Personalized nutrition diagnostics at the point-of-need

Seoho Lee, Balaji Srinivasan, Sasank Vemulapati, Saurabh Mehta and David Erickson

Micronutrient deficiency is widespread and negatively impacts morbidity, mortality, and quality of life globally. On-going advancements in nutritional biomarker discovery are enabling objective and accurate assessment of an individual’s micronutrient and broader nutritional status. The vast majority of such assessment however still needs to be conducted in traditional centralized laboratory facilities which are not readily accessible in terms of cost and time in both the developed and developing countries. Lab-on-a-chip (LOC) technologies are enabling an increasing number of biochemical reactions at the point-of-need (PON) settings, and can significantly improve the current predicament in nutrition diagnostics by allowing rapid evaluation of one’s nutritional status and providing an easy feedback mechanism for tracking changes in diet or supplementation. We believe that nutrition diagnostics represents a particularly appealing opportunity over other PON applications for two reasons: (1) healthy ranges for many micronutrients are well defined which allows for an unbiased diagnosis, and (2) many deficiencies can be reversed through changes in diet or supplementation before they become severe. In this paper, we provide background on nutritional biomarkers used in nutrition diagnostics and review the emerging technologies that exploit them at the point-of-need.

Introduction

Micronutrient deficiency is prevalent worldwide and is associated with an increased risk of developing numerous health complications.1-3 Iron deficiency for example is responsible for many of the 2 billion cases of anaemia, which in turn contributes to 20% of all maternal deaths.4 Vitamin B12 deficiency is the leading cause of cognitive decline in the elderly, and affects a high percentage of the vegetarian and vegan populations whose dietary constraints prevent them from a sufficient B12 intake.5-7 Vitamin A deficiency is also widespread and is the primary cause of blindness globally.4 It is reported that 77% of the US adults are considered to be vitamin D insufficient,8 and face increased risks of developing bone diseases,9 cardiovascular diseases10 and infections.11-13

Tackling micronutrient deficiencies has been identified by the Copenhagen Consensus as one of the most cost-effective intervention to further global development and progress.14 Furthermore, numerous international agencies have identified the development of tools to efficiently measure micronutrient deficiencies as a priority (e.g. Gates Foundation15). Despite these, effective intervention for micronutrient deficiency has been a challenge as this often requires information about the micronutrient status of the population at the individual level. Unfortunately, most people around the globe have limited knowledge about their micronutrient status which is largely due to the difficulty in obtaining nutritional status measurements. The current standard methods of nutritional analysis need to be performed in centralized lab facilities, and thus are time consuming, labour intensive and can be even more challenging in resource-limited settings where the risks of micronutrient deficiencies are typically higher.16 The problem is further complicated by the fact that deficiencies are often asymptomatic, resulting in patients who are unaware of the severity of their deficiencies until complications develop.

We believe that recent advancements in “lab-on-a-chip (LOC)” devices can be applied to nutritional diagnostics and help tackle the micronutrient deficiency problem by making deficiency and tracking tests more accessible. The LOC devices have been used to carry out complex sample processing and biochemical reactions on-chip, effectively serving as portable laboratories at the point-of-need (PON) settings.17-19 In addition to being globally prevalent and having significant health consequences, micronutrient deficiency has two unique traits that make their PON diagnosis particularly appealing. First, unlike many other biomarkers found in bodily fluids, the healthy ranges for nutritional biomarkers have
been well studied and well defined, which enables one to often make more explicit and unbiased nutritional diagnosis. Second, many cases of micronutrient deficiencies can be reversed through simple changes in diet (e.g. supplementation, food fortification and food diversification) and/or behaviour modification before the condition becomes severe. This means that the effect of deploying a simple PON tool that can inform people of their micronutrient deficiencies can also make relatively simple therapeutic recommendations and the outcomes can be tracked with far fewer confounders.

In this paper, we outline the role of nutritional biomarkers in nutritional analysis and the factors that may complicate their detection, and the PON technologies that have emerged to help address the world’s micronutrient deficiency problem. We then discuss the challenges and opportunities facing the PON nutrition diagnosis at the consumer level.

Nutritional biomarker considerations for diagnostic device design

Nutritional biomarkers are biochemical indicators found in bodily fluids that provide objective and specific means to assess one’s micronutrient status. They play a particularly important role in the diagnosis of micronutrient deficiencies for which patients are often asymptomatic and therefore not observable using common screening approaches based on anthropometric indicators (i.e. body measurements and clinical symptoms). Since the concept of nutritional biomarkers was first introduced more than 30 years ago by Solomons et al., the importance of nutritional biomarkers in assessing one’s nutritional status has been well recognized and investigated. Prior to reviewing the technological state of the art, we first present a selection of standard biomarkers that can be used as detection targets of common micronutrient deficiencies. We then discuss how the diagnosis reliant on the biomarker levels alone could lead to misleading diagnosis, as each biomarker level could be affected differently by various factors including the presence of inflammation or infection, risk profile of the patient and timing relative to dietary exposure. While our review focuses on detection of micronutrient deficiencies from bodily fluids, interested readers may refer to a recent review article on the role of LOC devices and microfluidics technology in macronutrient analysis (i.e. carbohydrate, protein, and fat) and nutrient analysis in food samples. When using nutrient content/intake from food or dietary supplements to assess one’s nutritional status, their interplay with human hosts for varying degrees of nutrient absorption, distribution, metabolism and excretion becomes crucial; interested readers are encouraged to refer to recent work in nutrigenetics and nutridynamics.

Standard biomarkers. Selecting nutritional biomarkers that accurately and reliably reflect the nutrient exposure, status and effect is challenging and there is currently a limited availability of these biomarkers. This has been identified by the Institute of Medicine (IOM) as a knowledge gap that requires further research, and is being addressed by the National Institute of Child Health and Human Development (NICHD) through the creation of the Biomarkers of Nutrition for Development (BOND) program. Despite the challenges, the tremendous progress in the areas of nutritional biomarkers, medicine, metabolism and genetics has led to the discovery of useful nutritional biomarkers over the years. Table 1 briefly outlines the standard biomarkers that have been studied and widely accepted for use in the status assessment of micronutrients such as iron, zinc, vitamins A, D, and B₁₂ (Table 1). A more comprehensive information can be found elsewhere.

Sample types and naturally occurring form of biomarkers. Following the selection of a proper biomarker, the PON device should consider the sample type in which the biomarker reference values are defined. As such they should often be able to process blood samples, which are complex to work with but contains the richest level of physiological information. Nonetheless, there are other bodily fluids like urine, saliva, breast milk, and sweat that could be suitable for assessment of certain biomarkers (see Table 1). Further, many nutrients are under homeostatic control in blood and their concentration in blood may not be indicative of true status or reflect shorter time periods of intake, necessitating analysis in other body fluids or tissues. For example, decreased levels of folate in serum (<7 nM) may indicate vitamin B₉ deficiency, however deficiency is more accurately diagnosed by looking for RBC folate levels below 305 nM. As there exist different processing requirements for each sample type, the diagnostic device should be developed accordingly. Another key consideration should be on what natural form the biomarkers are found in within the sample. For example, blood 25-hydroxyvitamin D [25(OH)D] exist 95–99% bound to the vitamin D binding proteins (VDBP). As this hinders the immunoassay interactions that most vitamin D tests are based on, an essential sample processing to liberate the 25(OH)D from the VDBP has to be incorporated into the diagnostic device design.

Presence of infection or inflammation. Biomarker levels of certain micronutrient deficiencies will be affected by the presence of infection or inflammation in the patient. The failure to take this into account has caused major problems in the current methods used to identify vitamin A and iron deficiencies. Being acute phase reactants, the RBP and ferritin concentrations change (i.e. RBP levels decrease and ferritin levels increase) during an infection or any underlying inflammation. Therefore without accurate assessment of inflammation, the estimates of iron and vitamin A deficiency are biased – false overdiagnosis of iron deficiency and underdiagnosis of vitamin A deficiency. For the detection of such micronutrients, the PON device design should eliminate false outputs by incorporating inflammation and infection markers such as C-reactive protein (CRP) or α₁-acid glycoprotein (AGP).

Risk group. Health consequences of some micronutrient deficiencies could represent a higher threat for certain population groups based on gender, age, race, or location. As such, cut-off values and reference ranges of nutritional biomarker levels may be defined differently for each population group.
For example, iron deficiency is defined by serum ferritin concentrations below $12 \, \text{ng\,mL}^{-1}$ and $15 \, \text{ng\,mL}^{-1}$ in children less than five years of age and adults, respectively. Such understanding should be incorporated in the design of the diagnostic device for more accurate diagnosis of one's nutritional status.

**Timing relative to dietary exposure.** Biomarkers can be classified into short term (representing intake over past hours/days), medium-term (representing intake over weeks/months) and long-term markers (representing intake over months/years). As such the knowledge of the user's recent/usual micronutrient intake, as well as acute/chronic exposure should be incorporated into the operation timing of the nutritional diagnostic devices. For example, analysis of selenium in urine provides information on recent intake, and that in hair or toenails gives a more reliable assessment of long-term selenium status.

**State-of-the-art PON devices for nutritional biomarkers**

Currently there is a need for the development of accurate and reliable PON devices for nutrition diagnostics that can: (1) detect nutritional biomarkers directly from bodily fluids with a matching performance to that of the gold standard methods, where the device’s limit-of-detection and sensitivity can provide an in-depth, quantitative information about one’s nutritional status, and (2) multiplex the detection to a handful of sample types. Realization of such aims in PON has
been the idea behind the development of LOC devices or microfluidic based diagnostics technology, and there recently have been demonstrations of such devices for the rapid biochemical detection of some nutritional biomarkers including: iron, vitamins A, B7, B12 and zinc. In the following sections we review the technological state of the art, while categorizing them by the underlying biochemical detection principle. While immunoreactions represent the standard method of detection for many, special molecular characteristics of some biomarkers enable their detection through other approaches using electrochemical, affinity-based and chemiluminescent reactions. We also discuss how the recent advancements of smartphone technology could facilitate the PON deployment of many of these LOC sensor systems.

**Immunosensing of nutritional biomarkers in PON.** Immunoreactions (i.e. specific binding interactions between the biomarkers and their respective antibodies) have been the underlying detection principle of many gold standard methods including enzyme-linked immunosorbent assays (ELISA) and immunofluorescence assays (IFA). The biomarker-to-antibody interactions are easily transferrable onto the miniaturized features on LOC platforms, which have led to their widespread use in the PON detection of nutritional biomarkers.

**Labelled immunosensors.** Labelled immunoassays are characterized by the use of detection labels on the antigen or antibody for producing discernible signals in an immunoreaction, and may be further classified by the specific label type (e.g. fluorescent, colorimetric, enzymatic, chemiluminescent etc.). Fig. 1A shows a disposable microfluidic fluorescent immunoassay for the 5-plex detection of ferritin, CRP and other blood proteins in human serum samples. In this work, in-parallel detection of 5 different target molecules is achieved on-chip by the incorporation of: the micromechanical values for controlling the reagent flow by pressure, the microchannels for directing the reagents within the chip, and the microchambers for hosting the fluorescent sandwich immunoassay. The device can sensitively measure serum ferritin levels in the range of 350–3500 pg ml\(^{-1}\), which could be combined with its CRP measurements to provide an accurate iron deficiency diagnosis. While this represents a promising step towards a PON detection of multiple nutritional biomarkers, there remained major limitations including the absence of on-chip filtration mechanism for blood samples, and the requirement for a laboratory-scale microscope and computing platform for the fluorescent signal analysis. To overcome these challenges in the near term, this system could be integrated with recent demonstrations of portable fluorescent reader systems, as well as on-chip blood filtration systems based on various microfluidic strategies.

Rapid diagnostic tests (RDTs), widely known for their lateral flow detection principles, are another example of a labelled immunosensor where gold nanoparticles (AuNP) are used as the colorimetric detection labels in an immunoreaction. They have been successfully deployed at the consumer level for the diagnosis of various diseases and medical conditions (e.g. ref. 56), but their application in nutrition has been limited. The recent development of a RDT for vitamin D (Test4D™; Nanospeed Diagnostics) demonstrates the growing demand for these tests which are rapid, cheap, and capable of accepting whole blood samples directly for autonomous processing, transport and biochemical analysis. Here the Test4D™ can effectively act as a PON binary test using 32 ng ml\(^{-1}\) of 25(OH)D levels as the vitamin D deficiency cut-off. More recently our group has demonstrated a novel lateral flow assay for vitamin B12 (Fig. 1B) that achieves the detection of sub-ng ml\(^{-1}\) levels of vitamin B12 through the use of silver enhancement and the “spacer pad” that increases the duration of a

![Fig. 1](https://example.com/fig1.png)

**Fig. 1** Immunosensing of nutritional biomarkers (A) microfluidic fluorescent immunoassay for the 5-plex detection of ferritin, CRP and other blood proteins (B) lateral flow colorimetric immunoassay for vitamin B12 detection in PON (C) ITO-based label-free electrochemical immunosensor for RBP detection. Figures adapted with permission from: (A) ref. 50 Copyright 2009 John Wiley and Sons, (C) ref. 62 Copyright 2014 John Wiley and Sons.
key immunoreaction.\textsuperscript{57} This has the potential to extend the applicability of RDTs for the detection of other key nutritional biomarkers that are physiologically found in sub-ng m\textsuperscript{-1} concentrations (e.g. vitamin B\textsubscript{12} or biotin; 140–365 pM). Despite their exceptional suitability for PON applications and the recent performance advancements, the RDTs are typically limited in two main ways. First is the binary nature of the results which are insufficient for fully describing one’s micro-nutrient status. Second is the simple one-directional nature of the sample/reagent flow which hampers the multiplexing of distinctly different biomarkers that demand varying levels of sample processing, biochemical reaction and/or signal amplification.

\textit{Label-free immuno}sensors. Detection of nutritional biomarkers can also be achieved using label-free immunosensors based on the transduction of biomarker-to-antibody binding events into measurable signals that may be electrochemical, optical, piezoelectric or thermometric.\textsuperscript{56,58,59} Among the methods based on different transduction schemes, electrochemical label-free immunosensors have shown the biggest potential for PON sensing applications as they are low-cost, highly sensitive, biocompatible, and easily miniaturized.\textsuperscript{60,61} Recently a disposable, label-free electrochemical immuno}sensor for vitamin A deficiency diagnosis was reported.\textsuperscript{62} As shown in Fig. 1C, indium tin oxide (ITO) surface with an immobilized layer of anti-RBP acted as the transducer that changed its impedance in response to the binding events between the anti-RBP and the sample RBP. Analysing the impedance data using the single frequency impedance technique, \c{S}im\c{s}ek \textit{et al.} quantified purified RBP in artificial serum samples with a limit-of-detection of 2.5 ag mL\textsuperscript{-1}. While the presented immunosensor required laboratory infrastructure for its operation, ultrasensitive electrochemical immunosensors that are better suited for PON nutrition diagnostics may be developed by integrating the sensors into compact microfluidics systems as demonstrated in other works for the detection of non-nutritional biomarkers.\textsuperscript{63,64}

\textbf{Chemical sensing of nutritional biomarkers.} Special molecular characteristics of some nutritional biomarkers offer additional sensing strategies to be exploited even when the associated antibodies are non-existent or not readily available. These are often minerals and water-soluble vitamins that can easily be incorporated into: a part of known protein–ligand affinity interactions, or a chain of chemical reactions as reactants or catalysts. In the following sections we discuss how these principles have been used to enable the PON detection of biotin (vitamin B\textsubscript{12}), zinc and cobalt(\textit{ii}) ions in vitamin B\textsubscript{12}.

\textit{Protein–ligand affinity sensors.} Extremely high affinity between biotin and streptavidin has been well known and exploited in numerous purification and sensing applications by tagging biotin to target proteins and subsequently capturing them with streptavidin modified with various reporters (e.g. enzymes, fluorophores).\textsuperscript{65} For the special case where biotin is the target molecule itself, its detection using streptavidin can be more convenient and direct. Dimov \textit{et al.} has previously demonstrated a “stand-alone self-powered integrated microfluidic blood analysis system (SIMBAS)” which used the biotin-streptavidin affinity pair to quantify pM of biotin levels in whole blood.\textsuperscript{66} As shown in Fig. 2A, the PDMS-based SIMBAS system has 3 key parts: the filter that extracts plasma from whole blood samples by sedimentation, detector that captures sample biotin \textit{via} an immobilized layer of streptavidin, and suction chamber that sucks in and drives the sample flow with the vacuum pressure created by degassing. For operation, the authors ran SIMBAS with blood samples spiked with various concentrations of fluorescently tagged biotin, and demonstrated quantification of biotin down to 1.5 pM after 10 min. The effective use of microfluidics in SIMBAS has led to numerous advantages including: the low sample volume requirement of 5 \textmu l, rapidity, self-
powered blood sample processing and flow activation, and the ability to run 5 in-parallel tests. Despite the strengths, the key limitation of the SIMBAS is that it required the fluorescent labels to be on the biotin for quantitation, whereas the naturally occurring biotin in human blood will be without labels. Furthermore, the future application of the SIMBAS to other biomarkers necessitates the detection using other affinity pairs that likely have weaker interactions than those between biotin and streptavidin, which will evidently lead to reduced sensor performance. For this prototype SIMBAS system, the requirement for a microscope to analyse fluorescence signals at the end hampered its deployment for PON quantification of biotin, which could be addressed by its integration with portable fluorescent reader systems as previously discussed.

**Electrochemical sensors.** Several nutritional biomarkers of the ionic form may be detected using an electrochemical principle known as anodic stripping voltammetry (ASV). Recently, a disposable copper-based electrochemical sensor that uses the ASV method for the PON detection of zinc in serum was demonstrated. As shown in Fig. 2B, the zinc in the sample undergoes two steps, namely: preconcentration step in which zinc is electroplated on a copper electrode of the electrochemical sensor, and stripping step in which zinc is oxidized to generate current that can be measured and correlated with sample zinc concentrations. Their novel sensor showed a limit-of-detection of 140 nM zinc and operated in the physiologically relevant range of 10−15 μM zinc with a total analysis time under 15 min. Furthermore, the presented sensor has a number of attributes that make it attractive for PON applications including: the choice of low-cost copper as the new electrode material, sensor integration with miniature potentiostat/galvanostat system, and disposability. However, the sensor operation in this work was limited by the laboratory-based sample extraction, and could be advanced by incorporating microfluidics to perform sample processing on-chip as previously mentioned. Additionally, copper as a working electrode may not be as robust as the carbon-based electrode choices.

**Enzymatic sensors.** On the other hand, some nutritional biomarkers may be known catalysts of reactions that emit detectable signals of various forms including chemiluminescence. Recently, a continuous-flow microfluidic LOC device based on luminol-peroxide chemiluminescent reactions was demonstrated for the determination of vitamin B12. In this work, Lok et al. exploited the finding that the cobalt(ii) ions in vitamin B12, when liberated via acidification, are effective catalysts for the oxidation of luminol in the presence of hydrogen peroxide (H2O2) under alkaline conditions. The chemiluminescent signal produced could be correlated to the cobalt(ii) ion concentrations, which are directly correlated to the sample B12 concentrations. As shown in Fig. 2C, the microchip device contains two passive micromixers with 5 inlets (correlating to vitamin B12 sample, hydrochloric acid (HCl), sodium hydroxide (NaOH), luminol and H2O2), and double spiral microchannels as optical detection regions. In operation, sample B12 and HCl were inputted into the micromixer in order to free the cobalt(ii) ions from the B12 via acidification, subsequently followed by the supplies of NaOH, luminol and H2O2 which initiated the chemiluminescent signal production. While the device has demonstrated an excellent LOD of 0.368 pg mL\(^{-1}\) B12, the performance was only demonstrated using B12 supplements and egg yolk which were processed and prepared off-chip as samples. Furthermore, the requirements for laboratory-scale detector system and infusion pumps represented hurdles for its PON vitamin B12 determination. Beyond incorporating the portable chemiluminescence analyzers, this system could benefit from the recent advancements in on-chip microfluidic pumping strategies based on pneumatics, electrokinetics, magnetohydrodynamics and acoustics. In Table 2, we summarize the different biosensing technologies for the PON detection of the nutritional biomarkers presented in Table 1, along with their main challenges and future outlook. While there may exist numerous other biosensing schemes that can be used to detect these biomarkers, we present the technologies that we believe to have high prospects for enabling PON applications.

**Smartphone enabled sensing of nutritional biomarkers in PON.** Detection of nutritional biomarkers in PON settings is being enabled by the technological advancements in LOC sensor systems based on various biochemical phenomena, however obtaining quantitative results from these systems still requires the sensor outputs to be read and interpreted using a sophisticated instrument. While recent technological advancements have successfully transformed some of the laboratory based instruments into portable units, the cost of such systems become prohibitively high for their widespread adoption by consumers who typically only require sporadic nutrition diagnostic measurements. The smartphone technology can be a convenient yet powerful alternative to these instruments, as the vast majority of the complexity required to make and interpret such measurements is already embedded in smartphones. Furthermore, as smartphones are becoming increasingly ubiquitous both domestically and abroad smartphone based PON devices could face significantly lowered barriers to entry into the consumer diagnostics market. The efficacy of smartphone based platforms in nutritional analysis was previously demonstrated by our group for vitamin D deficiency diagnosis. In that work, we presented the “vitamin D AuNP immunoassay device (vitaAID)” system shown in Fig. 3A which used the smartphone’s imaging and computational capabilities to read and analyse colorimetric signals from a novel AuNP-based immunoreaction on the vitaAID test strips. The system accurately quantified serum 25(OH)D with an accuracy of 15 nM and a precision of 10 nM without the use of conventional laboratory equipment (i.e. spectrophotometer). More recently we presented the NutriPhone system, a mobile platform for personalized diagnosis of vitamin B12 deficiency. This system improved upon the limitations of the vitaAID system by incorporating a custom RDT for vitamin B12 capable of processing and analysing whole
blood samples on-chip in less than 15 min. The effectiveness of the NutriPhone system was validated in a domestic human trial in which it was used to correctly diagnose the B12 status of human participants. Here the use of smartphone technology enabled our system to obtain quantitative B12 information from conventionally qualitative RDT systems in PON settings. As such detection principle could be applied to other RDT systems, NutriPhone system could be expanded for the diagnosis of other micronutrient deficiencies simply by developing additional RDT technologies.

Fig. 3B demonstrates another example in which a simple paper based microfluidics technology was empowered to perform complex PON nutrition diagnostics by being integrated with the smartphone technology. In this work, Lee et al. presented the “electronics enabled microfluidic paper-based analytical device (EE-μPAD)” which consists of: a paper-based microfluidics device for immunodetection of ferritin, RBP and CRP in whole blood samples, and an optoelectronics system that measures light transmission through the detection regions on the paper-based device and wirelessly transmits the data to a smartphone for processing the data and communicating the results. Outsourcing of the computing power and connectivity of smartphones enable the EE-μPAD to assess vitamin A, iron and inflammation status by allowing the quantification of RBP, ferritin and CRP, respectively, in the physiologically relevant ranges. The efficacy of the EE-μPAD was further demonstrated through a clinical trial where it showed comparable performance to the gold standard ELISA method in assessing whole blood samples. The partial reliance on smartphones wherein sensing is achieved by a separate optoelectronics component adds costs to the EE-μPAD, however presents benefits including its universal compatibility with different types

### Table 2  Summary of challenges and future perspectives for biosensing of nutritional biomarkers at the PON

<table>
<thead>
<tr>
<th>Biosensing technology*</th>
<th>Target biomarker</th>
<th>Main challenges</th>
<th>Future perspectives</th>
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<td><strong>Immunosensors</strong></td>
<td></td>
<td>Large performance dependence (i.e. accuracy, sensitivity, reproducibility, cross-reactivity) on quality of antibodies, and the difficulty in obtaining high quality antibodies; Probability for steric hindrance of antibody binding (e.g. VDBP interfering in vitamin D immunoassays)</td>
<td>Immunoassay performance standardization through adoption of External Quality Assurance (EQA) schemes;76 Heavier reliance on monoclonal antibodies (vs. polyclonal antibodies) and their recombinant production;68 Incorporation of sample pre-treatment (e.g. liberation of 25(OH)D from VDBP)</td>
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<td>Microfluidic enzyme-linked immunosorbent assay (ELISA); fluorescent immunoassays; lateral flow immunoassays; label-free electrochemical immunosensor</td>
<td>RBP 25(OH)D</td>
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<td>Vitamin B12</td>
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<td>Ferritin/transferrin</td>
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<td>Folate (Vitamin B9)</td>
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<td>CRP/AGP70</td>
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<td><strong>Affinity pair sensors</strong></td>
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<td>Requirement for biotin pre-treatment as demonstrated in a recent work36</td>
<td>Assay scheme modification (e.g. pre-treatment of streptavidin instead), adaptation of electrochemical methods29</td>
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<tr>
<td>Biotin-to-streptavidin pair</td>
<td>Biotin (vitamin B1)</td>
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<td>Electrochemical sensors</td>
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<td>Poor long-term stability and susceptibility to interferents of copper electrode; High cost of gold/platinum electrode</td>
<td>Investigation into use of new electrode materials such as graphite23 or carbon</td>
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<td><strong>Anodic stripping voltammetry</strong></td>
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<td>Biosensor optimization (e.g. optimization of thin-film thickness in a recent work29)</td>
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<td><strong>Enzymatic sensors</strong></td>
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<td>High pH and/or temperature dependence76</td>
<td>Incorporation of performance calibration in working pH and temperature ranges</td>
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<td><strong>Chemiluminescent</strong></td>
<td>Cobalt(i) ions</td>
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Fig. 3 Smartphone enabled sensing of nutritional biomarkers (A) vitaAID system for PON diagnosis of vitamin D deficiency (B) EE-μPAD system for diagnosis of vitamin A and iron deficiencies and inflammation. Figures adapted with permission from: (A) ref. 89 Copyright 2014 The Royal Society of Chemistry, (B) ref. 90 Copyright 2015 John Wiley and Sons.
of smartphones, and easier automation of the device operations.

Summary and future perspectives

Micronutrient deficiencies are globally prevalent and responsible for the suboptimal health of the world population, most of whom lack access to proper nutrition diagnostic systems. Development of rapid, cheap and easy-to-use system for accurate evaluation of nutritional biomarkers in PON settings has the potential to address the challenges with current laboratory based methods, and allow for more effective management of micronutrient deficiencies. The on-going advancements in nutritional biomarkers are establishing ways to more accurately diagnose micronutrient deficiencies, which can foster the development of such diagnostic systems.

Various systems for PON diagnosis of numerous micronutrient deficiencies have been realized by miniaturizing different biochemical sensing approaches on LOC devices, which necessarily incorporate microfluidics and/or filtering mechanisms to process and handle the complex bodily fluids on-chip. While most of the aforementioned work are still limited to laboratory use due to the persisting requirements for sophisticated analytical tools and off-chip sample processing, we have shown that the technological solutions (e.g. portable analyzer systems) to these challenges have already been demonstrated for other applications, and could be incorporated in the near future to enable developments of truly PON tools for nutrition diagnostics. Despite the clear roadmap for the development of these PON devices, major challenges remain in their deployment to the consumers market as adoption of portable analytical tools that are necessary for making accurate nutrition diagnostics can significantly increase the device cost. Fortunately, new generation of analyzer systems based on smartphone technology with growing ubiquity, powerful sensing, computing and communication capabilities are replacing such equipment in PON settings. So far, in the field of PON nutrition diagnostics, smartphone technology has been coupled with simple paper-based test strips, empowering them to diagnose several micronutrient deficiencies based on quantitative biomarker measurements. However, smartphone technology can also enable other types of LOC devices such as fluorescent or chemiluminescent based systems for PON nutrition diagnostics, as has been demonstrated in recent works for similar applications. Beyond their roles as analytical systems, the smartphone based PON diagnostic systems have the potential to effectively store and communicate micronutrient status data for better epidemiological understanding and management of the micronutrient deficiencies.

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