passive head up tilt test between a control group, a vitamin B12 deficient group, and a group with diabetes mellitus [16]. They found that the deficient group had comparable autonomic neuropathy to the diabetic group. Our exploration of observable impacts of deficiencies on autonomic functioning found three main affected areas relating to biofluid composition and physiological effects: cardiac functioning, sleep, and general symptoms.

2.3.1 Cardiac Function

One main aspect of autonomic functioning affected by nutrient imbalance is cardiac functioning. Several studies explore the interaction between heart rate variability (HRV) and micronutrient deficiencies. Components of HRV are associated with parasympathetic (PNS) and sympathetic nervous system (SNS) activity [154]. High-frequency (HF) bands reflect PNS activity and correspond to the respiratory cycle, while low-frequency (LF) bands reflect PNS, SNS, and baroreceptor activity. Vitamin B12 deficiency is one of the most documented in terms of impact to HRV, with evidence that it lowers HRV overall, impacting sympathetic indices the most [8, 166, 102, 16]. Supplementation of B12 was also demonstrated to return HRV indices to a comparably normal state [8]. Deficiency of vitamin D was found to lower HRV as well [102]. Calcidiol (25(OH)D) levels, a form of vitamin D, was shown to be associated with the ratio of LF to HF HRV power [108]. This metric is sometimes called sympathovagal balance and is intended to be a measure of 'balance' between SNS and PNS activity, but there has been debate over this interpretation [154]. Iron-deficiency anemia (IDA), an advanced form of iron deficiency, has more conflicting evidence of HRV impacts, with some studies finding no difference in HRV indices versus the control [171] while others were able to find a difference in the IDA group [75, 188].

Impacts of micronutrients on blood pressure have also been studied [32, 16, 168]. The supplementation of potassium, magnesium, zinc, vitamins C, D, B6, and a decreased intake of sodium and selenium can "positively modulate blood pressure levels" [32]. The aforementioned study involving responses to a head up tilt test in vitamin B12 deficient people found a drop in systolic blood pressure 60 beats after the test [16]. This finding aligns with previous work suggesting that a dip in blood pressure when standing up from sitting or lying down is a symptom of vitamin B12 deficiency [168].

2.3.2 Sleep

Another area of research is the role of micronutrient status in sleep. Sleep duration is associated positively with iron, zinc, and magnesium and negatively with copper, potassium, vitamin A and vitamin B12 levels [74, 18]. Sleep quality increases with zinc, magnesium, and vitamin B9 status and is negatively associated with vitamin B12 status [31, 71, 18]. There are conflicting findings for iron. One study reports that iron status is not proven to be correlated with sleep quality [73], while another claims that supplementation had positive effects on sleep disorders [98].

2.3.3 General Symptoms

Deficiencies can be classified as either clinical or subclinical based on their severity [167]. Most deficiencies result in symptoms of general fatigue, lethargy, irritability, muscle pain, weakness, and headaches. Clinical deficiencies often have more distinguishable symptoms, while subclinical deficiencies are limited to the above non-specific ones. Deficiencies of vitamin C, B vitamins, iron, magnesium, and zinc have been linked to fatigue more so than others [167, 9]. While energy and fatigue are more subjective and can can rely on subject-reporting, there are some established and validated assessment methods such as the Multidimensional Fatigue Inventory [158] and the SF-36 Vitality Scale [181]. A comprehensive reference for physiological symptoms associated with deficiency is lacking in literature, so we provide one in Tables 14, 15, and 16 within the Appendix.

3 Biofluid Analysis Methods for Micronutrient Status Assessment

This section describes how biofluid analysis has been leveraged to assess micronutrient status in humans. These assessment methods target particular biomarkers within a biofluid that are indicators of micronutrient status. Reliable

biomarkers are a research challenge themselves (which AI techniques may address [36]), but clinical literature shows that micronutrient biomarkers are more established and specific than macronutrient biomarkers [43]. Most micronutrients have one or two specific biomarkers associated with their circulating status that are considered to be the 'gold standard' for status assessment. Although some gold standards are still debated, their existence makes the evaluation of novel assessment methods more straightforward.

3.1 Clinical Biochemical Analysis

Clinical biochemical analysis involves laboratory testing of biomarkers (biological compounds) found in urine, blood, or other biosamples [142]. Results can be influenced by several factors and need to be interpreted in the context of other factors of the patient's health. Additionally, biochemical testing is often time and resource-intensive. Despite this, biochemical analysis describes clinical gold-standard methods for quantifying the circulating micronutrient status in the body [189, 69]. These methods can be roughly separated into assays and liquid chromatography (LC)-coupled spectroscopy. Assays work on the principle that specific micronutrients are needed for the growth of certain bacteria, and this growth can be measured to indicate the amount of a micronutrient present in a sample [189]. Assays were previously the widely-accepted gold standard, but improvements in LC and spectroscopy highlight their relatively poor precision and accuracy. These flaws have relegated assay methods to be used mostly during screening or in resource-constrained testing, except for some micronutrients, where they remain the standard. A common assay applied in studies is the enzyme-linked immunosorbent assay (ELISA). An ELISA test is used for measuring antibodies in blood, and is a useful clinical screening tool for further testing [88]. While not the gold-standard for the assessment of micronutrient status, ELISA tests can yield valuable results for studies that are time or resource limited.

Modern gold-standards overwhelmingly apply LC-coupled spectroscopy [189, 69] (Tables 17 to 19). LC is defined as "a separation process used to isolate the individual components in a mixture" [29]. High-performance LC (HPLC) uses pressure to facilitate the separation process, reducing the time required. It is commonly coupled with mass spectroscopy (MS) in gold-standard approaches [189, 69]. Spectroscopy is the "investigation and measurement of spectra produced by matter interacting with or emitting electromagnetic radiation" [2]. Every molecule reacts to the applied radiation in a unique way that allows us to "detect, determine, or quantify the molecular and/or structural composition of a sample" [2]. MS is the most important subfield of spectroscopy to understand for biochemical analysis. It measures the mass-to-charge ratio of the molecules in a sample as a way to determine and quantify the composition of molecules in the sample [2]. This is done by vaporizing the molecules in a sample into gas-phase ions, which are then sorted by their mass-to-charge ratios. We will discuss other forms of spectroscopy and their utility for micronutrient status assessment further in Section 3.4.

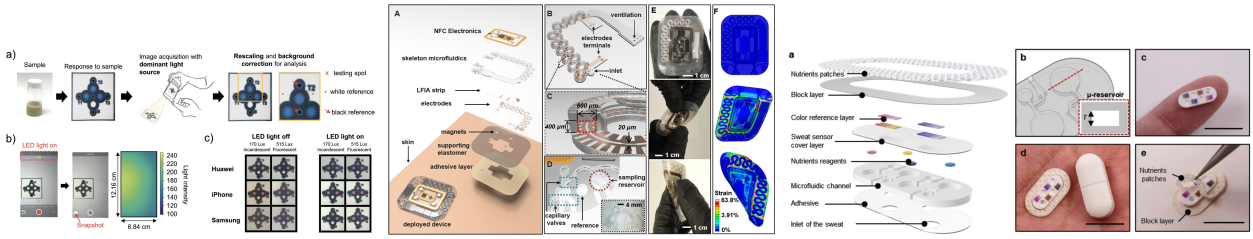
Matrices are the biosamples that are the subjects of the aforementioned methods of analysis. Most are blood based, but in a few cases urine is used in the gold-standard (mostly for water-soluble vitamins) [69]. Blood matrices are whole blood, washed red blood cells, plasma, and serum. Whole blood is blood as it is from the vein. Washed red blood cells are red blood cells that have been separated from the other components of blood such as plasma, platelets, and white blood cells [81]. Plasma is obtained by adding an anticoagulant to whole blood and placing it in a centrifuge [165]. Serum is obtained similarly to plasma, except the blood is allowed to clot before centrifuging.

As we proceed to review the non-clinical methods of biofluids analysis (Sections 3.2 to 3.5), we will be paying attention to what each approach claims to assess, the method by which they conduct this assessment, how their solution was evaluated, and the clinical relevance of their implementation. This last element includes the target biomarker and the concentrations of that biomarker that are evaluated. A clinically-relevant method should closely align with the gold-standard on these factors (Tables 17 to 19). To aid future work that may wish to integrate or innovate on a particular method, we explicitly mention when a study does not demonstrate this agreement. We also provide tables that group together similar works and summarize the pertinent details and quantitative results of their evaluation, if available.

3.2 Assay-Based Technology

One of the largest areas of work applicable to micronutrient status explored by this review is quantitative assays. Although we cover many different types of quantitative assays, a comprehensive review of lateral flow quantitative assays by Urusov et al. [174] provides a robust background for how these devices work. Lateral flow (immunochromatographic) assays indicate that a target compound is either present in the sample or present in excess of a particular threshold, usually via staining on the test membrane. These types of assays are useful for tests that benefit from quick conclusions (e.g. pregnancy tests). The most prevalent approach for extracting quantitative information from lateral flow assays is via optical signal registration [174]. This method involves the analysis of absorbed and reflected light from the test surface and the staining upon it, similar to the practice of spectrophotometry. The test/control ratio is a common metric used to quantify the magnitude of this staining relative to a control or reference area. Some commercially-available

devices are limited to automatically confirming the presence of the test line, while others can use line intensity to calculate analyte content. Urusov et al. [174] note that portability has become a recent focus in this market, and this is not just limited to specialized devices in a portable form factor. Smartphones have been successfully used for optical signal registration, even with fluorescent labels that decrease detection limits. Some manufacturers provide their own smartphone apps for quantitative analysis, but controlling for lighting and positioning is a challenge. To address this issue, another approach is the use of a standardized scanning device to collect image data and the off-device analysis of the image by specialized software. More experimental approaches, such as magnetic and electrically conductive labels, have also surfaced but have yet to mature. Tables 1, 2, and 3 present a summary of assay-based methods developed for micronutrients.



(a) (b) (c)

Figure 1: (a) A smartphone camera and LED flash can be used to analyze results from a colorimetric assay. Used with permission from [91]. (b) The design of a colorimetric sweat sensor using microfluidics. Used with permission from [85]. (c) The design of a multiplexed sweat sensor using microfluidics. Used with permission from [86].

3.2.1 Sweat-Based Colorimetric Sensors

Colorimetrics uses reagents to react with an analyte and change color to indicate concentrations of target substances. This color can be analyzed by a camera, such as in a smartphone as demonstrated in Figure 1a by Kong et al. [91]. Most sweat-based sensors use microfluidic devices for the collection of sweat [76]. These devices use very tiny valves and channels to "capture and store sweat on the surface of the subject's skin" via the natural pressure of sweat glands [76]. Sekine et al. [149] demonstrate a microfluidic skin patch with fluorometric probes that target chloride, sodium, and zinc in sweat. A smartphone attachment was designed to take light from the camera's flash and pass it through an excitation filter, allowing only a particular wavelength through. This excites the fluorometric probes, which emit light that can be captured by the smartphone camera. An emission filter is used to only let fluorescence emission wavelengths through. Similar to other quantitative assays, normalized intensity can be calculated against a reference and used to determine target biomarker concentration. Calibration curves for each nutrient were determined by known concentrations in spiked synthetic sweat. The authors claim strong correlation and accuracy, but do not report exact results. Field studies were conducted where sweat was induced in human volunteers. Measured concentrations were compared to ion chromatography (chloride), atomic absorption spectrometry (AAS; sodium), and inductively coupled plasma mass spectrometry (ICP-MS; zinc). Again, no statistical analysis of results is presented and it appears zinc measurement was the most inaccurate and subject to the most variance.

A paper by Kim et al. [85] describes on-body colorimetric measurement of vitamin C, calcium, zinc, and iron using sweat as a biofluid (Fig. 1b). A bespoke colorimetric assay is used for each micronutrient, assessed with known concentrations in a buffer solution. Since temperature and pH can affect colorimetric results, the authors conducted tests within the normal range of body temperature and pH in sweat, finding only a slight shift in results. Uniquely, micronutrients can be supplemented transdermally through the patch itself. Multiple on-body tests were conducted. Sweat was induced by a sauna before and after supplementation (either orally or transdermally). Patch-based measurements were found to be correlated with ICP-MS results. Although the paper claims that "sweat chemistry correlates, at least semiquantitatively, to plasma chemistry" for these micronutrients [85], this claim is based on the time dynamics of concentrations after supplementation rather than a comparison to a gold-standard status assessment (Tables 17 and 19). Furthermore, the clinical literature points out that that vitamin C and iron concentrations in sweat have been shown to have little to no correlation with levels in blood [14, 13]. Therefore, this device provides insights into the rate of excretion of these micronutrients rather than their status. There is no analysis of possible measurement bias induced by transdermal supplementation at the point of sweat collection.

3.2.2 Sweat-Based Multiplexed Sensors

Multiplexed analyses combine and analyze data from assays with multiple sensors such as electrocardiogram (ECG), temperature, electrodermal activity (EDA), HRV data and more. Thus, these multiplexed sensors can enable more

complex physiologic monitoring and diagnosis. Kim et al. [86] developed an on-body biosensing platform that can collect and analyze cortisol, glucose, and vitamin C in sweat using microfluidics (Fig. 1c). The device includes a lateral flow assay for cortisol and fluorometric assays for glucose and vitamin C. Assay results are imaged with a smartphone (with special lenses in the case of fluorometry) and are analyzed to yield quantitative results. There are also electrodes for sweat rate and EDA, with Near-Field Communication (NFC) and Radio Frequency (RF) to power them and communicate results. The focus in the paper is on stress indicators. Field-testing of the device involved subjecting participants to "intensive work periods" (interrupted sleep schedule and caffeine intake) for 7 days then rest (regular schedule) and daily vitamin C intake (for 2 participants) for 14 days [86]. Spikes in vitamin C associated with intake could also be observed. The authors also claim they were able to assess changes in circadian rhythms through cortisol, but no trends in glucose were found.

For a more in-depth review of the nuances of creating wearable multimodal sensors with sweat collection and analysis capabilities, we direct the reader to [187].

Table 1: Assay-Based Methods Using Sweat

Method	Form Factor	Targets	Analytes	Evaluation	Control	Results	Notes	Source
Fluorometry	Wearable patch	Chloride, sodium, and zinc	Sweat	Human subjects	Ion chromatography (chloride), AAS (sodium), ICP-MS (zinc)	Zinc measurement was the most inaccurate and subject to the most variance	Statistical analysis of results is not presented	[149]
Colorimetry	Wearable patch	Vitamin C, calcium, zinc, and iron	Sweat	Human subjects	ICP-MS on diluted sweat samples and supplementation	Correlations with ICP-MS of 0.926 for Vit C, 0.743 for calcium, 0.895 for zinc, and 0.963 for iron, time dynamics of measurements after supplementation were in line with those of blood	Sensor can also supplement micronutrients transdermally	[85]
Fluorometric and lateral flow assays	Wearable with smartphone analysis	Cortisol, glucose, and vitamin C	Sweat	Human subjects	ELISA for cortisol, controlled stress and diet	Cortisol aligned with circadian rhythm changes and had R^2 of 0.7974 with ELISA, observed spikes in Vit C with intake, no trends in glucose	Measurement of sweat rate and EDA, with NFC and RF to power them and communicate results	[86]

3.2.3 Smartphone-based Quantitative Assays

Smartphones are increasingly being used as analytical platforms for quantitative assays. Often, the phone is either used to photograph the assay results for quantitative analysis [95, 96, 160, 152, 153, 47, 41, 137], or it communicates with a more specialized and standardized sensing device [103, 97, 176]. Lee et al. [95] demonstrate the use of smartphones to image and quantify vitamin D (calcidiol) levels from an immunoassay. After the sample is deposited on the test and it is incubated for a few hours, the assay is imaged using a custom smartphone accessory. Concentrations can then be quantified from the image by comparing the brightness of the detection region to the reference area. The device was evaluated using three levels of known concentrations that span from deficiency to sufficiency, but not excess (Table 18). Although this range is debated. Results were compared to an ELISA test. Motivated by the asymptomatic nature of Vitamin B12 deficiency, the same group developed a smartphone-based assay method for B12 quantification [96]. The ratio of test to control line intensities extracted from the smartphone image are used as an input to a 4-parameter logistic curve to output an estimated vitamin B12 concentration. 12 human subjects provided whole blood samples from a finger prick, and assay results were compared to an Immulite 2000 immunoassay system. On these samples, the Nutriphone failed to accurately determine B12 levels above 441 pg/mL. Unlike the Immulite, this solution appears to be insensitive to the upper spectrum of B12 insufficiency and sufficiency. In addition, while the authors do not specify the form of vitamin B12 targeted by their device, the reported molecular weight is closest to the non-gold-standard cyanocobalamin. The authors suggest that future work should aim to be more effective at lower limits of detection and better account for interferents in whole blood. Iron, as ferritin, has likewise been targeted by this group [160]. Their assay was evaluated in-lab with known concentrations of ferritin in spiked buffer (n=27) and serum samples (n=12) to optimize performance. Human trials were also conducted and results were compared to the Immulite 2000.

Serhan et al. [152] had a similar goal of using a smartphone-based assay to measure total iron in serum (Fig. 2). This paper focuses on total iron instead of ferritin (the clinically-accepted biomarker for imbalance; Table 19) because it is "the most direct metabolite in the [iron] panel" [152]. Total iron can provide valuable insights into iron status, thus it is a worthwhile target even if it is not the gold-standard [118]. This is the first work to consider the risks of excess iron, purposefully designing the assay to be sensitive to both deficiency and excess with a "dynamic range of 50-300

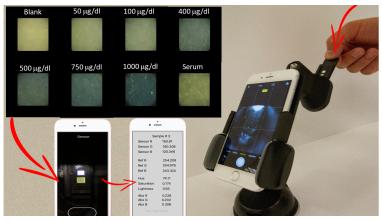


Figure 2: The use of a smartphone-based assay for the quantitative assessment of iron in serum. Used with permission from [152].

μg/dL" [152]. 20 finger-prick samples were taken, and assay results were compared to optimized multi-well plate spectrophotometry. Additional analysis found that their approach had a coefficient of variance of 10.5%, compared to 2.2% for the lab tests. One unique form of validation here that did not appear in other work was specificity testing using interferent analytes. The same first author improved their method and developed a new system to measure total iron levels from whole blood, consisting of an iOS smartphone application, a 3D printed sensing chamber, and a vertical flow membrane-based sensor strip [153]. Their new approach filters out the cellular components of whole blood and carries out a colorimetric chelation reaction. A resulting color change is detected by the smartphone device within 5 minutes. The smartphone application's accuracy and precision were tested against a reference imaging software (ImageJ) for the same colorimetric sensing strip. They compared their iron detection technique to a spectrophotometry-based laboratory test for iron detection on 14 venous blood samples from 9 volunteers and found greater limit of detection (LoD) than the laboratory method (2.2 mcg/dL) The authors discuss future steps of expanding this tool toward measuring total iron binding capacity and saturation levels.

Ferreira et al. [47] develop an assay for urine, a biofluid that is less invasive than blood and more representative of status than sweat. Their paper presents a paper-based colorimetric assay for urinary iron and quantification of results from images. The assay itself contains four columns of five sample units each. This design allows for replicate results and outlier exclusion. Blank (water) samples were used to obtain baseline signal intensity for absorbance calculations and calibrate for urine color interference. Calibration curves were determined using iron standards in water and synthetic urine. It was found that the phosphate and citric acid in the synthetic urine significantly interfered with the slope of the calibration curve. The sensor was evaluated using volunteer urine samples (n=26) pre-treated with nitric acid. The results were compared to AAS and found to be similar. Dortez et al. [41] showcase another colorimetric assay for serum iron, quantified by smartphone images. Samples are preprocessed with anti-transferrin to strip iron (Fe3+) ions from the protein transferrin, which are then reduced to Fe2+ before the sample is deposited on the assay. A smartphone and light box were used to capture images of the test area, which are analyzed to yield quantified results. The assay was developed using iron standard solutions and evaluated using diluted serum spiked with Fe3+. It was found that the assay could analyze multiple samples simultaneously, allowing for auto calibration of test samples against a certified reference control. Prakobdi et al. [137] claims to present a low-cost, non-invasive saliva-based screening test for IDA using a nitrocellulose lateral flow system. The method uses a capillary flow-driven microfluidic device and tests iron's reaction with bathophenanthroline (Bphen) and ferrous (Fe2+) ions to measure iron (Fe3+) levels in spiked saliva. A color change is analyzed, and the results indicate a linear response in the 100-2000 mcg/dL range (falling far above the lower limit for 'normal' serum iron results [27]). The study used pooled commercial saliva, and it does not compare measured iron levels with a clinical gold standard for iron status assessment. In addition, we note the general ambiguity of whether saliva levels of iron are an accurate indicator of circulating blood levels (Section 2.2).

The smartphone moves into a supporting role in Lee et al. [97]. The authors implement a paper-based microfluidic immunoassay for vitamin A (as retinol binding protein or RBP), iron (as ferritin), and C-reactive protein (CRP) in a unique, card-like form factor. Most notably, it is able to analyze finger prick whole blood samples with minimal pre-treatment because of built-in plasma separation (similar to [153]). The device includes competitive assays for CRP and RBP and a sandwich assay for ferritin. Sandwich-based assays are more sensitive, while competitive assays are more effective for analytes of larger concentrations. CRP is a useful inclusion since both iron and vitamin A assessment are impacted by inflammation (Table 18). On-device light emitting diodes (LEDs) and photodetectors analyze the assay, sending results and various test metrics to a smartphone app over NFC. The added ability to store this data in a remote server makes the device a powerful tool for population-level screening. The device was evaluated on whole blood samples (n=95), each run 3 times, and compared to ELISA. 84.4% of sample was male, and 6 samples were spiked with ferritin, CRP, and RBP to assess a wider range of concentrations. In assessment, a physiologically-relevant cutoff of 15 mcg/L was set for ferritin deficiency (although this is insensitive to the deficiency upper limit of 30 mcg/L; Table 19). The decision to make this device single-use is perplexing, especially since a reusible version was used for testing.

Table 2: Assay-Based Methods Using Smartphones

Method	Form Factor	Targets	Analytes	Evaluation	Control	Results	Notes	Source
Quantitative immunoassay	Smartphone-based	Vitamin D (calcidiol)	Serum	Known sample solutions and human subjects	ELISA	Errors "at same order" as ELISA	Erickson Lab at Cornell; no statistical analysis of results	[95]
Quantitative immunoassay	Smartphone-based	Vitamin B12 (cyanocobalamin)	Finger prick blood sample	Human Subjects	Immulite 2000 Immunoassay	Correlation of 0.93 with control, 85% specificity and 60% sensitivity for deficiency detection	Erickson Lab at Cornell; used synthetic form of B12; poor accuracy outside of deficiency range	[96]
Quantitative immunoassay	Smartphone-based or desktop device (TIDBIT)	Iron (ferritin)	Finger prick blood sample	Human Subjects	Immulite 2000 Immunoassay	Correlation of 0.92 with control, sensitivity of 0.9 for deficiency detection	Erickson Lab at Cornell	[160]
Quantitative immunoassay	Smartphone-based	Total iron	Finger prick serum blood sample	Human Subjects	Multi-Well Plate Spectrophotometry	R^2 of 0.98 with control, CoV of 10.5%	Conducted specificity testing using interferent analytes; considered toxicity	[152]
Quantitative immunoassay	Smartphone-based	Total iron	Finger prick whole blood sample	Human Subjects	Laboratory developed test: spectrophotometry-based technique	Correlation plot with slope of 1.09, R^2 of 0.96, and a mean bias of 5.3%	Improved on control LoD; manually diluted some samples to represent a low iron concentration	[153]
Colorimetric assay	Paper-based	Total iron	Urine	Human subjects	AAS	RSD of 9.5%	Urine citric acid was found to interfere with results	[47]
Colorimetric assay	Paper-based	Iron (Fe3+)	Serum	Spiked, diluted from human subjects	Spiked known concentration	Error of 3.7% and RSD of 1%	LoD of 0.3 mcg/mLl	[41]
Colorimetric assay	Paper-based	Iron (Fe3+)	Saliva	Spiked pooled commercial saliva	Spiked known concentration	R^2 of 0.99	It is debated whether salivary iron reflects circulating status	[137]
Opto-electronic immunoassay	Card	Vitamin A (RBP), Iron (ferritin), and CRP	Whole blood	Human subjects	ELISA	CVs of 2.5% for ferritin, 10.8% for RBP, and 3.9% for CRP	Cutoff for iron deficiency was insensitive to upper limit; RBP is not the clinically-accepted biomarker for vitamin A status; Device transmits results to a smartphone over NFC	[97]
Multiplexed quantitative assay	Desktop device (TIDBIT)	Vitamin A (RBP), Iron (ferritin), and CRP	Serum	Human subjects	ELISA	R^2 of 0.56 for RBP, 0.92 for ferritin, 0.88 for CRP	Erickson Lab at Cornell; ferritin range does not cover anemia; RBP is not the clinically-accepted biomarker for vitamin A status; RMSE for RBP was 21 mcg/mL	[103]
Quantitative immunoassay	Desktop device (TIDBIT)	Vitamin D3 (calcidiol)	Serum and whole blood	Commercial standards; human subjects	Known solutions; LC-MS/MS	CoV with standards of 2.63% at 34 ng/mL and 11.2% at 0 ng/mL; R^2 of 0.91 for serum tests, 0.94 for finger prick tests	Erickson lab	[176]

We would also like to note that RBP is not the clinically-accepted biomarker for vitamin A in serum, which should be measured directly instead (Table 18).

In Lu et al. [103], the Erickson lab deviates from smartphone image analysis and moves to a bespoke device. Their new approach achieves simultaneous quantification of vitamin A (as RBP), iron (as ferritin), and CRP on a single test strip using multiple fluorescent markers and immunoassays. The paper proposes a reusable, standalone reader (the TIDBIT). Six blue LEDs with band-pass optical filters (458 nm) are used for fluorescent excitation of the assay tags. Interestingly, quantitative results are only presented to the user if they are within a "physiologically relevant dynamic range" of 2.2-20 μg/mL for RBP, 12-200 mcg/L for ferritin, and 0.5-10 μg/mL for CRP [103]. We note that the ferritin range sufficiently covers deficient status, but not anemia (Table 19). 43 human serum samples were purchased from a commercial vendor for testing and compared against ELISA. Evaluation results for RBP were poor, with no explanation of why this may be. This TIDBIT device is applied further in Vemulapati et al. [176], targeting vitamin D (as 25(OH)D3). A “novel elution buffer that separates 25(OH)D3 from its binding protein in situ” enables the assessment of finger prick blood with no pre-treatment [176]. In testing, the test/control ratio was highly correlated to vitamin D concentrations in standard solutions. Commercial serum standards highly correlated with assay results (4-parameter logistic curve), with coefficient of variance (CoV) of 2.63% at 34 ng/mL and 11.2% at 0 ng/mL. Human trials with serum (n=21) and finger prick whole blood (n=6) samples were conducted, and results were compared to LC-MS/MS measurements. The accuracy of deficiency detection for serum but not whole blood was assessed, with an area under the curve (AUC) of 0.836 for deficiency cutoffs of 20 ng/mL and 1 for 12 ng/mL. Only the latter aligns with the general clinical threshold for deficiency (Table 18).

3.2.4 Commercial Products

Commercially-available devices have arose in recent years to provide point of care (POC) testing for some micronutrients [4, 38, 48, 7]. One study has explored the utility of a commercial iCheck FLUORO device to assess vitamin A concentrations in human breast milk (breast milk vitamin A or BMVA) [4]. BMVA is critical since it is the primary source of vitamin A for breastfeeding children. If there is a vitamin A deficiency in breast milk, it is likely to cause developmental issues for a child. The authors collected breast milk samples and socio-demographic and anthropomorphic data from lactating mothers in the Mecha district, Ethiopia (n=104). This region was selected because prior studies applying this device for BMVA assessment recommended further investigation of populations at greater risk of vitamin A deficiency. Concentrations of vitamin A in breast milk were measured by iCheck FLUORO and compared to HPLC. The commercial device was found to overestimate low BMVA concentrations and had a weak overall correlation with HPLC results. Therefore, the paper concluded that studies that assess vitamin A intake among breast-feeding children in developing countries should not assume average BMVA. It is argued that devices like the FLUORO are needed to monitor BMVA status, especially for intervention programs that typically assume average BVMA. Still, they must be “reliable across a range of BMVA concentrations” [4].

Albrecht et al. [7] likewise studied the efficacy of the Quidel Inc Sofia fluorescent immunoassay for serum vitamin D (as calciol). The assay is analyzed by the Sofia Analyzer, a POC device for immunoassay analysis. Because only one sample had a concentration above 100 ng/mL, the authors recommend additional testing for concentrations above 80 ng/mL (well into the range of toxicity; Table 18).

Bloom Diagnostics is a desktop ’lab’ device that analyzes single-use qualitative test strips to quantitatively assess the status of in-vitro (in-body) biomarkers, similar to the Erickson Lab’s TIDBIT [176, 103, 38]. Tests available for Bloom include thyroid-stimulating hormone (TSH), ferritin, CRP, and estimated glomerular filtration rate with cystatin C. While Bloom approaches the goal of accessible nutrition assessment, its assays still require the user to collect a sample themselves. Depending on the test, this could involve a finger prick, coaxing the blood into a collection tube, and depositing it properly onto the assay. VitaScan (from the Erickson lab) is another commercial POC device that tests for iron deficiency, and they validate results against the clinical gold standard for in-body iron measurement [48]. The device is not yet released, but it is planned to assess vitamins B12, D, and A and CRP in the future. The method is still invasive as it utilizes blood obtained from a finger prick and also requires the user to obtain the sample themselves.

3.3 Electrochemistry-Based Methods

Electrochemical analysis is a method that has been applied in literature to quantify levels of micronutrients in biofluids such as saliva, sweat, tears, urine, and blood. Huang et al. [66] summarizes the basis of electrochemical sensors with respect to vitamins, but we see work applying these ideas to minerals as well. The concentration of vitamins in an electrolyte (water or fat/organic solution that allows for the transfer of electrons) can be quantified by measuring electrical properties at a working electrode. The most common measurement techniques are *voltammetry* and *amperometry*. Voltammetry applies a varying voltage to the electrolyte and measures the resulting current, while amperometry applies a constant voltage and measures the resulting current over time [61]. Below, we dive into novel methods that were

Table 3: Commercial Assay-Based Methods

Method	Form Factor	Targets	Analytes	Evaluation	Control	Results	Notes	Source
Fluorometry	Desktop device	Vitamin A (retinol and retinyl esters)	Breast milk	Human subjects	HPLC	Weak correlation (R^2=0.59, p<0.001), but mean difference was "not statistically different from zero"	Of major concern was the ability for the breast milk to satisfy the vitamin A requirements of children	[4]
Quantitative immunoassay	Desktop device	Iron (ferritin), other tests	Finger prick blood sample	Not published	Not published	Not published	Commercially available	[38]
Immuno-fluorescence	Desktop device	Vitamin D (cholecalciferol)	Serum	Human subjects	Abbott Alinity i immunoassay	R^2 of 0.89; SE of 0.16 at 10 ng/mL, 0.19 at 12 ng/mL, and 0.35 at 30 ng/mL	Standard error lower than control; recommends additional testing for excess status; target is not the clinically-accepted biomarker for Vitamin D	[7]

evaluated in biofluids, roughly divided into voltammetry and amperometry. For more detailed discussions, we direct the reader to recent reviews focusing on electrochemistry-based methods (e.g. [66, 126, 89, 147]). A summary of electrochemistry-based methods reviewed herein is found in Tables 4, 5, and 6.

3.3.1 Voltammetry

Voltammetry has been a popular method for the assessment of micronutrients in biofluids. Revin and John [143] propose a novel electrode for the simultaneous measurement of B2 (riboflavin), B9 (folic acid), and C (ascorbic acid) vitamins. Peak currents for each vitamin were well separated at mixtures of various concentrations. For vitamin C in particular, the tested linear range of the sensor was insensitive to the lower limit of sufficiency and below (Table 17). Additionally, the targets for vitamins B2 and B9 differed from their clinical gold-standard. Selectivity analysis showed that linearity in each vitamin was maintained even in the presence of elevated concentrations of all other vitamins. No interference from other common physiological interferents was found. Two plasma samples were tested before and after spiking with vitamin standards, with good recovery. Another electrode was developed by Jothimuthu et al. [77], targeting zinc. Interestingly, a sample pH of 6 was optimal for zinc assessment, which is more acidic than most biofluids (e.g. blood has a pH of 7.35-7.45). The zinc content in both acetate buffer and spiked, HCl-diluted serum was evaluated. A square wave voltammetric sensor was developed for the simultaneous measurement of glutathione (GSH), nicotinamide adenine dinucleotide (NADH) and folic acid (vitamin B9) [141]. A single urine and serum sample was collected for evaluation and spiked with known amounts of the targets. The authors did not present serum results.

Kim et al. [84] designed a square wave anodic-stripping voltammetric sensor has been used to monitor zinc in sweat during physical activity. The sensor itself is wearable, printed on tattoo transfer paper. The assessment was conducted with standard zinc solutions in a buffer medium. On-body experiments demonstrated the sensor's ability to assess zinc in cycling-induced sweat. A linear calibration equation was used to quantify zinc concentrations, which were found to be close to the physiological range of zinc in sweat. Again, no gold-standard assessment methods for individual zinc status were used for comparison. However, this is less of a concern since this work explicitly focuses on providing insights into zinc excretion through sweat rather than determining in-body status.

Gao et al. [51] targeted zinc as well as copper using voltammetry. A wearable electrochemical sensor was created to assess Zn, Cd, Pb, Cu, and Hg ions in sweat and urine. Uniquely, the sensor incorporates skin temperature measurement for calibration and to compensate for the influence of temperature on electrochemical signals. This was important, as peak current was shown to increase with temperature. The device was developed with spiked synthetic sweat samples at concentrations an order of magnitude lower than is observed in blood (Table 19). Calibration curves demonstrate a linear relationship between peak current and concentration for the ions, but quantitative metrics are not reported. The authors conducted human studies for on and off-body measurements with ICP-MS as a control. The measured and controlled concentrations for Zn and Cu were similar, but statistical analysis of the results was not conducted. In Stanković et al. [162], a novel "boron-doped diamond electrode" for vitamin B12 (as cyanocobalamin) quantification is studied. Interference analysis showed a 10% signal change in the presence of a "10-fold excess of vitamin B6" [162]. The sensor was evaluated in four spiked urine samples, diluted, and pH adjusted to 2. In this case, the electrode targeted cyanocobalamin, which is the synthetic form of vitamin B12.

Sempionatto et al. [150] assumes a glasses form factor to conduct electrochemical analysis of tears, assessing the concentration of glucose, alcohol, vitamins B2, B6, and C. Tears were induced with menthol sticks before being collected and analyzed by the glasses. The sensor itself uses square wave voltammetry (SWV) for vitamin measurement, demonstrated only as a proof of concept. After a baseline was acquired with the sensor, tears were induced and analysis

was conducted every 30 minutes for 2 hours after taking a multivitamin. Peak potentials emerged for each vitamin and were verified with known concentrations of vitamins added to baseline tear samples. Because this was a proof of concept, no comparison to gold standards with blood or efforts to quantify in-body vitamin levels were made.

A microfluidic, graphine-oxide-based sensor chip has been applied for the quantification of ferritin in serum [52]. Notably, the sample must be pumped through the sensor, where cyclic voltammetry is performed continuously with an external potentiostat. An evaluation was conducted with spiked serum samples (which were not sensitive to deficiency) and compared to ELISA. The sensor overestimated concentrations <100 mcg/L by $\sim$10% and underestimated the larger concentration by $\sim$4%.

Sun et al. [163] focuses on low-cost and reusability in vitamin C assessment. Their device uses cyclic voltammetry to determine whether the vitamin C content present in the sample is normal or deficient (<4.93 mg/L). This deficiency threshold is greater than what is reported by the clinical literature, exceeding even the limit for sufficiency (Table 17). Interestingly, their device is also self-powered, using vitamin C as a biofuel. In a trial for scurvy detection (n=22), the device correctly determined the 4 deficient individuals (ground truth by HPLC). The authors also demonstrated its potential to screen for patients exhibiting a medical condition.

On-body electrochemical sensing of vitamins B6 (pyridoxine), C (ascorbic acid), D3 (calciol), and E (alpha-Tocopherol), as well as 9 amino acids and several macros in sweat, is enabled by Wang et al. [180]. Of the biomarkers targeted for each vitamin, only the target vitamin C aligns with the clinical gold-standard biomarker. Sweat is passively sampled using iontophoresis in a watch form factor. Voltammetry is used to detect vitamins indirectly. Quantitative results are not reported, but concentrations of vitamins appear to linearly correlate with peak height current density. The authors note the flexibility of this approach to the measurement of numerous other biomarkers. Human trials were conducted with healthy volunteers and patients but only targeted amino acids. This exemplifies the tendency of research to ignore micronutrients.

Lokesh Kumar et al. [101] developed a manganese dioxide nanoparticle–bimetallic metal-organic framework composite to detect vitamin D3 in spiked human plasma. Voltammetry measurements were compared to a gold standard for vitamin D detection, HPLC with ultraviolet detection (HPLC-UV), and obtained similar values. Seker et al. [148] designed a touch-based sensor that simultaneously monitors zinc and ascorbic acid (vitamin C) levels after supplementation. The technique measures fingertip sweat and uses SWV for zinc detection and potentiometric measurement for ascorbic acid detection. Lastly, Shi et al. [157] assessed an NFC-powered sensor for riboflavin (B2) in sweat. Selectivity testing was conducted by the addition of common sweat molecules into the standard, which did not significantly influence results. Uniquely, a pH sensor was incorporated into the device to account or the influence of pH on measurements. Human trials involved subjecting participants to exercise (n=1) or heat stress (n=2) to induce sweat after supplementation. Sweat samples were analyzed by the device and compared to HPLC sweat measurements for the exercise trials and fluorescence spectroscopy for the heat-stress trials. The time-dynamics of the device results followed the general trend of the control analysis methods, exhibiting more variance compared to urine results.

3.3.2 Amperometry

Vitamins B2, B9, C, and D, as well as the mineral iron, have been assessed in biofluids using amperometry [106, 151, 190, 145, 191, 122]. Maiyalagan et al. [106] confront a major limitation of glassy carbon electrode-based sensors for vitamin B9 (folic acid): interference from vitamin C. Their nanofiber-modified electrode successfully avoided this interference. In evaluation, two serum samples were collected and evaluated before and after spiking with 4.41 mcg/L of folic acid. The peak current increased accordingly, allowing for greater than 99% recovery. Vitamin C is targeted by Sempionatto et al. [151], who deployed amperometry and immobilized ascorbate oxidase in a tattoo-based form factor. Tests on human subjects focused on the temporal characteristics of the current response, finding peak response in sweat 90 minutes after supplementation and a return to baseline 180 minutes after, in line with the plasma response of vitamin C. Tears and saliva are noted as other possible biofluids, with tears yielding a similar temporal profile (albeit with different peak currents). The authors also experimented with providing different amounts of vitamin C through both supplements and orange juice, claiming the response from sweat samples to increase in line with increasing vitamin C content. Crucially, no statistical analysis was conducted on the results of the experiments, such as correlations between intake and measured current. There are also no comparisons made to clinical reference methods of vitamin C assessment, though this is stated as a subject for future work. A wearable, electrochemical device to measure vitamin C levels in sweat, urine, and blood was proposed by Zhao et al. [190]. The device was used in a study where 6 male participants, aged 20-30, were given vitamin C as emergen-C brand supplements. The sensor is wearable, but no on-body measurements were made. Instead, urine and induced sweat were collected three hours post-intake and measured with the device. Blood samples were collected from a single participant in a separate study, analyzed with the device, and compared to results from urine and sweat. This research does not compare device results to any gold standard for vitamin C assessment; instead, it relies on intake, which (as we will see in Section 4) is a poor equivalent

Table 4: Electrochemistry-Based Methods Using Voltammetry

Method	Form Factor	Targets	Analytes	Evaluation	Control	Results	Notes	Source
Voltammetry	Benchtop	Vitamins B2 (riboflavin), B9 (folic acid), and C (ascorbic acid)	Plasma	Human subjects	Spiked known concentrations	>99% recovery of spiked concentrations	Tested in 7.2 pH buffer, which is slightly lower than pH of blood (∼7.4); direct riboflavin is not the gold-standard biomarker for vitamin B2; folic acid is the synthetic form of folate; Linear range for vitamin C did not reach below the lower limit for sufficiency	[143]
Anodic stripping voltammetry	Benchtop	Zinc	Serum	Human subjects	Spiked known concentrations	Peak current decreased with concentration, but were lower in magnitude than buffer	A sample pH of 6 was necessary for optimal performance	[77]
SWV	Benchtop	Vitamin B9 (folic acid), GSH, and NADH	Urine and serum	Human subjects (urine only)	Spiked known concentrations	Accurate recovery of spiked concentration	Simultaneous determination of targets; unclear whether pre-existing urine composition biased recovery; claims serum evaluation but this is not presented; folic acid is the synthetic form of folate	[141]
Square wave anodic stripping voltammetry	Wearable tattoo	Zinc	Sweat	Zinc stock solutions	Known zinc solutions	R^2 of 0.999 for measured current vs stock solutions, LoD of 0.05 mcg/mL	Zinc content in actual sweat from single participant was close to physiological range	[84]
Square wave anodic stripping voltammetry	Wearable patch	Zn, Cd, Pb, Cu, and Hg ions	Sweat and urine	Human subjects	ICP-MS	Similar to control, but provided no statistical analysis	Included temperature sensor to account for the influence of skin temperature on peak current	[51]
SWV	Benchtop	Vitamin B12 (cyanocobalamin)	Urine	Diluted from human subjects	Spiked known concentrations	98-105% recovery	Cyanocobalamin is the synthetic form of B12	[162]
SWV and chronoamperometry	Eyeglasses	Vitamins B2, B6, and C, alcohol, and glucose	Tears	Human subjects	Breathalyzer BAC, commercial glucometer, vitamin supplementation	Correlations of 0.852 with BAC, 0.7 with glucometer, distinct voltage peaks for each vitamin	Glucose and alcohol was main focus, vitamin assessment included as a proof of concept; exact form of each vitamin is not known	[150]
Cyclic voltammetry	Benchtop	Iron (ferritin)	Serum	Spiked from human subjects	ELISA	R^2 of 0.966 and lower linearity than control; tended to overestimate concentrations <100 mcg/L	Range of spiked concentrations was not sensitive to deficiency; studied the impact of pH and interferent compounds	[52]
Cyclic voltammetry	PoC	Vitamin C	Serum	Human subjects	HPLC	R^2 of 0.984 ($p<0.001$) and 100% accuracy in deficiency detection	Targets scurvy (extreme deficiency) but the threshold was set above sufficiency	[163]
Voltammetry	Wearable patch	Vitamins B6 (pyridoxine), C (ascorbic acid), D3 (calciol), E (alpha-Tocopherol), and other macronutrients and amino acids	Sweat	Not reported for vitamins	Not reported for vitamins	Observable linear relationship between vitamin concentration and peak height current density	Only vitamin C aligns with clinical standard, quantitative results for vitamins were not reported	[180]
Voltammetry	Benchtop	Vitamin D3	Plasma	Spiked from human subjects	Spiked known concentrations and HPLC-UV	LoD of 1.9 ng/mL; RSD of 0.3-2.6% and recovery of 96-102%	Exact form of D3 (gold-standard 25(OH)D3/calcidiol or calciol) not reported	[101]
SWV and potentiometric measurement	PoC	Zinc and vitamin C (ascorbic acid)	Fingertip sweat	Human subjects	Supplementation	Both micros could be analyzed over time simultaneously	No comparison to clinical assessment of status or statistical analysis of results; Vitamin C range far exceeded physiological concentrations in plasma	[148]
Differential pulse voltammetry	Wearable patch	Vitamin B2 (riboflavin)	Sweat	Human subjects	HPLC	R^2 of 0.9783 with sweat HPLC and 0.87 with urine fluorescence spectroscopy	Urine included as comparison to sweat status; incorporates pH sensor to control for influence of pH on measurements; direct riboflavin is not the gold-standard biomarker for Vitamin B2 status	[157]

for in-body status due to individual differences in micronutrient absorption. In addition, we note that the vitamin C supplement, emergen-C, contains several other nutrients that could influence the results.

One paper proposes simultaneous measurement of vitamins C and D from a single saliva sample [145]. Their sensor combines an electrocatalytic vitamin C (ascorbic acid) amperometric assay and competitive vitamin D (25(OH)D3) immunoassay. Vitamin D is the clear focus of the paper, the vitamin C sensor gets little to no attention aside from some analysis of potential cross-talk between the two sensors. The sensor was applied in a study that supplemented vitamins to 3 participants and used the device to analyze saliva samples at increasing time intervals from intake. No gold-standard assessment method was used to evaluate the sensors, although the authors advocate for this evaluation and the development of truly quantitative sensors in future work. Another flexible, electrochemical sweat biosensor for vitamin C used polyaniline film modified with phytic acid [191]. The biosensor was validated with synthesized vitamin C samples of known concentrations. Human subjects were given supplements and had their sweat collected 3 times over 90 minutes. The sensor detected a general increase in current from the sweat samples over time, with variation across subjects. Saliva tests were also conducted. Peak current occurred 60 minutes after supplementation, in line with results from [151]. Another group designed a finger-actuated wirelessly-charging wearable that measures vitamin C and levadopa (a central nervous system agent) levels from sweat [122]. The system has a microfluidic chip with a self-driven pump and anti-reflux valve, a flexible wireless circuit board, and an associated smartphone app. They ran a study with five healthy participants whose sweat was collected and measured after exercising and ingesting vitamin C tablets as well as fava beans [122]. The results were not compared to clinical results as a gold standard baseline.

Table 5: Electrochemistry-Based Methods Using Amperometry

Method	Form Factor	Targets	Analytes	Evaluation	Control	Results	Notes	Source
Amperometry	Benchtop	Vitamin B9 (folic acid)	Serum	Human subjects	Spiked known concentrations	>99% recovery of spiked concentration	Robust against interference from ascorbic acid; sensor performance peaked at pH 7.2, slightly lower than pH of serum; folic acid is the synthetic form of folate	[106]
Amperometry	Wearable tattoo	Vitamin C (ascorbic acid)	Sweat, tears, and saliva	Human subjects	Supplementation	Similar time dynamics to plasma for tears and sweat		[151]
Amperometry	Wearable sensor chip	Vitamin C (ascorbic acid)	Sweat, urine, and blood	Human subjects	Supplementation/ intake, blood measurements with the same sensor	Urine and sweat measurements increased after intake, with inter-trial variation. Correlations with blood sensor measurements were 0.81 for sweat and 0.72 for urine	Used emergen-C as a vitamin C supplement, which contains several other micronutrients	[190]
Electrocatalytic vitamin C amperometric assay and competitive vitamin D immunoassay	PoC	Vitamins C (ascorbic acid) and D (calcidiol)	Saliva	Human subjects	Vitamin supplementation	Observable rise and drop in levels over time	No quantitative results; saliva sample must be pretreated; Vitamin C has little evaluation	[145]
Amperometry	Wearable sensor chip	Vitamin C (ascorbic acid)	Sweat and saliva	Stock solutions and human subjects	Known solutions and supplementation	R^2 of 0.99 and LoD of 0.0299 mcg/mL for stock solutions, was able to detect general increase in current after supplementation		[191]
Chronoamperometry	Wearable sensing system	Vitamin C (ascorbic acid) and Levodopa	Sweat	Human subjects	Vitamin supplementation	Detection correlation coefficients of both exceed 0.99; both sensors have a wide linear detection range of 0-17.6 mg/L and 0-1000 μM, respectively, and low detection limits of 0.05 mg/L and 17.9 μM, respectively.	The system is wireless, battery-free, flexible, finger-actuated, and self-pumping	[122]

3.3.3 Others

Two techniques do not fit neatly into the above categories: body impedance measurement scanning [65] and electroacupuncture [3]. Heo et al. [65] focuses on analyzing solely vitamin D (calcidiol) status using body impedance.

They explored the correlation between vitamin D levels in blood, body composition, blood parameters from checkup, and arm impedance (from wrist to elbow) to calibrate an impedance measurement frequency for vitamin D [65]. The motivation was that "body fat accumulates vitamin D," and body fat can be measured by impedance measurement [65].

Vitastiq is the most immediately relevant device for micronutrition insights, with the company claiming that the device can non-invasively measure 26 different vitamins and minerals [3]. The basis of the device's technology is electroacupuncture. It measures the electrical resistance at an 'acupuncture point' and compares it to a 'calibration point'. These points are very specific, and the user is taken through a tutorial to locate them properly [92]. Minimal details about how the device functions and electroacupuncture provide insight into micronutritional status are available. The information the user receives is only qualitative, and consumer reviewers noted a lack of confidence in the results [92]. As such, the manufacturer notes that Vitastiq is not a medical device and should only be used as an indicator of "general vitamin trend" [3].

Table 6: Other Electrochemistry-Based Methods

Method	Form Factor	Targets	Analytes	Evaluation	Control	Results	Notes	Source
Arm impedance measurement scan	Non-mobile, clinical device	Vitamin D (calcidiol)	Impedance measurement at 21.1 Hz	Human subjects	Vitamin D status by blood test (method unspecified)	R^2 of 0.75 (regression with Vitamin D level)	Also evaluated regression models with medical checkup and body composition analysis data	[65]
Electroacupuncture	PoC	26 different vitamins and minerals	Electrical resistance on the surface of the skin	Not published	Not published	Not published	Should only be used as an indicator of "general vitamin trend"	[3]

3.4 Spectroscopy-Based Methods

Spectroscopy is the most common method of gold-standard micronutrient status assessment. However, there is still a lot of work to do to make spectroscopic approaches more accessible and less invasive. We begin with some definitions of common terms in the field. *Spectroscopy* is "the investigation and measurement of spectra produced by matter interacting with or emitting electromagnetic radiation" [2]. *Spectrometry* is the application of spectroscopy; the way in which quantitative measurements are obtained. When we speak of a *spectra*, it means any measurement that is a function of wavelength or frequency. A sample will absorb or emit these spectra when electromagnetic radiation of a known wavelength is applied. Spectra are measured by a detector, the *spectrometer*. Because the level of radiation applied is known, analyzing the resultant spectra after it interacts with the sample provides information about the sample.

We have previously described MS and LC-coupled spectroscopy, the types of spectroscopy used by most gold-standard clinical biochemical analyses (Section 3.1. As mentioned, these methods are expensive, non-specific, complex, and often require an invasively-collected biosample. While there have been strides to make MS more accessible [35], we focus on alternative spectroscopic techniques that may yield micronutrient insights.

Categorized under emission spectroscopy, *fluorescence spectroscopy* has been demonstrated for the measurement of primarily B vitamins in non-biological samples such as multivitamins and energy drinks, although vitamin B1 was measured in urine [189]. The B vitamins continue to get attention in *near infrared (NIR) spectrophotometry* (750-2500 nm wavelength), where their measurement has been reported as well [189]. One review recognizes the ability of *vibrational spectroscopy*, which includes *infrared (IR)* and *raman spectroscopy*, to act as a tool for biofluid analysis in precision nutrition [40] (Fig. 3). However, like other studies in nutrition, this review has few considerations for micronutrients and the approaches covered are largely concerned with general nutritional status or macronutrients. Tsiminis et al. [170] notes the potential of raman spectroscopy for measuring vitamin B12, though this has yet to be realized at in-body concentrations due to the low sensitivity of raman spectroscopy. In biosamples, spectrophotometry was applied to measure vitamin C [69]. Spectroscopic skin tests and raman spectroscopy have also been noted as promising techniques in the assessment of provitamin A carotenoid status in the body [69]. Measurement of several water-soluble vitamins in synthetic mixtures and dosage forms was achieved with *derivative and multivariate spectrophotometry* [189]. Derivatives of UV spectrophotometry (185-400 nm wavelength) have also been particularly useful in the analysis of caffeine and B vitamins in energy drinks.

3.4.1 Accessible Spectroscopy

Significant progress has been made to make spectrometry as a study more accessible and compact [35]. Major subfields of spectrometry (visible, Raman, mid-IR, NIR, MS, and hyperspectral imaging) have seen the development of portable

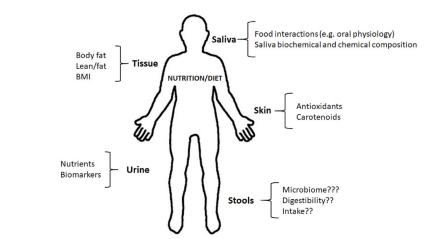


Figure 3: Vibrational spectroscopy targeted at saliva, tissue, skin, urine, and stools holds potential nutritional insights. Used with permission from [40]

or handheld devices. In some cases, such as visible, near-IR, and hyperspectral imaging, these can even be smartphone-based. This paves the way for more accessible, noninvasive techniques. A summary of spectroscopy-based methods is found in Table 7.

There was one application of spectrometry for vitamin D (calcidiol) measurement in our interest area of accessible approaches [178]. With human serum samples in mind, their sensor used surface plasmon resonance (SPR) together with smartphone-based spectrophotometry to assess vitamin D content. The general design of the sensor involved an optical waveguide to direct light from the smartphone flash through one or more SPR sensors, a diffraction grating, and finally into the smartphone camera where a spectra of pixel intensities can be extracted. The device was evaluated on spiked serum samples. As the concentration decreased, the center of mass of the spectra shifted right, allowing for the detection of these concentrations. The paper claims a comparable LoD to gold standard methods of LC-MS, but no quantitative estimates of vitamin D concentration or statistical analysis of the results are provided.

Some benchtop approaches to spectroscopic analysis of biofluids for micronutrient assessment have also been demonstrated [132, 133, 19, 116]. Peterson et al. [132] present a photonic crystal-based sensor for ferritin assessment. Photonic crystals are designed to accumulate a target biomolecule on their surface, which changes their reflected peak wavelength value (PWV) under a spectrophotometer. The authors subjected their sensor to robust evaluation, utilizing human liver ferritin, commercial serum controls (Liquichek), and three different ELISA tests. The developed sensor held up against the ELISA tests, but with a higher LOD that doesn't cover the lower end of deficiency, into anemia (26 mcg/L). Bias by Bland-Altman analysis was similar to that of the BioVendor ELISA, and recovery from known serum controls was >94%.

The same first author identifies a specificity issue with their previous assay [132] and employs iron-oxide nanoparticles to minimize non-specific signals [133]. This time, the goal was soluble transferrin receptor (sTfR) quantification from serum, an indicator of iron supply to tissues [6]. Biomolecule interaction on the assay was quantified using the Biomolecular Interaction Detection system from SRU Biosystems Inc. The authors compared results from their assay to ELISA and the previously developed photonic crystal assay. The authors claim that the bias of the assay was not "statistically different from the reference ELISA tests" [133].

Moving from iron to vitamin C, Bi et al. [19] demonstrates the immobilization of ascorbate oxidase in a microfluidic channel, enabling the quantification of vitamin C with UV-visible spectroscopy. During analysis, the biosample is diluted in phosphate buffered saline (PBS) and "pumped through the microfluidic channel". A serum sample was obtained by a single healthy, female volunteer for evaluation. The sample was pretreated to remove proteins , and a few drops were added to the sensor. Even with extensive pretreatment, there was evidence of interference at 280 nm, close to the analysis peak of vitamin C at 266 nm. No gold standard measurement was provided for comparison. Mughal et al. [116] mix different electrolytic solutions with plasma and serum, and when paired with a novel reduced graphene oxide, vitamins K1, K2, B6, and D3 can be individually identified using UV-visible spectrophotometry. Though this method did not compare measured levels with clinical values.

3.5 Biofluid Analytic Methods

A common approach to estimating nutritional status is by making predictions from pre-existing biofluid analysis or demographic data using ML. Such methods have been applied to derive micronutrient-specific insights [169, 94, 82, 131, 138, 104]. A summary of biofluid analytic methods can be found in Table 8.

Table 7: Spectroscopic Methods

Method	Form Factor	Targets	Analytes	Evaluation	Control	Results	Notes	Source
SPR-coupled spectrophotometry	Smartphone-based	Vitamin D (calcidiol)	Serum	Spiked from human subjects	LC-MS	Claim comparable LoD to control, spectra shifted right with decreasing concentration	No quantitative vitamin D estimates or statistical analysis	[178]
Photonic crystal	Benchtop	Iron (ferritin)	Serum	Liquichek control sera	Known ferritin concentrations and multiple ELISA tests	Comparible recovery (>94%) and bias (by Bland-Altman analysis) to best-performing ELISA	LOD higher than cutoff for anemia, lower end of deficiency	[132]
Sandwich iron-oxide nanoparticle immunoassay	Desktop device	Iron (STfR)	Serum	Liquichek control sera	ELISA	SD of 0.45 mcg/mL vs ELISA		[133]
UV-vis spectrophotometry	Benchtop instrument	Vitamin C (ascorbic acid)	Serum	Human subjects	None	Within phsyiologically-relevant concentrations	Strong focus on the effectiveness of the immobilization technique, not vitamin C measurement	[19]
UV-vis spectrophotometry	Benchtop instrument	Vitamins K1 (phylloquinone), K2 (menaquinone), B6, D3 (cholecalciferol)	Serum/plasma	Human subjects	Various sensing techniques (SWV, HPLC-MS/MS (ESI), SWAdSV, Thermal wave transport analysis, DP AdSV, SWASV, DPV, Electrochemical, Colorimetric aptasensor)	Limits of detection of vitamins K1, K2, B6, and D3 are 0.075, 0.1, 0.12, and 0.15 ng/mL, respectively. Limits of quantification are 0.29, 0.3, 0.38, and 0.48 ng/mL for vitamins K1, K2, B6, and D3, respectively.	Clinical gold-standard biomarkers are not used for all vitamins (except for K, where LoD is too high for deficiency); used bismuth nanoparticle embedded polypyrrole nanocomposite (rGO/pPy/Bi NC) as an optical sensing material	[116]

3.5.1 Single Micronutrient Malnutrition Detection

Some studies focus on detecting deficiency of a specific micronutrient. Two such papers target iron status [105, 138], while a third targets vitamin D [131]. Luo et al. [105] used hospital outpatient data collected over three months to predict whether a patient had normal or abnormal ferritin (iron) status. The collected data included age, sex, ferritin test results (used as targets), and other 'predictor' tests that were conducted in the main hospital lab only (n=5128). These authors considered the broader clinical usefulness of ML-powered insights, claiming that "predicted ferritin results may sometimes better reflect underlying iron status than measured ferritin" [105]. This conclusion was based on dual independent review by pathologists on 26 selected cases where measured and predicted ferritin were 'highly discrepant'. They propose that predicted levels could be used to flag lab-measured ferritin for further review. Pullakhandam and McRoy [138] use gradient boost on NHANES complete blood count (CBC) data to classify and explain IDA (n=19995). They found that the most critical features for IDA are low levels of hemoglobin, higher age, and a higher red blood cell distribution width.

For the prediction of vitamin D deficiency, Patino-Alonso et al. [131] applied ML to anthropomorphic data of older Europeans (35-75 y/o; 50/50 males/females) given anthropomorphic features. 501 participants contributed their "waist circumference (WC), body mass index (BMI), waist-to-height ratio (WHtR), body roundness index (BRI), visceral adiposity index (VAI), and the Clinical University of Navarra body adiposity estimator (CUN-BAE) for body fat percentage". Vitamin D as 25(OH)D was measured by immunoassay and the threshold of deficiency was set to be 20 ng/mL (34.7% prevalence). We note that this threshold more closely aligns with *insufficiency* (Table 18). Logistic regression analysis found that the most significant features differed by sex. All but CUN-BAE were associated with vitamin D deficiency in males, while only CUN-BAE was associated in females. ML models for deficiency prediction were trained on each feature individually. The authors discovered that Naive Bayes was the top performer by AUC for WC, BMI, WHtR, and BRI but was bested by logistic regression for VAI and CUN-BAE.

3.5.2 Multiple Micronutrient Detection

Since it is rare for micronutrient imbalance to occur in isolation, researchers have studied the ability to predict malnutrition of multiple micronutrients [169, 94]. Truijen et al. [169] focus on micronutrient malnutrition in older populations, citing how malnutrition in older adults is often diagnosed too late despite the existence of screening methods. The goal of the study was to classify each sample as having either no micronutrient deficiency or one or more deficiencies among vitamins C, B6, B12, selenium, and zinc, confirmed by blood tests. These particular micronutrients

were selected because they interact less with each other (we add that B-vitamins do interact; Table 11), were among the most prevalent deficiencies, had clinically relevant cutoff points for deficiency. Logistic regression was applied to routine biochemical and diagnostic data from 9 years of United Kingdom NDNS for ages $\geq$50 (n=1518). This dataset suffered from ethnic disparities, with the authors noting limited generalizability to ethnic groups due to $\geq$95% of NDNS participants being white. Kurstjens et al. [94] aimed to develop an ML algorithm to assess risk of low body iron storage (ferritin plasma levels) in anemic primary care patients using CBC and CRP tests. Two algorithms were developed, each based on laboratory ferritin results from different chemistry analyzers (from Siemens and Roche). Interestingly, the two most important features were both derived from CBC test results (Table 8). The authors took an important step to consider how such a model could assist a clinician by asking professionals to indicate whether a patient had low ferritin based on CBC and CRP, with and without algorithm results. The found that the algorithm alone was more accurate than both scenarios. Detection of low vitamin B12 and B9 levels were also considered, but this yielded poor results with AUCs of 0.52 and 0.57 respectively.

3.5.3 Adjusting Biomarkers for Inflammation

When conducting biochemical analysis for micronutrient assessment, a common issue is the impact of inflammation on biomarker measurement. One R package aims to solve this problem and improve interpretability by adjusting biomarkers of micronutrients in the context of inflammation [104]. The package implements inflammation adjustment for retinol-binding protein, serum retinol, serum ferritin, sTfR, and serum zinc, using acid glycoprotein (AGP) and/or CRP as biomarkers for inflammation. The authors have also published a paper describing a procedure on when and how to apply their technique [104].

Table 8: Analytic Methods

Method	Targets	Data	Important Features	Ground Truth	Results	Notes	Source
Logistic regression	Normal or abnormal ferritin	Hospital outpatient data	"total iron-binding capacity, mean cell hemoglobin, and mean cell hemoglobin concentration" [105]	Ferritin test results	AUC of 0.97	Predictions could be used to flag lab ferritin for review	[105]
Gradient boost	IDA	US NHANES (CBC and serum ferritin) dataset and Kenyan nutrition dataset	Low blood level of hemoglobin, higher age, and higher red blood cell distribution width	Serum ferritin	PR AUC of 0.87 and recall/sensitivity of 0.98 and 0.89 for original and test dataset, respectively	Heavy class imbalance (4.9% IDA vs. 95.1% non-IDA)	[138]
Logistic regression, naive bayes, and random forest	Vitamin D deficiency	Anthropormorphic measurements of older Europeans	CUN-BAE for females, all others for males	Blood 25(OH)D by immunoassay	Max AUC of $\sim$0.53 for all features; LR best for VAI and CUN-BAE, NB for all others	Did not assess predictive ability of multiple features at once	[131]
Logistic regression	Presence of micronutrient deficiency	UK NDNS, ages $\geq$50	Low protein, energy intake, TC, hemoglobin, HbA1c, ferritin, vitamin D and high CRP	Blood test results for Vitamins C, B6, B12, selenium, and zinc	AUC of 0.79	$\geq$95% of NDNS participants were white	[169]
Random forest	Classify low body iron storage (plasma ferritin)	CBC and CRP tests in anemic primary care patients	Mean corpuscular hemoglobin and mean corpuscular volume	Ferritin results from two laboratory chemistry analyzers (two separate models)	AUC of 0.9 and 0.92 for each model, models were more accurate than professionals with and without access to results	Attempted Vitamin B12 and B9 deficiency detection with poor results (AUCs of 0.52 and 0.57)	[94]

3.6 Biofluid Analysis Limitations

The largest general limitation of existing biofluid-based assessments is that the most effective require an invasively-collected biosample. Methods that indicate in-body status of a micronutrient often utilize a blood sample for analysis. Clinical research on micronutrients suggests that sweat and saliva may not accurately reflect micronutrient status compared to blood, yet many methods continue to overlook this discrepancy. We also observe that biofluid-based assessments are also more specialized in nature. Frequently, a bespoke assay, device, or sensor is implemented for the assessment of only a single micronutrient. This is understandable considering the inherent difficulties in detecting and quantifying micronutrients that have unique metabolic pathways, are present in such limited quantities, and play different roles in bodily function. We find that current methods do not consistently test the clinical biomarker for a given micronutrient (e.g. measuring Vitamin D via calciol instead of the gold-standard calcidiol/25(OH)D). Even when the proper biomarker is targeted, the device itself may be evaluated on concentration intervals that are not pertinent to the spectrum of deficiency to excess (e.g. the linear range of a sensor may be able to indicate sufficient status, but the LoD is too high to infer deficiency). Some research fails to include a clinically-relevant biochemical test (e.g. ELISA or HPLC) for their target micronutrient as a scientific control. Such oversights actively limit the clinical value, and

therefore the real-world utility, of the proposed solution. Therefore, we have taken note of when this is the case, both in the main body of the text and the above tables.

Moving to the specific approaches, we find that clinical biochemical analysis is invasive, expensive to analyze, and the methods of analysis and thresholds for imbalance are debated [142]. Additionally, biomarkers are generally sensitive but not specific, and their analysis requires considering an extensive list of factors that alter the ability of a marker to indicate nutrient status (such as inflammation, disease, or medications) [43].

Although there are several wearable, smartphone, and point of care assay devices, their accessibility in some cases is limited by specialized assay chips and most require invasive biofluids for analysis. Saliva and sweat have been proposed as alternatives to blood, but their ability to reflect in-body status of a given micronutrient is often unknown, debated, or disproved (Section 2.2). One survey offers additional insight into the issues faced by wearable sweat sensors: low sweat rates, sample evaporation, skin contamination impacting sweat content, and the difficulty to access fresh sweat [15]. Assays and electrochemical devices also suffer from being more specialized in nature (complicating manufacture and integration) and not utilizing more ubiquitous methods of health monitoring such as smartphones and smartwatches. Most work is limited to the measurement of one vitamin, claiming that simultaneous detection of multiple vitamins, especially both water and fat-soluble vitamins at once, is more limited. There is also a common need for a buffer or other solution to serve as an electrolyte for the electrochemical analysis of a vitamin. Like reagents in an assay, this adds complexity to the measurement process and creates a barrier to widespread adoption.

The field of spectroscopy shows great potential (especially IR and Raman), though we argue that there is not yet enough accessible, micronutrient-specific research that provides insights into in-body status. Most work in this area required benchtop analyzers instead of on-body approaches. With the latter however, one must take great care not to harm a user with the spectroscopic approach. UV spectroscopy, for example, requires the application of UV light and could cause skin damage.

Prediction from clinical health data is able to combine and analyze a large breadth of features, but this data is often insufficient for micronutrition. The lack of micronutrition data availability poses a grand limitation for the ability to make strides in analytic techniques with AI/ML. As a result, Brown et al. [21] and others urge for more micronutrition data. Additionally, the insights provided by these solutions are limited to a small set of micronutrients and/or indicate only a binary deficiency status, rather than a continuous one.

4 Physiological Analysis Methods for Micronutrient Status Assessment

This section outlines methods that assess micronutrient status by detecting associated physiological features. Physiological assessment is just as important as biofluid assessment for micronutrition, with many arguing that the two should be considered jointly. Despite this, physiological analysis receives significantly less attention in emerging research.

4.1 Clinical Evaluation and Physical Examination

An important component when clinically diagnosing a nutritional imbalance is analyzing the medical history of the patient and conducting a physical exam [142, 68]. When considering a patient's medical history, the assessor looks for factors that influence nutritional intake, needs, and absorption like medication, diseases, impairments in physical functions, and operations. A nutrition-focused physical exam (NFPE) is "an objective method of detecting clinical signs and symptoms of nutritional deficiencies" [142]. A typical NFPE examines muscle mass, fat stores, fluid retention, and specific nutrient deficiencies. The first three involve assessment of an individual's “bulk and tone”, “bone visibility”, and a “spongy” feel. Muscle strength and function is also assessed by handgrip strength testing. A standalone study on the NFPE goes into more detail on its methodology and efficacy [68]. Common regions of interest (ROIs) for nutrient deficiencies are the skin, nails, scalp, eyes, and mouth. Different symptoms in these regions have been shown to have associations with nutrient deficiencies (mostly in micronutrients). For example pallor, or white coloring on the nails, indicates a possible iron, protein, and vitamin B12 deficiency. Such symptoms and their relations to individual deficiencies are summarized in Tables 14, 15, and 16. Additionally, some regions such as the skin, mouth, hair, and tongue have high cellular turnover, meaning symptoms of deficiency can manifest relatively quickly in these regions. This characteristic could be key to the early detection of clinical deficiencies. Due to its subjectivity, an expert is often necessary to perform a NFPE. It is also important to repeat the exam periodically to take changes over time into account.

4.1.1 Dietary History

Nutritional status is commonly determined by the balance between food intake and energy expenditure, as reported by dietary history [142]. Dietary history is another component of assessment, and possibly one of the most flawed. It looks at a person's intake patterns, habits, and other variables such as culture, allergies, diet, and more. To analyze

this balance, the assessor must have an idea of an individual's nutritional requirements, as well as their dietary intake. The gold standard for determining the former is indirect calorimetry, which provides insights into a person's energy expenditure, and therefore primarily macronutrient use. The latter half of the balance, dietary intake, attempts to quantify the amount of nutrients in one's diet. The authors of one study suggest that "the assessment of macronutrients (fat, carbohydrates, and proteins) is as important as the assessment of micronutrients (vitamins, trace elements)" [142]. While this should be the case, we see that micronutrients are often an afterthought in dietary logging. Because EDRs are not one-size-fits-all, estimates of nutrient requirements are useful to paint a fuller picture of an individual diet. Although indirect calorimetry and formulas considering energy expenditure have been shown to be effective for macronutrient requirements estimation, no such method exists for micronutrients [142]. Macronutrient intake can be correlated with micronutrient intake, however this is not fully indicative of micronutrient intake.

AI and ML have become prominent tools in gathering and analyzing dietary history data. Macronutrient status monitoring in particular has used AI to estimate energy expenditure from physical activity data [146]. Various work has used AI and dietary pattern data to predict the risk of diseases, such as breast cancer, colorectal cancer, and cardiometabolic diseases. This technology can also help study the effects of nutrient supplementation on disease treatment and prevention, or examine relationships between mineral levels and cardiovascular disease, diabetes, and schizophrenia. Dietary recommendations can be made on an individual level using information from nutritional databases and clinical expertise [146, 36]. Various types of data can be useful to a nutrition-focused AI model, even social media. As reported by Côté and Lamarche [36], several studies have gathered and analyzed nutritional behavior data from Twitter and Reddit to uncover dietary correlations and understand geographical and demographic differences in diet. Computer vision has also been used to identify and estimate nutrient intake from consumed food and extract eating behaviors from video [36, 146, 72]. Some researchers have designed smart neckbands that utilize AI and ML to track dietary intake and provide nutritional insights [129, 30, 83].

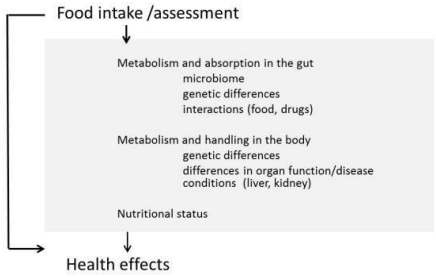


Figure 4: Having an interdependent perspective on all aspects of nutrition can lead to a better understanding of its health effects. Used with permission from [139].

4.2 Remote Nutrition Assessment

Since the COVID-19 pandemic, there has been a growing push for assessment methods that don't require a clinician to be physically-present [68, 109, 155]. These remote assessments use telehealth technology to carry out clinical evaluations and NFPEs. Many components of the NFPE remain the same in the remote setting, with increased reliance on activity tracking devices when assessing a patient's functional status. Observing a patient's living space during a telehealth call is another useful way a way to gain insights into nutritional intake, food availability, and quality of life. The visual inspection of ROIs and the analysis of health data is critical to conducting a remote NFPE. A video call or a collection of photos is especially helpful, as the nutritionist can visually inspect ROIs like they would in-person. It is recommended to start with the oral cavity since it has many early signs of deficiency [68]. Health tracking devices can be useful for a remote exam, providing the practitioner with biometric and physical activity data that can guide a telehealth visit and/or nutritional intervention. Consumer-level scales, bioelectrical impedance analysis (BIA) devices, blood glucose monitors, blood pressure monitors, sleep trackers, and activity trackers are readily available as sources of these data.

Verbal questioning can also be a valuable source of information for remote nutrition assessments, especially for less tech-savvy patients or when photo sharing or video calls are not possible. Researchers recommend asking the patient a series of specific questions to guide them through a virtual head-to-toe examination [109, 155]. Chizmar and Lewis [33] assessed the correlation between patient responses to verbal questions from a clinician and "physical signs of malnutrition" to validate assessments of nutrition without a physical exam. A set of four questions about eye and rib appearance, percieved skinniness, and intake changes from swelling were significantly associated with a diagnosis of malnutrition. These and other questions were further associated with other individual factors of malnutrition. As to be expected, this study has few specific implications for micronutrition, but it's findings are nonetheless useful for the

diagnosis of nutrition as a whole. It should be noted that of these 30 patients, 22 were white and 28 were male. This is critical, as female and non-white patients are disproportionately affected by the burden of malnutrition [134, 80].

In remote dietary assessment are app-based, most new innovations are app-based [109]. These methods can act as intake loggers: users can input their dietary history, and nutritional metrics can be calculated from food and nutrient databases. There is also potential for remote dietary assessment apps to connect with health-tracking devices that can provide additional context to a patient's dietary history.

4.3 Physiological Sensing

Physiological sensing may be useful for the automatic detection of physiological symptoms of deficiency (Section 2.3), aiding NFPE assessments. King et al. [87] reviews techniques for yielding health insights from multiple sensors and their applications. Applications here focus on aspects of health that are not directly related to nutrition, however the authors suggest several criteria for practical wearable monitoring, such as being "noninvasive, intuitive to use, reliable, and provide relevant feedback to the wearer" [87]. Witt et al. [184] describes interesting ways that raw sensor data from wearables can be used to explore health. Different sensors such as photoplethysmogram (PPG), ECG, accelerometer, EDA, and those collecting temperature and physical activity can give valuable insights into human physiology that can be associated with micronutrient imbalance. Some examples are insights into HRV, sleep quality, stress levels, and behavioral patterns. While not explicitly wearables-focused, Yokus and Daniele [187] provide some useful considerations for wearable devices that should be applied to future micronutrient assessing devices.

4.3.1 Electrodermal Activity (EDA)

EDA measures the change in skin conductance caused by sweat, which is an indicator of nervous system arousal [136]. EDA is a common sensing modality in wearables, but we feel it is unexplored in the context of micronutrition. We review the potential of EDA as a measure of the nervous system, which can be associated with micronutrient status (Section 2.3).

All eccrine sweat glands activate upon sympathetic nervous system arousal [136]. As a signal, EDA is based on how "sweat gland activity modulates the conductance of an applied current" [136]. EDA signal analysis can yield several interesting metrics, such as skin conductance responses, nonspecific skin conductance responses, and skin conductance levels. These give insight into sympathetic response and conductance measured. One study shows the potential of EDA, when used together with HRV, to yield insights into nervous system functionality [57]. The approach combines HRV point process models and EDA spectral analysis to better detect autonomic nervous system response to cold-pressor and emotional response tests. They report an accuracy of 73.08% for "automatic emotional valence recognition".

EDA's spectral component varies based on activity level [136]. Efforts have been made to separate high and low frequencies of EDA to isolate sensitive responses. Fingerprinting of EDA signals is an interesting concept, with one paper proposing a spectrogram-inspired multidimensional representation of EDA signals [28]. This approach allows the representation to emphasize finer signal changes, helping to distinguish between arousal levels and stress-eliciting environments.

4.3.2 Photoplethysmography (PPG)

Similarly to EDA, PPG has a lack of attention in its potential to derive micronutritional insights, yet it is even more prevalent in wearable and accessible devices. It is commonly found in pulse-oximiters, smartwatches, and other health sensors. PPG is a continuous signal of cardiac function obtained by measuring the light transmitted through or reflected by the skin (Fig. 5). As blood pulses, it is possible to observe subtle color changes corresponding to the pulse rate (PR), analogous to heart rate (HR). From PR, we can derive pulse rate variability (PRV), which can provide valuable insights into the autonomic nervous system (ANS) activity [99]. PPG can further derive HRV and blood pressure, thus giving insight into bodily functions. Other works have used raw PPG data to estimate BP via ML [114], and DL [127]. Some notable works that apply PPG include an open-source method for remote PPG using a consumer-grade webcam [175]. Smartphone cameras have also been used to conduct PPG through the fingertip, with the smartphone's flash as a light source [59].

4.3.3 Optical Sensors

McDuff [110] reviews how camera sensors can be used for non-invasive physiological measurement, which can allow for nutritional insights. The analysis of motion artifacts can reveal minute subtleties in body motion over time that are caused by various physiological mechanisms (e.g. breathing). Also, camera sensors can measure the intensity and wavelength of light absorbed and reflected by our bodies, especially skin (Fig. 5). Differences in measured light over

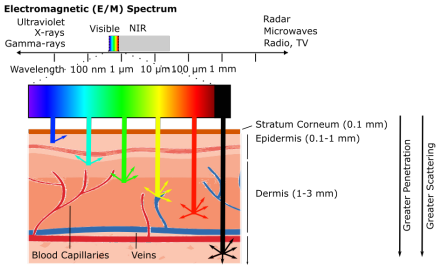


Figure 5: As light is applied to the skin, it is absorbed and reflected in different ways depending on its wavelength. Used with permission from [110].

time can be observed and associated with physiological signals (e.g. heartbeat) and status (e.g. low blood oxygen saturation).

The type of optical sensor has a large impact on the signals that can be derived from it [110]. RGB cameras are found in most smartphones and, therefore, are the most prevalent. These operate largely in the visible spectrum of light (400 to 700 nm wavelengths), but they can often detect some light in the NIR range. NIR cameras are able to detect light in the 700 to 1000 nm range, and thermal cameras can go fully into the infrared spectrum of 2000 to 14000 nm. Thermal cameras, as their name suggest, can provide unique information over other sensors such as body temperature and sweat gland activation. However, this comes with a higher cost and lower resolution. Finally, multi and hyper-spectral cameras allow for the measurement of multiple wavelengths of light at once. This can also be achieved by combining signals from multiple sensors.

Cameras have been extensively applied to the measurement of physiological vital signs [110, 111, 100, 117, 175, 59]. One survey describes 5 physiological vital signs where optical sensor measurements been applied: pulmonary activity, EDA, blood oxygen saturation, glucose status, and cardiac activity [110]. PPG signal caputure has been achieved with RGB cameras. NIR cameras are also useful for PPG in use cases like sleep monitoring that require environments with low visible light [110]. When measuring blood oxygen saturation, multi or hyper-spectral cameras are preferred. Measuring multiple wavelengths of light at once benefits the simultaneous measurement of oxy and deoxy-hemoglobin, and therefore blood oxygen saturation. Optical sensor-based techniques could be use to detect and investigate other physiological symptoms in the fingertip [62, 179, 173], mouth [183, 186], and eyes [164, 128, 93, 79].

Optical sensors have been successfully applied to noninvasively assess a major protein, hemoglobin. Hemoglobin allows for oxygen transport in red blood cells and is produced by iron, vitamin B9, and vitamin B12 [161]. A deficiency in these micronutrients can result in lowered hemoglobin, which manifests as anemia. A review of the state of the art emphasizes the need for an affordable and accessible method of hemoglobin measurement, which can be realized with commodity smartphone cameras [63]. Various work has used smartphone cameras as a way to estimate hemoglobin status noninvasively [63, 179, 62, 164]. They report that PPG signals derived from the fingertip and conjunctiva (skin behind the lower eyelids) under NIR light in 1070 and 850 nm wavelengths contain the most critical features to hemoglobin estimation. Wang et al. [179] had users place their fingertip directly onto a smartphone camera while it recorded video to determine hemoglobin status. Analysis found that the blue spectra of plasma was the most important for protein composition, and therefore hemoglobin estimation. Classifiers were built to identify hemoglobin status as “normal" or “anemic" in the context of demographic averages. Gold-standard blood hemoglobin measurements (Masimo Pronto optical device) were used as ground-truth. Because camera-based assessment can exhibit bias with differences in skin color, the authors carefully considered and reported the demographics of their study participants in addition to controlling for light absorption by skin tissue during experiments. A later paper devised a similar system, applying artificial neural networks (ANNs) and using only the smartphone’s flash as a light source [62]. By combining frames within the videos, the researchers were able to identify regions with high variation in predicted levels.

The use of the conjunctiva as an ROI is gaining popularity due to the fact that it, like the fingernail bed and palmar creases, has no melanin and is devoid of “epidermis, dermis or subcutaneous fat which could impede the transmission of light” to deeper vascular layers [164]. This means the blood vessels are easier to analyze and there may be less bias due to skin color. These properties have been leveraged by Suner et al. [164] to estimate hemoglobin concentration and screen for anemia using smartphone images of the conjunctiva. Spectral super-resolution (SSR) has been introduced to measure blood hemoglobin levels [128]. This method is based on a wealth of existing research reconstructing hyperspectral images from RGB signals. Statistical learning is applied to approximate a hyperspectral image of the conjunctiva from a simple smartphone camera image. Using the hyperspectral data, the hemoglobin content in blood can be computed more effectively than the RGB data alone. However, no analysis of skin color differences was conducted.

4.4 Physiological Analysis Limitations

The general limitation of physiology-based micronutrient assessments is that physiological symptoms are often only shown to be associated with deficiency, and not proven to be causally-related. This limitation could stem from the intricacy of micronutrients and the cost and complexity of running a clinical micronutrition study, which results in a lack of micronutritional data to analyze and mine for new insights. This echoes the limitation of a lack of micronutritional-related data that we found in biofluids analysis. The largest form of physiological micronutritional data are dietary surveys, which often prove to be unreliable [142]. This review finds a lack of technological advancements in physiology-based micronutrient assessments as compared to biofluid-based ones.

Physiological assessment of micronutrient status from the clinical perspective can be summarized by nutrition-focused physical examinations and dietary logs. Physical exams are subjective, qualitative, and require a trained professional. Dietary logs are the most popular and accessible. However, they provide sparse micronutrient information, rely heavily on self-reporting, and do not provide an objective measure for dietary intake in free-living individuals [142]. These accuracy and bias issues are especially a concern when estimating micronutrient intake, where precision is key. AI and ML tools for dietary logging do not adequately address it's fundamental shortcomings, and face additional problems of low explainability, a high potential for induced bias, a general lack of performance comparisons, and a persistent debate over where these methods should exist in the patient-clinician relationship [36, 146].

The remote approach to nutrition assessment relies on patient input and cooperation, as well as the patient's own eye for detail, more so than non-remote approaches [109, 155]. Remote nutrition assessments also inherit common telehealth problems, including wireless signal and camera/microphone quality issues. The accuracy of health tracking devices used to aid these assessments varies and they must be referenced consistently over time to provide meaningful information to the clinician. Remote dietary assessment methods are not immune from the limitations in dietary intake logging described previously. Even in light of these limitations, remote assessment methods can be as effective as traditional methods, while being more accessible [109].

Physiological sensing is the most accessible and noninvasive avenue for micronutrient status assessment. However, for physiological sensing to be accurate, the detected signals and symptoms must be placed in a clinical context. Otherwise, the thin line between association and causation is at risk of being blurred. We also note that current physiological analysis approaches fail to fully explore the variety of physiological symptoms of micronutrient imbalance (Section 2.3).

Techniques that use optical sensors benefit from the accessibility provided by the use of smartphones but their nutritional applications thus far are limited to macronutrients. They are also more susceptible to demographic biases in hardware, software, and datasets [110].

5 Discussion and Future Work

This review of the state of the art reveals several limitations and gaps that hinder the assessment of micronutrient status in individuals: (1) the lack of *clinical relevance* in innovative approaches, (2) the absence of *comprehensive* assessment techniques, and (3) the deficiency of *accessible* and *noninvasive* methods. To address these challenges, we propose high-priority, actionable responses for each limitation (Table 9). These future directions aim to transition micronutrient status assessment from the laboratory to everyday use, enabling valuable micronutritional insights in a manner that is both easily accessible and noninvasive. This approach would empower clinicians to get reliable insights into nutritional status, empower researchers to run cost-effective, larger-scale studies to analyze more data, and empower individuals to manage micronutrient malnutrition effectively. These are necessary steps to not just address micronutrient malnutrition when it occurs, but to be proactive in its prevention.

5.1 Clinically-Relevant Innovations

Generally, we find that current innovative approaches to biofluid analysis for micronutrient status assessment lack clinical relevance (Sections 3.2 to 3.5). To overcome this limitation and develop effective, meaningful assessment methods, future research could prioritize demonstrating clinical utility and shift focus toward clinical relevance as a primary goal. At a high level, this requires an interdisciplinary approach to technological research, and an understanding that novel, technological methods are best utilized as an aid to clinical expertise. Micronutrition is fundamentally a clinical field, so all future solutions must contextualize their work within a clinical application.

It is imperative to benchmark new approaches against established clinical standards. Such comparative validation is crucial to demonstrate the relevance and efficacy of a novel method, and to determine its potential as a viable substitute for the clinical gold standard. Only a limited number of the reviewed methodologies conduct such comparative analyses

Table 9: Limitations of Existing Micronutrient Status Assessment Methods and Opportunities to Address Them

Limitation	**Opportunity**
Limited clinical relevance	- Compare new approaches to the clinical gold-standard - Evaluate assessment performance in routinely-assessed patients - Adopt an interdisciplinary mindset when innovating - Understand and integrate clinically relevant biofluid samples - Measure clinically proven levels of circulating micronutrients
Lack of holistic and comprehensive approaches	- Employ precision nutrition by considering several types of data - Utilize multi-modal solutions - Gather many micronutrient statuses simultaneously - Collect data from diverse populations and make data available
Highly invasive and inaccessible	- Utilize commodity devices (smartphones, smartwatches) to collect data - Make designs open-source - Render insights actionable to non-experts - Bypass the need for biofluid samples by using wearables - Leverage less invasive biofluids such as urine

with state-of-the-art clinical practices. To aid in this endeavor, gold-standard methods for the assessment of each micronutrient are depicted in Tables 17, 18, and 19 in the Appendix. We note that ELISA tests are sufficient in most cases, as long as the standard protocol is followed.

Since this validation can be difficult and expensive (Section 3.1), there is potential for studies to apply new assessment methods among patient populations that are routinely assessed by clinicians. Often, the clinical gold standard is already being used to determine the status of these individuals. Thus, there is an opportunity to collaborate with medical professionals working in these populations, who may provide access to clinical test results for comparative analysis as well as valuable feedback. Selecting populations at risk for imbalance, such as candidates for bariatric surgery or individuals with diabetes, may be useful because they are frequently surveyed and assessed for nutritional status pre and post-treatment.

Researchers could also understand and integrate clinical literature about the relevance of different biofluid samples (Section 2.2), utilizing them where appropriate. For example, several reviewed papers claim to measure in-body micronutrient status from sweat, despite clinical evidence that sweat is an inaccurate representation of in-body status [14, 13]. Clinical relevance can be further improved by assuring novel methods can measure the same biomarkers used by clinicians across the spectrum of deficiency to excess.

5.2 Comprehensive Approaches through Individualized and Multi-Modal Solutions

Micronutrition is a broad and complex field requiring a holistic and individualized approach (Section 1). To develop effective and applicable technologies, it is essential to embrace precision nutrition assessment and multi-modal sensing. Precision nutrition leverages technology to account for individual differences in diet, metabolism, demographics, lifestyle, and more [124, 159, 90, 192]. This allows nutritional diagnoses and interventions to be fine-tuned to a specific patient. Future micronutrient assessment methods could integrate elements of precision nutrition by considering these individual differences. AI and ML will be powerful tools in this endeavour, allowing for the consideration of the varied aspects of micronutrition during an assessment.

Multi-modal sensing is a promising opportunity to incorporate precision nutrition into new assessment methods. Current methods conduct biofluid analysis (Section 3) or physiological sensing (Section 4) in isolation. New technologies can combine these approaches with conventional health data, such as physical activity, demographics, and other clinical information. A multi-modal approach that leverages both biofluid and physiological analyses can reveal more relevant insights into nutritional status, alleviate over-reliance on subjective assessments (like dietary reports and physical exams; Section 4.1), and ultimately create a comprehensive overview of an individual's health.

Multi-modal sensing also allows for the analysis of multiple micronutrients simultaneously, a functional necessity since micronutrient imbalances rarely manifest alone [12]. Current biofluid analysis methods often focus on assessing a single micronutrient, but precision nutrition emphasizes the need to consider all aspects of an individual's health to gather the most relevant insights. Micronutrients interact in complex ways (Tables 11, 12, 13), and there is opportunity for more methods that target several micronutrients concurrently to understand their comprehensive effects on individual health.

If it is not feasible for a single device to measure multiple micronutrients, future work could combine several devices or measurement modes reviewed here together in a unified solution.

We urge calls by Brown et al. [21] for more micronutrition data, as progress towards precision assessment can be accelerated by access to data on individual nutrition prognoses (Section 3.5). Innovative micronutrient status assessment devices and other health sensors could be utilized during clinical studies and interventions to collect the necessary data for assessing micronutrient status in a personalized manner. Additionally, studies could analyze micronutrient status changes within and between individuals over time, leading to important insights and discoveries. Useful data for research and analysis includes patient demographic information, previous clinical history, and all nutritional screening or assessments conducted through the course of treatment. Images of physical symptoms, biochemical analysis results, and data from a wearable device such as a FitBit could be critical to valuable insights. Increased data availability could enable clinicians to gain greater insights and discoveries, allow for applying data-hungry AI and ML models [36], and pave the way for early detection of micronutrient imbalance. Diversity across cultures, gender, race, and other demographic and socioeconomic factors is essential to the global value of new research in precision micronutrient assessment. The impact of such factors should be carefully analyzed and reported, creating a more well-rounded understanding of nutrition that minimizes bias. To achieve this, accessible PoC devices will be needed to collect data at scale, while AI and ML tools will be useful to mine for insights.

5.3 Accessible and Noninvasive Point-of-Care Devices

This work highlights the critical need for accessible and noninvasive methods to assess micronutrient status. Accessible assessment can both aid in data generation, through POoC sample analysis [90], and be a byproduct of it, through a greater general understanding of micronutrition. Our review of existing methods reveals that current technologies have yet to fully address these limitations. The solution to this issue involves leveraging commodity hardware, open-source designs, prioritizing ease-of-use for non-experts, and reducing invasiveness in assessment techniques.

As technology becomes increasingly available to the general public, they can be used to power accessible micronutrient status assessment methods. Interfacing with commodity smart devices as analyzers or additional data sources will continue to be valuable for future work. Smartphones and smartwatches have powerful built-in sensing capabilities with the potential to reveal physiological micronutrition insights. Their utility can be expanded by adding accessories like multiplexed electrochemical sensor chips or microfluidic pumps to perform micronutrient status analyses on biofluids.

Improving ease of use for non-clinicians could significantly enhance accessibility, allowing these devices to be used at home or in the field. Methods that allow users to gain important insights into their micronutritional status and understand the impact on their overall health should be both understandable and actionable for the general user, without needing to consult a medical professional. Although this level of accessibility is a future goal, achieving it would provide individuals with the most direct way to obtain micronutritional status and related insights to take control of their health.

To be more accessible, future methods should be minimally invasive. Current methods strongly rely on invasively-collected biofluid samples, such as serum and plasma. One promising approach is to utilize alternative biofluids, such as urine, which is less invasive to obtain and clinically relevant for assessing micronutrient status (Section 2.2). An even less invasive strategy is to bypass biofluid samples altogether, leveraging on-body devices such as wearables. For example, some research has shown promise in developing on-body spectroscopic devices [35, 182], and others have demonstrated the simultaneous quantification of multiple micronutrients from the spectra of a complex sample using novel ML approaches [112, 113]. Accessible, noninvasive approaches could allow for continuous monitoring of micronutrient status, providing valuable insights and offering a novel approach to micronutrient assessment.

6 Conclusion

This article provides a comprehensive review of accessible and noninvasive in-body micronutrient status assessment methods. We take a critical look at a wide variety of research in the field, based on analyses of both biofluids (Section 3) and physiology (Section 4). These current methods, such as assays, electrochemistry, spectroscopy, optical sensors, and AI & ML, are evaluated in the context of their performance and overall clinical relevance. Several tables are contributed throughout the paper and in the Appendix, intended to gather the most relevant information about micronutrients and their assessment together into an intuitive reference. Our major contributions to micronutrient status assessment as a research area include (1) general background information on micronutrients for a non-clinical audience, (2) synthesis of existing technological and clinical micronutrient status assessment methods, split into biofluid and physiological-based techniques, (3) recommendations for future directions to develop accessible and noninvasive assessment methods, and (4) a unique focus on clinical relevance throughout.

The review synthesized existing micronutrient status assessment methods, split into analyses based on biofluids and physiology. We find that biofluid-based assessments benefit from established biomarkers of micronutrient status and more attention on micronutrients in general, but suffer from highly specific methodologies that frequently rely on blood matrices and lack a comparison to a reliable clinical standard of in-body assessment. Assessments that consider physiology must combat the associative nature of physiological symptoms, the bias inherent in diet self-reporting, as well as the sparseness of micronutrient-specific clinical research or technological advancements. By taking a unique, clinical perspective, this review reveals that no technological solution attempts to consider the holistic nature of micronutrition. Where the clinical standard is to consider both biofluid and physiological analyses together, the focus of current research is predominantly on the application of either biofluid or physiological analysis for the assessment of one single micronutrient at a time.

This review concludes with a discussion of the limitations and gaps in current micronutrient status assessment methods, highlighting actionable opportunities for future research. Three specific opportunities are outlined that require immediate attention to expedite progress in this field. As summarized in Table 9, these opportunities address gaps related to limited clinical relevance, the absence of holistic and comprehensive approaches, and the high invasiveness and inaccessibility of current methods. These challenges can be overcome through clinically relevant innovations, comprehensive individualized multi-modal solutions, and the development of accessible and non-invasive PoC devices. Advancing towards these goals would enable individuals to effectively combat the 'hidden hunger' of micronutrient malnutrition using accessible, non-invasive methods.

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A Tables

Table 10: Alphabetized List of Abbreviations in Main Text

Abbreviation	Definition	Abbreviation	Definition
AAS	atomic absorption spectrometry	LoD	limit of detection
AGP	acid glycoprotein	MILCA	mutual information least dependent component analysis
AI	artificial intelligence	ML	machine learning
ANN	artificial neural network	MS	mass spectroscopy
ANS	autonomic nervous system	NADH	nicotinamide adenine dinucleotide
AUC	area under the curve	NDNS	National Diet and Nutrition Surveys
BAC	blood alcohol content	NFC	near-field communication
BIA	bio-electrical impedance analysis	NFPE	Nutrition-Focused Physical Exam
BMI	body mass index	NHANES	National Health and Nutrition Examination Survey
BMVA	breast milk vitamin A	NIR	near infrared
BP	blood pressure	PBS	phosphate buffered saline
Bphen	bathophenanthroline	PNS	parasympathetic nervous system
BRI	body roundness index	PoC	point of care
CBC	complete blood count	PPG	photoplethysmogram
CoV	coefficient of variance	PR	pulse rate
CRP	C-reactive protein	PRV	pulse rate variability
CUN-BAE	Clinical University of Navarra body adiposity estimator	PWV	peak wavelength value
CVD	cardiovascular disease	RBP	retinol binding protein
ECG	electrocardiogram	RDA	Recommended Dietary Allowance
EDA	electrodermal activity	RF	radio frequency
EDR	estimated daily requirement	RMSE	root mean square error
ELISA	enzyme-linked immunosorbent assay	ROI	region of interest
GSH	glutathione	SNS	sympathetic nervous system
HF	high-frequency	SPR	surface plasmon resonance
HPLC	high-performance liquid chromatography	SSR	spectral super-resolution
HPLC-IR	HPLC with IR detection	sTfR	soluble transferrin receptor
HPLC-UV	HPLC with UV detection	SWV	square wave voltammetry
HR	heart rate	TSH	thyroid-stimulating hormone
HRV	heart rate variability	UL	Upper Limit
ICA	independent component analysis	US	United States
ICP-MS	inductively coupled plasma mass spectrometry	UV	ultraviolet
IDA	iron-deficiency anemia	VAI	visceral adiposity index
IR	infrared	WC	waist circumference
LC	liquid chromatography	WHO	World Health Organization
LED	light emitting diode	WhtR	waist-to-height ratio
LF	low-frequency		

Table 11: Characteristics of Micronutrients: Water-Soluble Vitamins. Information from [12, 34, 115, 17, 119]

	Overview				Interactions Impacting Status		
Micronutrient	Purpose	Storage	Risk of Excess	High Risk Populations	Micronutrients	Diseases (decrease)	Medications (decrease)
Vitamin B1 (thiamin); 3 forms (TMP, TTP, TPP)	Critical to energy metabolism and cell development, functionality	Small amounts in liver	Lack of evidence	Older adults	Absorption decreased by magnesium, folate deficiency	Alcoholism, Inflammatory bowel diseases, Obesity post bariatric surgery, chronic renal failure, critical illness, HIV/AIDS, diabetes	Furosemide, Fluorouracil
Vitamin B2 (riboflavin); 2 coenxyme derivatives (FMN and FAD)	Critical to energy metabolism, cell development and functionality, and metabolism of fats, drugs, and steroids (maintains homocysteine levels)	Small amounts in liver, heart, kidneys	Lack of evidence	Vegitarian athletes, pregnant and lactating women and their infants, people who are vegan and/or consume little milk, people with riboflavin transporter deficiency	Absorption decreased by copper, zinc, iron, manganese intake; deficiency associated with those of folate, pyridoxine, nacin	Alcoholism, Chronic intestinal failure	None
Vitamin B3 (niacin); 2 forms (NAD and NADP)	Critical to energy metabolism, NAD is needed in over 400 enzyme reactions	Some excess in red blood cells	Yes (in supplementation)	Those with undernutrition	Status decreased by inadequate riboflavin, pyridoxine, and/or iron intakes	Hartnup disease, carcinoid syndrome	Antidiabetes, isoniazid and pyrazinamide
Vitamin B5 (pantothenic acid)	Critical to energy metabolism, breaking down and making fats	Red blood cells and tissues	Lack of evidence	Those with a pantothenate kinase-associated neurodegeneration 2 mutation			

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	Overview				Interactions Impacting Status		
Micronutrient	Purpose	Storage	Risk of Excess	High Risk Populations	Micronutrients	Diseases (decrease)	Medications (decrease)
Vitamin B6 (pyridoxine); 3 forms (pyridoxine, pyridoxal, pyridoxamine)	Involved in a wide variety of enzyme reactions, protein metabolism, and cognitive development (maintaining homocysteine levels)	Majority bounded to Albumin	Yes (in supplementation)	Those with autoimmune disorders	Poor status associated with low concentrations of other B-complex vitamins	Alcoholism, Inflammatory bowel diseases, chronic renal failure, malabsorption (celiac, Crohn's, etc), homocystinuria	HIV therapy/treatment, therapies inhibiting vitamin activity, cycloserine, antiepileptics, theophyline
Vitamin B7 (biotin)	Critical to the metabolism of proteins, fats, and carbohydrates into energy	Most stored in liver	None	Those with biotinidase deficiency, chronic alcohol exposure, and pregnant and breastfeeding women		Alcoholism, chronic intestinal failure	Anticonvulsants
Vitamin B9 (folate)	Used to create DNA and RNA, facilitate cell division, as well as to metabolize amino acids (conversion of homocysteine)	15-30 mg with 50% in liver, rest in blood and body tissues	Yes (masks B12 deficiency)	Women of childbearing age, pregnancy, MTHFR genetic polymorphism	Absorption decreased by zinc deficiency, bioavailability increased by Vitamin C, excess can mask B12 deficiency	Alcoholism, chronic intestinal failure, Chronic (atrophic) gastritis, obesity post bariatric surgery, chronic renal failure	Methotrexate, antiepileptics, sulfasalazine
Vitamin B12 (cobalamin) [5]	Critical to CNS development and functionality, RBC formulation, DNA synthesis, conversion of homocysteine	80% in liver; 1-5 mg (thousands times more than daily consumption); can last 2-5 years, 1-3 by some sources	None	Women, elderly, black people, those with low socioeconomic status, who have had gastrointestinal surgery, are vegitarian/vegan	Absorption decreased by excess vitamin C	Alcoholism, chronic intestinal failure, chronic (atrophic) gastritis, Liver diseases, obesity post bariatric surgery, critical illness	Gastric acid inhibitors, metformin
Vitamin C (ascorbic acid)	Required in synthesis of collagen and neurotransmitters, used in protein metabolism, and critical to immune function	High concentrations in cells and tissues, WBC, eyes, adrenal glands, pituitary gland, and brain; total content 300 mg (near acute deficiency) to 2g	Yes (mild nausea, diarrhea, cramps)	Smokers, those with low food variety, any disease causing oxidative stress	Shown to regenerate other antioxidants (ex vitamin E)	Alcoholism, chronic (atrophic) gastritis, obesity post bariatric surgery, critical illnesss	Chemotherapy/radiation, 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors

Table 12: Characteristics of Micronutrients: Fat-Soluble Vitamins. Information from [12, 34, 115, 17, 119]

	Overview				Interactions Impacting Status		
Micronutrient	Purpose	Storage	Risk of Excess	High Risk Populations	Micronutrients	Diseases (decrease)	Medications (decrease)
Vitamin A	Critical for vision, cell growth, immune and reporductive functions	Most in liver (about 6 months), some in eyes	Yes	Infants, pregnant women in low/middle income/developing countries	Absorption decreased by zinc deficiency	Alcoholism, chronic intestinal failure, Inflammatory bowel diseases, liver diseases, obesity post bariatric surgery, cystic fibrosis	Orlistat, retinoids (results in toxicity)
Vitamin D; 2 forms: 25(OH)D (calcidiol) and 1,25(OH)D (calcitriol) [144]	Bone groth and strength, absorption and control of calcium, reducing inflammation	Fatty tissue and liver	Yes	Breastfed infants, adults 20-39, those with kidney/liver dysfunction, with dark skin, limited sun exposure, conditions limiting fat absorption	Magnesium is critical to activation and binding, function is heavily interwound with Calcium	Alcoholism, chronic intestinal failure, chronic (atrophic) gastritis, Inflammatory bowel diseases, liver diseases, obesity post bariatric surgery, chronic renal failure, critical illness	Orlistat, statins, steroids, thiazide diuretics
Vitamin E (alpha-tocotrienol form)	Function as antioxidants, aid in immune, cell signaling, metabolic processes	Liver (alpha-tocotrienol form)	Lack of evidence (UL of 1000 mg in adults)	Infants, those with fat malabsorption, dieting		Alcoholism, chronic intestinal failure, inflammatory bowel diseases, liver diseases, obesity post bariatric surgery	Anticoagulant, antiplatlet, simvastatin, niacin, chemotherapy/radio treatment

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	Overview				Interactions Impacting Status		
Micronutrient	Purpose	Storage	Risk of Excess	High Risk Populations	Micronutrients	Diseases (decrease)	Medications (decrease)
Vitamin K	Involved in blood clotting and bone metabolism	Low blood and tissue stores, carried in lipoproteins	None (except for K3)	Newborns and those with fat malabsorption	Excretion stimulated by excess Vitamin D, absorption decreased by excess Vitamin E and A	Alcoholism, chronic intestinal failure, inflammatory bowel diseases, obesity post bariatric surgery, chronic renal failure, bleeding disorders	Antibiotics and anticoagulants, bile acid sequestrants, orlistat

Table 13: Characteristics of Micronutrients: Minerals. Information from [12, 34, 115, 17, 119]

	Overview				Interactions Impacting Status		
Micronutrient	Purpose	Storage	Risk of Excess	High Risk Populations	Micronutrients	Diseases (decrease)	Medications (decrease)
Iron [58, 55]	Essential to oxygen transport through hemoglobin, energy metabolism, physical growth, neurological development, cell functioning, and hormone synthesis	60% in blood hemoglobin, rest as ferritin in liver, spleen, bone marrow, muscles	Yes (especially those with hemochromatosis and elderly)	Infants, young children, teen girls, those who are pregnant (especially if Mexican-American or black), premenopausal, in food-insecure households, have increased menstaral bleeding	Absorption increased by Vitamin C intake, Absorption decreased by zinc, calcium, manganese intake and copper deficiency	Chronic intestinal failure, chronic (atrophic) gastritis, inflammatory bowel diseases, obesity post bariatric surgery, critical illness, cancer, heart failure	Levodopa, levothyroxine, proton pump inhibitors
Copper	Cofactor in energy production, iron absorption, neuropeptide activation, and synthesis of connective tissue and neurotransmitters	50-120mg total, 95% carried by ceruloplasmin; 2-3 months in skeleton and muscle; tightly regulated, only 1mg/d loss in bile	Yes	Those who are pregnant	Absorption decreased by high zinc	Chronic intestinal failure, obesity post bariatric surgery, chronic renal failure, critical illness, celiac disease, menkes disease	
Zinc [130]	Physical growth and development, cellular metabolism, and immune functions	85% in skeletal muscle and bone, 0.1% in plasma where 70% of that is bound to albumin; 1.5g women, 2.5g men total	Yes	Children, teens, exclusively breastfed infants, those who are pregnant, vegitarian/vegan, have eating disorders, malabsorption, gastrointestinal disorders	Absorption decreased by high calcium/iron	Alcoholism, chronic intestinal failure, inflammatory bowel diseases, liver diseases, obesity post bariatric surgery, chronic renal failure, critical illness, sickle cell disease, HIV	Antibiotics, penicillamine, piuretics
Iodine	Thyroid gland function, protein synthesis, metabolism and enzyme activity	70-80% in thyroid gland; 15-20 mg total	Yes	Infants, those who are pregnant, use uniodized salt, are in regions with iodine-deficient soils	Absorption decreased by iron intake and selenium deficiency		Anti-thyroids, angiotensin-converting enzyme inhibitors, potassium-paring diuretics
Selenium	Reproduction, thyroid hormone metabolism, and DNA synthesis through selenoproteins; also acts as antioxidant	28-46% in skeletal muscle, most in selenomethionine form	Yes	Kidney dialysis patients, those in selenium deficient regions		Inflammatory bowel diseases, liver diseases, chronic renal failure, critical illness, obesity	Cisplatin
Magnesium	Regulates several chemical reactions, including blood glucose and blood pressure regulation, DNA, RNA, and protein synthesis, proper muscle and nerve functioning, bone development, and calcium and potassium ion transport	Approx 25g; 50-60% in bone, <1% in serum (tightly controlled), rest in soft tissue	Yes	Elderly	Absorption increased by Vitamin D	Alcoholism, gastrointestinal disease, bariatric surgery, T2D	Bisphosphonates, antibiotics, diuretics, proton pump inhibitors

Table 14: Physiological Symptoms of Micronutrient Deficiencies: Water-Soluble Vitamins. Information from [123, 37, 39, 140, 142, 115, 17, 119]

Micronutrient	Eye	Nail	Oral	Disease	Autonomic	Misc	Timeframe
Vitamin B1	Disability in eye movement (ophthalmoplegia)			Cardiomyopathies/ heart failure, Sarcopenia		Correlated with fatigue	Stores depleted within 20 days of insufficient intake
Vitamin B2	Conjunctiva inflamation/grittines (angular blepharitis), Redness/fissures in eyelid corners (Angular Palpebritis), conjunctiva redness/iritation, swollen/sticky eyelid, photophobia		Bilateral cracks/redness at corners of lips/mouth (angular cheilosis), dry/swollen/ulcerated lips (cheilosis), redness in lips and tongue, swollen/inflammed/smooth tongue (glossitis), Atrophied papillae			Correlated with fatigue	
Vitamin B3	Redness/fissures in eyelid corners (Angular Palpebritis), conjunctiva redness/iritation, swollen/sticky eyelid		Bilateral cracks/redness at corners of lips/mouth (angular cheilosis), dry/swollen/ulcerated lips (cheilosis), redness in lips and tongue, swollen/inflammed/smooth tongue (glossitis), Atrophied papillae, inflamed gums (gingivitis)	Pellagra		Correlated with fatigue	Biomarkers indicate insufficiency far before clinical symptoms appear
Vitamin B5					Sleep issues, fall in diastolic bp and lability of systolic bp	Correlated with fatigue, numbness/burning in extremities	
Vitamin B6	Conjunctiva inflamation/grittines (angular blepharitis), conjuntiva pallor, Redness/fissures in eyelid corners (Angular Palpebritis), conjunctiva redness/iritation, swollen/sticky eyelid	Excessive thinness, hapalonychia	Bilateral cracks/redness at corners of lips/mouth (angular cheilosis), swollen/inflammed/smooth tongue (glossitis), dry/swollen/ulcerated lips (cheilosis), Atrophied papillae, redness in lips and tongue	Anaemia, cardiomyopathies/heart failure	Supplementation improves blood pressure, reported to help regulate SNS	Correlated with fatigue	Borderline and mild status may not present symptoms for months or years; Radler and Lister [140] say "deficiency often occurs within 2 months of inadequacy"
Vitamin B7	Excessive dryness, excessive thinness, brittleness			Multiple sclerosis		Correlated with fatigue	
Vitamin B9	conjuntiva pallor	Central ridges	redness in lips and tongue, swollen/inflammed/smooth tongue (glossitis), inflamed gums (gingivitis), dry/swollen/ulcerated lips (cheilosis), Aphthous Stomatitis (canker sores), inflamed/burning mouth, Atrophied papillae	Anaemia, diabetes mellitus		Correlated with fatigue	
Vitamin B12 [5]	conjuntiva pallor	Pallor, clubbing (Koilonychia), transverse white lines (Muehrcke's lines), excessive dryness, darkness in nails, curved nail ends, central ridges, longitudinal melanonychia	Bilateral cracks/redness at corners of lips/mouth (angular cheilosis), swollen/inflammed/smooth tongue (glossitis), dry/swollen/ulcerated lips (cheilosis), pallor, Aphthous Stomatitis (canker sores), inflamed/burning mouth, Atrophied papillae, redness in lips and tongue, bleeding gums, tooth loss, tooth cavities	Anaemia, Osteoporosis, sarcopenia	Deficiency lowers HRV measurements, levels negitively correlated with sleep duration, levels positively correlated with sleep movement and self-assessed quality, night sweats, oxidative stress	Correlated with fatigue	Clinical symptoms can take years (typically 2-5) to appear because of storage levels, glossitis may present initally
Vitamin C		Splinter hemmorage, excessive thinness, hapalonychia	Intraoral mucosa and tongue inflammation, inflamed gums (gingivitis), thrush, tooth loss, tooth cavities	Scurvy	Supplementation improves blood pressure, helps regulate SNS	Correlated with fatigue	Deficiency can occur after 3-6 months of poor intake, signs of scurvy appear within 1 month of <10mg/day intake

Table 15: Physiological Symptoms of Micronutrient Deficiencies: Fat-Soluble Vitamins. Information from [123, 37, 39, 140, 142, 115, 17, 119]

Micronutrient	Eye	Nail	Oral	Disease	Autonomic	Misc	Timeframe
Vitamin A	Bitot's spots, yellowish lumps around eyes (xanthelasma), cornea softening (kerotomalcia), night blindness	excessive dryness, excessive thinness, leukonychia, hapalonychia		Obesity (beta-carotene), measles	Depletion led to increased norepinephrine and epinephrine in heart and spleen of rats	Heavily associated with antiox-idants and immune processes;	Plasma retinol lowers only after storage in liver and eyes are nearly depleted, then Xerophthalmia (progressive eye dryness leading to night blindness) develops after that
Vitamin D [144]		Beau's lines, longitudinal melanonychia, excessive thinness, hapalonychia	inflamed gums (gingivitis)	Cancer cachexia, car-diomyopathies/heart failure, Chronic obstruc-tive pulmonary disease, osteoporosis, sarcopenia, critical to formation of hypocalcemia, depression	Deficiency lowers HRV measurements, calcidiol deficiency lowers resting sympathovagal balance, calcitriol deficiency to worse reactions to stress, supplementation improves blood pressure		
Vitamin E				Obesity			
Vitamin K				Osteoporosis		Impaired clotting and bleeding	

Table 16: Physiological Symptoms of Micronutrient Deficiencies: Minerals. Information from [123, 37, 39, 140, 142, 115, 17, 119]

Micronutrient	Eye	Nail	Oral	Disease	Autonomic	Misc	Timeframe
Iron [58, 55]	conjuntiva pallor, Redness/fissures in eyelid cor-ners (Angular Palpebritis), conjunctiva redness/iritation, swollen/sticky eyelid, blue-tinted sclera	Pallor, clubbing (Koilonychia), transverse white lines (Muehrcke's lines), brittle-ness, excessive dryness, exces-sive thinness, darkness in nails, curved nail ends, central ridges, Onycholysis, onychorrhexis	Bilateral cracks/redness at corners of lips/mouth (angular cheilosis), pallor, swollen/inflammed/smooth tongue (glossitis), Atrophied papillae, dry/swollen/ulcerated lips (cheilosis), thrush, inflamed/burning mouth, redness in lips and tongue	Anaemia, cardiomy-opathies/heart failure, osteoporo-sis	Disrupts opti-mal function of endocrine and immune systems; positively correlated with sleep quality (dis-puted); IDA affects temp regulation and HRV (HRV disputed); low levels associated with higher HR	Status has rela-tion to energy levels and fatigue according to some sources; critical to oxygen binding; weak-ness; impaired cognitive func-tion	Multiple phases: depletion of stores (mild deficiency, can take several months), iron-deficiency erythropoiesis (erythrocyte production), then iron deficiency anemia (IDA)
Copper	conjuntiva pallor			Anaemia, chronic obstructive pulmonary disease, fatty liver disease, osteoporosis	negitively correlated with sleep quality, reported to help regulate sns	Abnormal lipid metabolism	Some weeks to develop and not readily recognized, Usually manifests in acute conditions
Zinc [130]	Conjunctiva infla-mation/grittines (angular blephari-tis)	Beau's lines, onychorrhexis, leukonychia, brittleness	Changes in taste (inconsis-tently observed), dryness (Xerostomia), inflamed gums (gingivitis)	Alcoholic hep-atitis, cancer cachexia, chronic obstructive pulmonary dis-ease, obesity, osteoporosis, sarcopenia, increased pneu-monia risk	Deficiency linked to increased blood pressure, positively correlated with sleep quality, reported to help regulate SNS, critical to ANS functionality according to some sources	Light evidence of relationship be-tween low dietary zinc and un-ideal metabolic re-sponse, correlated with fatigue	Symptoms after "several months of low levels"

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Micronutrient	Eye	Nail	Oral	Disease	Autonomic	Misc	Timeframe
Iodine		clubbing (Koilonychia)		Goiter, hypothyroidism		Critical to metabolic function	Hypothyroidism occurs when intake falls below 10-20 mcg/d, goiter appears fairly quickly
Selenium		excessive dryness, excessive thinness, pallor		Cardiomyopathies/ heart failure, chronic obstructive pulmonary disease, obesity	Intake reduces hypertrophy and oxidative stress, negitively effects blood pressure	Effects on metabolism	
Calcium		Beau's lines, transverse leukonychia, brittleness, excessive dryness, excessive thinness, onychomadesis, onychorrhexis, hapalonychia		Osteoporosis, rickets, osteomalacia, congestive heart failure, seizures		Hypocalcemia can be asymptomatic or have a wide range of symptoms; most common are numbness, tingling, muscle spasms	
Magnesium		Excessive dryness, excessive thinness, brittleness	inflamed/burning mouth	Cardiovascular disease, hypertension, metabolic syndrome, type 2 diabetes, depression, hypocalcemia, hypokalemia, seizures	Abnormal heart rhythms observed	Overt signs of clinical deficiency are not routinely recognized; correlated with fatigue, nausea, numbness, tingling, muscle spasms	

Table 17: Gold-Standard Methods of Assessing Micronutrient Imbalance: Water-Soluble Vitamins. Information from [69, 189, 115, 17, 119]

	Detection Standard				
Micronutrient	Method	Biomarker	Matrix	Intervals	Impact of Inflammation
Vitamin B1	Erythrocyte transketolase activity coefficient assay	Increase in erythrocyte transketolase activity	Washed red blood cells	Deficient: >25%; Insufficient: 15-25%; Sufficient: <15%	None on direct plasma levels
Vitamin B2	Erythrocyte glutathione reductase activity coefficient assay	Increase in erythrocyte glutathione reductase activity	Washed red blood cells	Deficient: >40%; Insufficient: 20-40%; Sufficient: <20%	Decrease in plasma levels (erythrocyte assays are more stable)
Vitamin B3	LC-MS/MS	Niacin metabolites (NMN and 2-pyr, limited representation of stores and recent intake)	Urine	Deficient: <5.8; Insufficient: 17.5-5.8; Sufficient: >17.5 mcmol/day	Lack of evidence
Vitamin B5	LC-MS/MS	Pantothenic acid (requires enzyme pretreatment)	Whole blood	Deficient: <0.22; Sufficient: 0.35-0.59 mg/L	Lack of evidence
Vitamin B6	HPLC or LC-MS/MS	pyridoxal phosphate (PLP)	Plasma or serum	Sufficient: >4.94 or >7.41 mcg/L plasma PLP (varies by source)	Decrease in plasma PLP, no effect on RBC concentration
Vitamin B7 [44]	LC-MS/MS for biotin; gel densitometry for MCC/PCC [107]	Biotin (less sensitive); holo-MCC and holo-PCC (only reliable markers)	Urine for biotin; WBCs for MCC/PCC	Sufficient: 4.4-31 mcg/day urinary biotin, 8.2 arbitrary units holo-MCC, 9.1 arbitrary units holo-PCC	None on biomarkers
Vitamin B9	LC-MS/MS	Folate	Serum for altered exposure and recent intake; Red blood cell for long term/3 month status and storage levels	Sufficient: >3 ng/mL serum, >140 ng/mL RBC	Lack of evidence
Vitamin B12 [5]	GC-MS	B12, confirmed with methylmalonic acid (MMA, also related to B2, B6, folate); no single 'gold standard'	Plasma or serum	Deficient: <200-250 pg/mL B12, >0.03 mg/L MMA (some debate over this)	Association with increased B12 levels
Vitamin C	HPLC	Ascorbate	Plasma (some claim serum should be avoided)	Deficient: 1.94; Insufficient: 2.11-4.05; Sufficient: 4.05 mg/L	Decrease in plasma ascorbic acid (rapid, decrease when CRP >10 mg/L, normal values not detected if CRP >40 mg/L)

Table 18: Gold-Standard Methods of Assessing Micronutrient Imbalance: Fat-Soluble Vitamins. Information from [69, 189, 115, 17, 119]

	Detection Standard				
Micronutrient	Method	Biomarker	Matrix	Intervals	Impact of Inflammation
Vitamin A	LC-MS/MS	Retinol (only sensitive to deficiency or excess in storage, affected by infection and protein/zinc deficiency). Best method is to indirectly measure reserves in liver over several days of administration	Plasma or serum	Severely deficient: <0.1; Deficient: 0.1-0.2; Sufficient: 0.3-1; Toxic: >1 mg/L retinol	Decrease in serum retinol (adjustment equations exist but are not universally applicable, BRINDA R package)
Vitamin D [144]	LC-MS/MS	25(OH)D (calcidiol)	Plasma or serum	Deficient: <12; Insufficient: 12-<20; Sufficient: 20-50; Toxic: >50 ng/mL (not definitively established/linked to clinical outcomes, varies based on assay and lab)	Decrease in plasma levels (all values below reference ranges with CBP >40 mg/L)
Vitamin E	LC-UV	Ratio of Vit E to total blood lipids	Plasma or serum	Insufficient: <5.17 mg/L Vit E, <0.8 mg Vit E/g total lipid; Sufficient: 8.6-13 mg/L Vit E (adults have higher levels)	Some effects (blood concentrations less interpretable at CRP >80 mg/L)
Vitamin K	Immuno-based assays	Plasma phylloquinone (usually for short term intake, no single 'gold standard'); prothrombin time (time to blood clot, only clinically relevant measure); variety of other 'functional' biomarkers	Plasma	Deficient: <0.15; Sufficient (fasting): 0.15-1 mcg/L	Status associated with lower inflammatory marker concentration

Table 19: Gold-Standard Methods of Assessing Micronutrient Imbalance: Minerals. Information from [69, 189, 115, 17, 119]

	Detection Standard				
Micronutrient	Method	Biomarker	Matrix	Intervals	Impact of Inflammation
Iron [58, 55]	Electro-chemilumi-nescence immunoas-say (ECLIA)	Ferritin for deficiency (first phase, evaluates storage, inflated by infection); Iron increase after supplementation for malabsorption; Hemoglobin used to confirm IDA	Serum	Anemia: <10, Deficiency: 10-30 mcg/L ferritin	Ferritin may be inflated, falsely normal/misleading (adjustment equations exist but are not universally applicable, BRINDA R package)
Copper	ICP-MS	Copper or ceruloplasmin (CP), neither reliable	Serum	Depletion: <50.8 (copper); Deficient: 50.8-76.2 (copper, high CRP); Sufficient: 63.5-158.9 mcg/dL (copper), 180-400 mg/L (CP)	Increase in plasma concentrations
Zinc [130]	Atomic Absorption Spec-troscopy (AAS)	Zinc (cut in half by SIRS, can be normal with clinical symptoms present, levels vary with time of day so it is recommended that albumin and CRP changes are taken into account)	Plasma or serum	Deficient: 70 women, 74 men; Insufficient: 70/74-80; Sufficient: 80-120 mcg/dL	Decrease in plasma levels (significant when CRP exceeds 20 mg/L, adjustment equations exist but are not universally applicable, BRINDA R package)
Iodine	ICP-MS	Iodine	Urine (24h or random), serum less recommended	Depletion: <20, NA; Deficient: 20-100, <40; Sufficient: 100-300 mcg/24hr urine, 40-100 mcg/L serum (levels should be higher in those who are pregnant or lactating)	Lack of evidence
Selenium	AAS, ICP-MS	Selenium (recent intake) or Selenoprotein P	Plasma or serum	Deficient: <60; Sufficient: >60; Toxicity: >474 to 948 mcg/L Se (intervals vary by source and population: women and black people have naturally lower concentrations)	Decrease in plasma levels proportional to inflammation, can be adjusted for
Magnesium	AAS	Magnesium	Serum (little correlation with overall status or tissue stores) and urine (after supplementation)	Deficient: <18.23; Sufficient: 18.23-23.1 mg/L serum Mg	