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*iron*Phone: Mobile device-coupled point-of-care diagnostics for assessment of iron status by quantification of serum ferritin

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ABSTRACT

Iron deficiency (ID) is an urgent public health problem that has devastating effects on maternal and child health. However, due to poor access and affordability, screening and diagnosis for ID is often limited to proxy hemoglobin measurements alone. Here, we report the development and validation of *iron*Phone, a mobile-device coupled portable diagnostics for quantification of serum ferritin concentrations, an iron status biomarker, within a few minutes, from a drop of fingerprick blood. The *iron*Phone diagnostic platform comprises of a smartphone accessory, an app, and a disposable lateral flow immunoassay test strip to quantify serum ferritin. For initial validation in the lab, we optimized and evaluated the performance of *iron*Phone with known ferritin concentrations in spiked buffer and serum samples. Following lab validation, we performed a human validation by collecting fingerprick whole blood samples from 20 participants to assess iron status using *iron*Phone and compared the results with the laboratory standard IMMULITE 2000 analyzer. Findings from the *iron*Phone for the buffer and spiked serum samples provided a calibration curve with R^2 values of 0.97 (n=27) and 0.93 (n=12), respectively. On comparison with the laboratory standard IMMULITE analyzer in whole blood samples, a correlation of 0.92 ($P < 0.0001$) was observed with a sensitivity of over 90% for predicting ID (ferritin < 15.0 μg/L) via the *iron*Phone, demonstrating its promise for iron status assessment at the point-of-care.

1. Introduction

Iron deficiency (ID) is the most prevalent micronutrient deficiency worldwide and is estimated to account for 50% of anemia cases (95% CI: 47–53%) (WHO, 2008, 2015). Pregnant women and young children are at increased risk of ID, in part due to increased nutritional requirements and inadequate intake and bioavailability. ID has been associated with deficits in neurodevelopment (Georgieff, 2008; Lozoff, 2011; Lozoff et al., 2006) and growth in children and work productivity in adults (Booth and Aukett, 1997), making screening and early diagnosis critical for public health programs.

The WHO recommended biomarkers for assessing iron status include hemoglobin, serum ferritin (Cook et al., 1974; Siimes et al., 1974; Simbauranga et al., 2015; Wang et al., 2010b), and soluble transferrin receptor (sTfR) (Dolka et al., 2007; Punnonen et al., 1997; Skikne et al., 1990) concentrations in combination with an inflammatory biomarker, such as C-reactive protein (CRP). However, most settings in the world where ID is widely prevalent do not have access or the laboratory capacity to measure these biomarkers, which hinders preventive and treatment interventions. Also, hemoglobin is often used as a proxy indicator for iron status, particularly in international settings or in national monitoring programs, although only half of anemia is due to iron deficiency, and ID can occur in the absence of anemia (WHO, 2015).

The traditional methods for iron status assessment, including ELISA and automated immunoassay analyzers such as IMMULITE 2000 Xpi (Siemens Medical Solutions USA, Inc.) require access to a clinic, cold chain, are time consuming, require skilled workers to perform these tests, and are expensive. Healthcare providers will benefit immensely by rapid and accurate diagnostic tools to improve both patient outcomes and workflow efficiency (Boppart and Richards-Kortum, 2014). The availability of a portable biosensor (Srinivasan and Tung, 2015; Turner, 2013) for point-of-care testing (POCT) (Louie et al., 2000; Price, 2001) will enable frequent screening and diagnostic

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testing and provide a means for cost-effective and early detection of ID, and inform appropriate interventions. Additionally, monitoring of iron intervention programs will become feasible with the availability of such a device. Access to simple, low-cost, and personalized nutritional diagnostics can further potentially transform individual health behavior and preventive interventions.

Current commercial POCT systems for assessment of ID are more suitable for centralized laboratories and their application in resource-limited settings is further limited by electrical power requirements and cost. Rainbow® Pulse CO-Oximetry (Masimo Corp, Irvine, CA), a portable device for clinical settings, provides noninvasive continuous measure of total hemoglobin level and can be applied to diagnose anemia based on hemoglobin levels. The Eurolyser smart 700/340 (Eurolyser Diagnostica GmbH) is another commercially available point-of-care system for clinical settings to diagnose anemia based on plasma ferritin and hemoglobin levels. At present, there are no commercially available POCT platforms for iron status assessment in home or field settings.

Lateral flow immunoassay (LFIA) is one of the most widely used rapid, inexpensive, and user friendly immunoassay technique (Posthuma-Trumpie et al., 2009) that has found widespread applications in detection of various targets, such as nucleic acids (Wang et al., 2010a), proteins (Mashayekhi et al., 2012), vitamins (Lee et al., 2016), viruses (Mashayekhi et al., 2010), bacterium (Terao et al., 2013), especially in resource-limited settings. Recently, there has been a surge in consumer health-related products that use smartphone apps and mobile electronic devices for monitoring personal health and self-testing. Mobile devices have become ubiquitous tools (Erickson et al., 2014) that are being widely applied to improve POCT by enabling mobile devices to perform assay readouts and electronic health record management.

Here we present the development and validation of *iron*Phone - a novel, mobile device-coupled LFIA-based POCT for quantification of serum ferritin concentrations from a fingerprick sample, and findings from a small-scale human validation study.

2. Materials and methods

The objective of this study was to develop *iron*Phone for serum ferritin detection and demonstrate how optical detection on a mobile device-coupled platform can achieve the limit-of-detection (LOD) of the ferritin assay to meet the required physiological range, without the need for any additional signal enhancement steps. The test uses a fingerprick blood sample and comprises of a LFIA test strip that can process whole blood samples without the need for any sample pre-processing, pre-store the necessary test reagents, and does not require any flow control devices or moving parts. Imaging of the ferritin LFIA test strips with a mobile platform, and computational image processing via the mobile application enables the *iron*Phone system to provide quantitative information from the traditionally non-quantitative LFIA test strips. The *iron*Phone platform also enables tracking changes in biomarker concentrations over time, providing user-free error tracking, and communication of test results via e-mail or short messaging service (Berg et al., 2015; Erickson et al., 2014). We first optimized the LFIA test strip design and experimentally determined the calibration curves with ferritin spiked buffer samples and ferritin standards in serum. We also validated the performance of *iron*Phone in a small human validation study. The development process is described in detail in the following sections.

2.1. Reagents and materials

Gold nanoparticles (InnovaCoat 20OD 40 nm Gold Conjugation Kit with gold nanoparticles (AuNP)) were purchased from Innova Biosciences Ltd. Purified ferritin, monoclonal and polyclonal antibodies to human ferritin were purchased from Scripps Laboratories. Rabbit anti-Mouse-IgG was purchased from Jackson ImmunoResearch Inc. Linearity FD Anemia Siemens Centaur (AUDIT MicroControls Inc.) were used for calibration with ferritin standards in serum. Amine-free phosphate buffer saline (PBS) buffer at 0.01 M pH 7.4, Tween 20, bovine serum albumin (BSA), borate buffer, and sucrose were acquired from Sigma-Aldrich. Glass fibre conjugate pad with dimensions 300×5 mm, Flow Plus 180 Membrane Cards, and cellulose fibre pad for absorbent pad were acquired from EMD-Millipore. Blood filtration membrane was purchased from MDI Membrane Technologies for use as sample pad. Ferritin ELISA kit was purchased from Ramco Laboratories, Inc.

2.2. Equipment

Equipment used in this study include: lateral flow reagent dispenser and legato 200 Dual Syringe Pump (Claremont BioSolutions LLC), paper trimmer from Dahle (Dahle North America, Inc.), IMMULITE 2000 analyzer (Siemens Healthcare Laboratory Diagnostics), and Synergy 2 Multi-Mode Microplate Reader (BioTek)

2.3. AuNP-anti-ferritin conjugate pad preparation

AuNP (Dykman and Khlebtsov, 2011; Quesada-González and Merkoçi, 2015; Rivas et al., 2014) is the most commonly used label in LFIA. Gold surface of the AuNP has very high affinity towards various biomolecules (Choi et al., 2010; Parolo et al., 2013; Zhu et al., 2014) with enhanced stability and biocompatibility, and can therefore be easily functionalized to conjugate them to various antibodies and enzymes. AuNP has optical properties that enhance sensitivity of analysis due to its intense ruby red color that can be detected even by naked eye or typically using readers to achieve lower detection limits in LFIA. The signal from AuNP can also be further enhanced by addition of silver solution (Anfossi et al., 2013) if required. The monoclonal anti-ferritin IgG produced in mouse was conjugated with 40 nm gold nanoparticles (AuNPs) by following the protocol provided in the InnovaCoat gold conjugation kit. Briefly, the stock antibody was diluted with the antibody diluent provided in the kit to obtain the recommended concentration of 0.1 mg/mL. The diluted antibody (volume 12 µL) was mixed with reaction buffer (volume 42 µL) provided in the kit, followed by addition of 45 µL of reaction buffer/antibody mixture to a mini vial of the AuNP in freeze-dried form, and allowed to incubate for 15 min. After the incubation step, 5 µL of quencher solution provided in the kit was added and mixed gently to stop the reaction. To remove any unbound antibody, 10 times the volume of the quencher (diluted to 1:10) was added to the conjugate and was centrifuged at 9000 g for 10 min. The supernatant was carefully removed, the final AuNP-anti-ferritin conjugates were reconstituted in 0.01 M TBS containing 2% BSA to obtain a 20 OD (optical density) conjugate solution. The conjugate solution was then stored at 4 °C until use. To prepare the conjugate pads for the ferritin assay, the AuNP-anti-ferritin conjugate solution was first diluted to 0.3 OD with conjugate buffer (2 mM borate buffer with 5% sucrose). The conjugate pads were soaked in the diluted conjugate solution for one minute, followed by oven drying at 37 °C for 12 h.

2.4. Ferritin LFIA strip assembly

The base layer of the LFIA strip is a membrane card with a 2 mm thick clear polyester film backing. The membrane card includes the nitrocellulose membrane and the adhesive sections which can be peeled to attach the conjugate, sample and absorbent pads. The test and control line antibodies were dispensed on the nitrocellulose membrane using the lateral flow reagent dispenser to dispense 1 mg/mL polyclonal anti-ferritin and 1 mg/mL anti-mouse IgG, respectively. The syringe pump was operated at a flow rate of 6.4 mL/min to obtain test and control line that are 1 mm wide and 3 mm apart. The