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Emerging Point-of-Care Technologies for Anemia Detection

Ran An[1], **Yuning Huang**[1], **Yuncheng Man**[1], **Russell W. Valentine**[1], **Erdem Kucukal**[1], **Utku Goreke**[1], **Zoe Sekyonda**[2], **Connie Piccone**[3], **Amma Owusu-Ansah**[3], **Sanjay Ahuja**[3], **Jane A. Little**[4], **Umut A. Gurkan**[1,2,5,*]

[1]Mechanical and Aerospace Engineering Department, Case Western Reserve University, Cleveland, OH, USA

[2]Biomedical Engineering Department, Case Western Reserve University, Cleveland, OH, USA

[3]Department of Pediatrics, Division of Hematology and Oncology, University Hospitals Rainbow Babies and Children's Hospital, Case Western Reserve University, Cleveland, OH, USA

[4]Division of Hematology & UNC Blood Research Center, Department of Medicine, University of North Carolina, Chapel Hill, NC, USA

[5]Case Comprehensive Cancer Center, Case Western Reserve University, Cleveland, OH, USA

Abstract

Anemia, characterized by low blood hemoglobin level, affects about 25% of the world's population with the heaviest burden borne by women and children. Anemia leads to impaired cognitive development in children, as well as high morbidity and early mortality among sufferers. Anemia can be caused by nutritional deficiencies, oncologic treatments and diseases, and infections such as malaria, as well as inherited hemoglobin or red cell disorders. Effective treatments are available for anemia upon early detection and the treatment method is highly dependent on the cause of anemia. There is a need for point-of-care (POC) screening, early diagnosis, and monitoring of anemia, which is currently not widely accessible due to technical challenges and cost, especially in low- and middle-income countries where anemia is most prevalent. This review first introduces the evolution of anemia detection methods followed by their implementation in current commercially available POC anemia diagnostic devices. Then,

[*]**Correspondence:** Umut A. Gurkan, PhD, Warren E. Rupp Associate Professor, Mechanical and Aerospace Engineering, Biomedical Engineering, Case Comprehensive Cancer Center, Case Western Reserve University, 10900 Euclid Ave., Glennan Building 616B, OH 44106, USA. umut@case.edu.

Author Contributions

RA and UAG conceived planned the organization of the review. RA, YM, and UG searched and collected the literature. RA, YM, EK, UG, and UAG discussed the structure and contents of the manuscript. RA, YH, RWV, ZS drafted the manuscript. RA, YM, and RWV created the figures. RA, YH, and UG created the tables. YM, EK, CP, UG, ZS, JAL, AOA, SA and UAG reviewed and edited the manuscript.

Conflicts of interest

RA, CP, JAL, UAG, and Case Western Reserve University have financial interests in Hemex Health Inc. JAL, EK, UAG, and Case Western Reserve University have financial interests in BioChip Labs Inc. UAG and Case Western Reserve University have financial interests in Xatek Inc. UAG has financial interests in DxNow Inc. Financial interests include licensed intellectual property, stock ownership, research funding, employment, and consulting. Hemex Health Inc. offers point-of-care diagnostics for hemoglobin disorders, anemia, and malaria. BioChip Labs Inc. offers commercial clinical microfluidic biomarker assays for inherited or acquired blood disorders. Xatek Inc. offers point-of-care global assays to evaluate the hemostatic process. DxNow Inc. offers microfluidic and bio-imaging technologies for in vitro fertilization, forensics, and diagnostics. Competing interests of Case Western Reserve University employees are overseen and managed by the Conflict of Interests Committee according to a Conflict-of-Interest Management Plan.

emerging POC anemia detection technologies leveraging new methods are reviewed. Finally, we highlight the future trends of integrating anemia detection with the diagnosis of relevant underlying disorders to accurately identify specific root causes and to facilitate personalized treatment and care.

Graphical Abstract

In this critical review, emerging non-invasive and minimally invasive point-of-care anemia detection technologies are discussed, highlighting future opportunities and the need for multiplexed approaches and integrated disease etiology identification.

1. Introduction

1.1. Global Scope of Anemia

Hemoglobin (Hb) is an iron-containing oxygen-transport metalloprotein in red blood cells (RBCs) that carries oxygen to tissues and transports carbon dioxide back to the lungs [1, 2]. Anemia, defined as low blood hemoglobin level, is a major global health problem. This disease affects about 25% of the world's population, with the heaviest burden borne by women and children, in countries throughout the world across all income levels [3]. Anemia has significant adverse health consequences and negative impact on social and economic development, which include reduced performance in school and work productivity [3]. Anemia causes numerous symptoms including weakness, fatigue, and dizziness in milder cases as well as life-threatening cardiovascular collapse in more severe cases [4, 5].

The Institute for Health Metrics and Evaluation reported, in the Global Burden of Disease (GBD) Study conducted in 2013, that anemia affected 27.0% of the world's population (1.93 billion people) [6]. Based on data collected between 1993 and 2005, anemia affected 41.8% of pregnant women, 30.2% of non-pregnant women, 47.4% of preschool children, and 25.4% of school age children (which equates to 56.4 million, 468.4 million, 293.1 million, and 304.6 million people in each sub-group respectively) [7]. Detailed definition of anemia has been provided by the WHO based on Hb level thresholds that are categorized into 5 distinct groups: adult males, adult non- pregnant females, adult pregnant females, male children, and female children depending on subject's gender, age, and pregnancy status (Table 1) [1].

1.2. Etiology of Anemia

The etiology of anemia can range from nutritional deficiencies (e.g., iron deficiency anemia) [8], infections (e.g., malaria, hookworm disease, HIV) [9, 10], chronic medical conditions that indirectly cause anemia (e.g., chronic kidney disease, inflammatory/ autoimmune disorders) [9, 10], hemoglobin or red cell disorders (e.g., sickle cell disease (SCD), thalassemia, myelodysplastic syndromes) [4], and pharmaceutical drug treatment (e.g., cancer chemotherapy) [4, 5]. A more extensive list of anemia etiologies is summarized in Table 2 by Kassebaum et. al. [11]. Among the major causes listed in Table 2, iron deficiency, malaria, and hemoglobin disorders constitute the leading causes for anemia.

Iron deficiency anemia can be caused by low dietary iron intake, chronic blood loss or loss of blood due to intestinal worm colonization (i.e., hookworm infection), and iron malabsorption, which is most prevalent in low- and middle-income countries [8, 12]. In addition, functional iron deficiency can also be caused by cancer due to inflammation induced sequestration/decreased utilization of iron and the blood loss from tumor sites, malignant invasion of normal tissue and bone marrow [13]. Malaria is also a major cause of anemia in endemic areas [14]. In high transmission settings, malaria induces anemia especially in young children. At all levels of transmission, malaria has been found to contribute to maternal anemia during pregnancy as well as to poor birth outcomes [14]. Inherited hemoglobin disorders such as SCD and thalassemia can cause hemolysis of red blood cells (RBCs) and severe, chronic anemia [11, 15].

Anemia can be mitigated by early diagnosis followed by timely intervention [16]. Effective treatment of anemia requires timely and accurate identification of the specific cause [17–19]. Misdiagnosis of the cause of anemia will lead to the incorrect intervention, which may result in severe clinical events [9]. For example, iron deficiency anemia can be treated by providing micro-nutrient supplementation. However, the WHO recommends that iron supplements be used only where malaria prevention and control systems are available [20]. Additionally, patients with hemoglobinopathies are at the risk of ron overload from hemolysis and recurrent multiple transfusions in patients with SCD, as well as from imbalanced iron homeostasis in patients with thalathemia [21–23]. Consequently, there is a significant need for rapid and accurate diagnosis of anemia and identification of the underlying disease etiology.

1.3. Anemia Detection and Anemia Cause Identification – Tests within Centralized Laboratory Environment and Unmet Needs at the Point of Care

Optimal management of anemia requires early diagnosis and monitoring using blood tests that measure hemoglobin levels [11, 24]. Even in high resource nations, anemia remains a major public health issue. Based on data collected between 2003 & 2012 for the US population, it was estimated 5.6 % met the criteria for anemia and 1.5% could be classified as moderate-severe anemia [25]. Laboratories in hospitals and clinical offices are utilized to aid in identifying the underlying cause of anemia in an individual patient [26, 27]. The current gold standard in laboratories for anemia detection and hemoglobin level measurement is complete blood count (CBC) using an automated hematology analyzer (AHA) on a venous blood sample in a centralized clinical hematology laboratory environment [26, 27]. The current gold standard laboratory methods for detecting 1) iron deficiency, 2) malaria, and 3) hemoglobin variant are 1) Levels of serum ferritin and transferrin, mean corpuscular volume, mean corpuscular Hb level [12], 2) microscopy visualization [28], and 3) High Performance Liquid Chromatography (HPLC), respectively. These detection methods are expensive and labor intensive, typically taking an extended period of time before results are available and are not accessible to patients all over the world, especially in low- and middle resource settings, where anemia is the most prevalent. In a 2019 report, the WHO listed diagnosis and monitoring of anemia, iron deficiency, malaria and SCD as essential in vitro diagnostic (IVD) tests for primary care use in low and middle income countries [29, 30]. POC technologies have been developed for detecting

iron deficiency [31–33], malaria [34–36], and SCD [37–39] and can be found in previous published review articles.

The purpose of this review is to provide a useful comprehensive resource to researchers working on developing novel, clinically useful POC technologies. We aim to provide an overview of the methodologies for measuring Hb level and to review the current commercially available as well as emerging technologies for anemia detection. Section 2 introduces a list of methods and theoretical foundations for minimally-invasive and non-invasive anemia detection. Section 3 reviews current commercially available technologies (both minimally invasive and non-invasive) and provides a summary of the reported performance from recent publications. Section 4 reviews minimally invasive technologies (4.1) using electrical impedance (**4.1.1**) and optical detectors (**4.1.2**), as well as non-invasive technologies using optical detectors including smartphone-based technologies (4.2). In Section 5, we provide recommendations for the development and testing of future POC anemia detection platforms. The objective of this review is to provide a summary of (1) major Hb measurement methods, (2) current commercially available (minimally invasive and non-invasive) technologies and (3) emerging technologies (minimally invasive and non-invasive) for anemia detection. Based on the reviewed literature, we attempt to recommend practical solutions to develop and test integrated technologies for screening, monitoring and diagnosis of anemia and identification of the major underlying causes for the disease with high reproducibility, sensitivity, and specificity.

2. Methods

Combinations of key words including ‘anemia’, ‘anaemia’, ‘hemoglobin’, ‘haemoglobin’, ‘point-of-care’, ‘microfluidics’, ‘non-invasive’, ‘near-infrared’, and ‘photoplethysmography’ were searched using Google Scholar and PubMed. For commercially available technologies, the maximum and minimum reported value of sensitivity, specificity and Bias ± standard deviation for each technology were summarized along with the test population, threshold Hb level used for determining anemia, and reference method (Table 3). Commercially available technologies evaluated by less than 3 publications were introduced but not included in the performance summary. Similar information was also summarized for the emerging technologies reported after 2015, although only single set of test result was available for most of these studies (Table 4). Finally, we summarized test time and cost for commercially available technologies (Table 5).

3. Hemoglobin Level Measurement Methods

3.1. Minimally invasive Methods

Various methodologies have been previously developed for Hb level measurement and anemia detection. Minimally invasive anemia detection methods require blood sampling using minimally invasive techniques such as finger pricks. The cyanmethemoglobin, or hemiglobincyanide (HiCN) method, is currently used as a reference method for evaluating new instruments and alternative methods for Hb level measurement in a standardized guideline provided by Clinical and Laboratory Standards Institute titled CLSI H15-A3 [40] and International Committee for Standardization in Haematology (ICSH) [41]. Vanzetti’s

azide methemoglobin method demonstrates comparable sensitivity and specificity for Hb level measurement to HiCN method and provided the foundation of the first generation of POC hemoglobin devices [42]. Other minimally invasive methods including Tallquist method [43], Sahli's method [44], Alkaline Hematin Detergent method [45], and Copper-Sulfate Specific Gravity method [46]. These methods, although mostly being inexpensive, simple and rapid, suffer from one or multiple deficiencies including significant measurement errors [42, 47], lower sensitivity and reliability [48–51], relying on subjective visual judgement [42], lacking quality control, or requiring biohazardous chemical disposal [46, 52, 53]. Currently, these methods are mostly used in low- and middle-resource settings, where expensive and complex methods are impractical due to limited resources [42].

3.2. Non-invasive Methods

Compared to minimally invasive methods, Hb level measurement based on non-invasive techniques is advantageous for obvious reasons of eliminating the need for a minimally invasive finger prick or venous blood draw. To date, the majority of commercialized non-invasive Hb level measurement devices rely on spectrophotometry or photoplethysmography (PPG) to identify the spectral pattern of Hb in underlying blood vessels to determine Hb level. The working principle of both methods is based on the Beer-Lambert law, $I_0 = Ie^{-\alpha \cdot C \cdot D}$, where I_0 is output light intensity, I is incident light intensity, α is the light absorption coefficient, C is the concentration of Hb, and D is the light path.

In spectrophotometry, near infrared (NIR) light transmit through or reflect from tissue and blood differentially depending on the properties of a living tissue [54]. The variation of these light transmission or reflection properties are associated with the shape, volume, refractive index of Hb, and angular distribution of scattered light, which captures the absorption properties of blood and tissue and enables non-invasive Hb measurement [55]. Light absorption (or light attenuation) is correlated to oxygenated Hb and deoxygenated Hb by modified Beer-Lambert Law, $A_\lambda = \ln\frac{I_0}{I} = \alpha_\lambda \cdot C \cdot D \cdot DPF + G$, where A_λ is the light absorption (in optical densities) at specific light wavelength, λ, DPF is the Differential Pathlength Factor, and G is a parameter of scattering [56]. One specialized spectrophotometer, CO-oximeter, has been applied by commercially available technologies for measuring total hemoglobin concentration based on the fact that hemoglobin and all of its derivatives including oxygen-carrying hemoglobin, non-oxygen-carrying normal hemoglobin, carboxyhemoglobin and methemoglobin are colored proteins which absorb light at specific wavelengths and thus have a characteristic spectrum [57, 58].

Photoplethysmography (PPG) signal consists of alternating current (AC) and direct current (DC) components [59]. The AC component represents the light absorption by changes of pulsatile component of artery blood, while the DC component represents the light absorption by tissues, non-pulsatile component of artery blood and continuous venous blood [60]. The absorption by tissue (T), plasma (P), and Hb under certain wavelength λ can be then expressed as: $I_{0,\lambda} = Ie^{-(\alpha_T \cdot C_T + \alpha_P \cdot C_P + \alpha_{Hb} \cdot C_{Hb}) \cdot D}$; and the AC and DC components are expressed as: $AC_\lambda = Ie^{-(\alpha_T \cdot C_T) \cdot D_T - (\alpha_P \cdot C_P + \alpha_{Hb} \cdot C_{Hb}) \cdot D_1}$, and $DC_\lambda = Ie^{-(\alpha_T \cdot C_T) \cdot D_T - (\alpha_P \cdot C_P + \alpha_{Hb} \cdot C_{Hb}) \cdot D_2}$, where D_1 is the path length for Hb and

plasma in AC signal (pulsatile component of artery blood), D_2 is the path length for Hb and plasma in DC signal, and D_T is the path length for tissue. The ratio between the AC and DC components, $\frac{AC_\lambda}{DC_\lambda} = e^{-(\alpha_P \cdot C_P + \alpha_{Hb} \cdot C_{Hb}) \cdot \Delta D}$, where $\Delta D = D_1 - D_2$ is the path length that affects only the Hb and plasma under wavelength of λ, is thus calibrate toward the effect of tissue and can be used to determine Hb level [59, 61]. The readers may refer to published reviews for a comprehensive understanding on non-invasive methods for Hb measurement [62].

4. Commercially Available Point-of-Care Testing Technologies for Hb Level Measurement

Performance of commercially available technologies including WHO Haemoglobin Colour Scale, Hemocue®, NBM-200, and Pronto-7® have been evaluated by multiple groups, while fewer publications are available for evaluation of other technologies. Performance of emerging technologies have been mainly reported by the inventors in the publications where the technology was reported. The majority of identified publications have reported mean bias (or mean difference) and standard deviation (or 95% confidence interval, or standard error) when comparing the Hb level measured using the reported/tested technology to the Hb level measured by various reference methods, as well as sensitivity and specificity for anemia detection. Fewer publications included the correlation of Hb level measured by reported/tested technology and reference methods (i.e., R^2, R, or Pearson correlation coefficient), and even fewer included the receiver operating characteristic (ROC) curves. Furthermore, large discrepancies exist among various publications reporting the performance of the same technology. For example, one report published by the WHO found that the WHO Haemoglobin Colour Scale to have 95.0% sensitivity and 99.6% specificity in determining the degree of anemia [63]. However, another study published by Lindblade et al. reported 24.0% sensitivity and 97.0% specificity [64], while one other study published by Madakshira et al. reported 99.3% sensitivity and 27.7% specificity [65]. These discrepancies exist in the published studies for most methods, probably due to the variations among test population (adults vs. children, male vs. female, females with and without pregnancy, healthy donors vs. patients with various of diseases), reference method (Cyanmethemoglobin method and a range of different models of AHAs), applied threshold hemoglobin level for defining anemia, as well as testers (researchers, clinicians, nurses, and laboratory technicians). In this review, we compare the performance of the most extensively tested technologies by summarizing the minimum and maximum values of reported sensitivity, specificity, as well as mean bias (mean difference) and standard deviation, along with the test information including test population and the applied reference method (Table 3). Reported performance for other technologies will be individually discussed, due to the limited data available.

4.1. Commercially Available Minimally Invasive Technologies

The World Health Organization (WHO) hemoglobin color scale (HCS) (Fig. 1a) is a quantitative method developed as a commercial kit by WHO under license agreement by COPAC (Oststeinbek, Germany). HCS determines Hb level by comparing blood induced

color change on a chromatography paper with the standard card containing 6 standard colors which correlates to Hb levels from 2 to 14 g/dL displayed in increments of 2 g/dL [66–68]. The performance of HCS has been evaluated in numbers of publications as summarized in Table 3 [51, 63–65, 69, 70]. Overall, WHO color scale is an inaccurate method to screen anemia [42, 71].

HemoCue developed various models of hemoglobin photometers (HemoCue® Hb 201 DM System, HemoCue® Hb 201$^+$ System, HemoCue® Hb 301 System (Fig. 1A)) for POC Hb measurement. HemoCue® System consists of a photometer to determine hemoglobin level by converting hemoglobin to azidemethemoglobin at 565 nm with compensation for turbidity at 880 nm [72, 73]. HemoCue® is by far one of the most extensively evaluated technology for anemia detection [74–83]. According to these studies, HemoCue demonstrated overall satisfactory performance for detecting anemia, while still hold variability among operators [84] and groups of tests [83] and test environment such as humidity [75]. Additional articles are available that review literature on the performance of HemoCue® as compared with laboratory reference tests [85].

Other commercially available minimally invasive technologies for Hb level measurement including Diaspect™, HemoControl, Mission® Plus Hb, URIT Hemoglobin meter, and AnemoCheck™ have also been evaluated, although fewer test data are available than HCS and HemoCue®. The DiaSpect™ system and HemoControl (Fig. 1C) by EKF Diagnostics utilizes multichromatic sensor which measures the whole blood absorbance report Hb level within 5 seconds [86–88]. Both of the technologies have been tested by a few studies as summarized in Table 3 [86, 89–92]. Mission® Plus Hb (Fig. 1D) developed by *ACON* Laboratories uses the principle of reflectance photometry. Hb is converted to methemoglobin on a disposable test strip and measures Hb level measurement, and Hematocrit [93]. We only identified two studies for evaluating the performance of this technology [93, 94]. AnemoCheck™ (Fig. 1E) developed by Sanguina, LLC uses a reagent solution containing hydrogen peroxide and 3,3',5,5'-tetramethylbenzidine that react with 10 μL of blood sample [95]. The blood sample and reagent reacts within a small vial, and the Hb level is measured by comparing the reactant color change to a standard color scale [95–97]. A modified AnemoCheck-LRS can be used in limited-resource settings to permit testing and diagnosis of severe anemia (roughly 2 – 8 g/dL) which can be common in this setting [95, 97, 98]. Further tests would be necessary to better evaluate these technologies under various conditions on different test populations. Overall, minimally-invasive anemia detection technologies demonstrate reasonable reproducibility and accuracy for Hb level measurement as well as sensitivity and specificity for anemia detection. However, blood sampling may cause discomfort to the patient and carry an infection risk.

4.2. Commercially Available Non-invasive Technologies

Pronto-7® developed by Masimo (Fig. 2A) allows intermittently records and displays spectrophotometry measurements [99, 100]. There are recent studies that have been conducted to evaluate the performance of Pronto-7® [81, 99, 101–110] as summarized in Table 3. Multiple publications have suggested to take general caution on making clinical decisions based on these measurements alone [99, 100, 106, 108, 111, 112].

Haemospect® (Fig. 2B) developed by MBR Optical Systems measures Hb level leveraging transcutaneous reflection spectroscopy [58]. The device projects white light into underlying tissue and the transmitted and reflected light are broken down to its separate wavelengths by a spectrometer to calculate the subject's Hb level [112]. Compared to Pronto-7® and NBM-200, fewer evaluation studies have been conducted on Haemospect® as summarized in Table 3 [58, 79, 111–117]. The observed sensitivity ranges from 3.5% [79] to 94.6% [115]. Additional studies on the performance of Haemospect® for Hb level measurement and anemia detection may further verify the validity of the system. Additionally, Bosch has recently developed a portable Hemoglobin Monitor Solution in 2021. We have not identified any clinical evaluation studies on this new technology as of this review.

NBM-200 developed by OrSense (Fig. 2C) utilizes a reusable, ring-shaped pneumatic sensor probe to perform optical absorption measurements of temporary blood-flow occlusion on a subject's finger. Hb level is calculated using a portable desktop monitoring system from a multi-wavelength light source generated by the occluded blood within 60 seconds [88, 111]. This method is similar to normal spectroscopy, except it has a high signal-to-noise ratio due to the obstruction of blood flow, which improves sensitivity of Hb concentration and pulse rate measurements [118–120], while it is intended to spot check Hb level instead of continuous monitoring. The performance of NBM-200 has been evaluated by multiple studies [77, 79, 89, 90, 105, 107, 110, 120–124] as summarized in Table 3. Similar to Pronto®–7, it is also suggested that general caution should be taken if this technology is used to prescreen blood donors for eligibility [77].

5. Emerging Technologies for Hemoglobin Level Measurement and Anemia Detection

In this section, we introduce the emerging approaches and technologies for anemia detection. We categorize the emerging technologies into minimally invasive, including electrochemical impedance and optical based methods, as well as non-invasive methods, including near-infrared, photoplethysmography, and smartphone-based technologies. We attempt to summarize the key perspectives or components for each technology that the researchers have focused on to improve for each category. We also refer the readers to previously published review articles for further information under each sub-category.

5.1. Emerging Minimally Invasive Anemia Detection Technologies

5.1.1. Emerging minimally invasive Anemia Detection Using Electrical and Electrochemical Sensors—Electrical and electrochemical sensors enable the estimation of hematocrit and Hb level by measuring key electrical parameters including impedance, capacitance and electric current of blood constituents. Electrochemical sensors are normally comprised of a working electrode, counter electrode, and with a third reference electrode [125], where WE measures electrical response of the applied blood sample, the CE supplies the required current, and the RE tracks the electrical signal.

StatStrip Hb/Hct developed by Nova Biomedical leverages the electrochemical method for measuring Hb level and hematocrit (Hct) using 1.6 μL of blood in 40 seconds [126]. As

of the time of this review, this technology is not yet available in the USA or Canada thus we included it under ‘emerging technologies’. Chakraborty et al have developed a silicon based microfluidic device employing a two electrodes system for measuring Hct levels (Fig. 3A) [127]. Punter-Villagrasa et al. developed a three electrodes system for measuring Hct levels based on the electric impedance (Fig. 3B) [128, 129]. Phillips et al. demonstrated a non-invasive platform to measure Hb level using a customized capacitance sensor (Fig. 3C) [130]. Overall, these technologies are still at relatively early stages and have only been tested using either small sample sizes or Hb solutions instead of actual clinical blood samples.

Ho et al. developed a unique system employing electrochemical detection of both Hb level and RBC mean corpuscular volume (MCV) [131]. The working principle of this technology relies on the detection of decrease in staircase current caused by collision of RBC to electrode surface, which hinders electrochemical oxidation of an electroactive redox species, potassium ferricyanide (Fig. 3D). This technology uniquely enables measurement of RBC MCV in addition to Hb concentration, which can potentially allow identification of anemia-related diseases caused by abnormal RBC size such as megaloblastic anemia and microcytic anemia.

Electrical and electrochemical based technologies have the potential to provide anemia detection platforms that are portable, label free, easy to use, high throughput, and without complex optical instrumentation [132]. However, some of the current electrochemical platforms still cannot achieve high reproducibility, accuracy and stability as published by the CLSI guidelines (EP05-A3 [133] and EP25-A [134]) [135]. Additionally, variations in other blood components such as white blood cells (WBCs), platelets, plasma proteins and plasma ions may affect the accuracy of the reading thus additional blood pre-processing is required. Readers are referred to recently published review articles for additional information regarding electrochemical sensors [132, 136, 137].

5.1.2. Emerging Minimally Invasive Anemia Detection Using Optical Sensors

—Optical-sensing based anemia detection relies on the sensitive detection of photon emission from blood cells, hemoglobin or reactant from hemoglobin such as methemoglobin. Researchers have employed microfluidic technologies to improve anemia detection with various perspectives including blood sample processing, mixing, detection wavelength selection, whole blood processing, and assay multiplexing. Kim et al. developed a disposable polymethyl methacrylate microfluidic cuvette with a single sidewall microchannel at customized channel depth and a dry film resist as adhesive to efficiently blood sampling by capillary action [138]. Plevniak et al. developed microfluidic auto-mixer, iPOC^{3D}, using 3D printing. The developed iPOC^{3D} automatically mix whole blood with reagents including 3,3’,5,5’-tetramethylbenzidine and hydrogen peroxide (H_2O_2) via capillary force, which is then captured and analyzed calorimetrically using smartphone camera for Hb levels measurement (Fig. 4A) [139]. These sample processing techniques can simplify sample preparation, eliminate the requirement of laboratory infrastructure and external off-chip operation, and increase detection accuracy by preventing air trapping and cell aggregation.

While Hb levels in lysed blood samples can be measured relatively easily solely based on light absorption, anemia detection using unprocessed whole blood can further simplify sample preparation steps and more importantly, can potentially allow additional blood tests for determining the causes of detected anemia with the same sample [140]. Taparia et al. and Halder et al. developed microfluidic systems for anemia detection using non-lysed whole blood samples instead of lysed hemoglobin [140, 141]. The membrane of intact RBCs in non-lysed blood causes light scattering which create deviation from the linear relationship between light absorption and hemoglobin concentration. To maintain the accuracy of Hb measurement, Taparia et al. incorporated parameters including absorption and scattering coefficient and fractional hematocrit in a more generalized model and enabled hemoglobin level measurement based on the calibrated non-linear fitting curve [140]. Halder et al. associated hemoglobin level with the difference between the absorption at 570 nm (isosbestic point of oxy- and deoxy-hemoglobin) and 630 nm (deoxy-hemoglobin) [141].

Zhu et al. developed three types of microfluidic cartridges that can be individually adapted to the same manifold which can incorporate smartphone for imaging the cartridges (Fig. 4B) [142, 143]. Each microchip enables one type of measurement including: (1) WBC counting based on fluorescent labeling, (2) RBC counting using pre-diluted RBCs, and (3) Hb level measurement using lysed blood. The capability of performing WBC and RBC counting in addition to Hb level measurement is highly significant. Future efforts on eliminating sample pre-processing steps or integrating with whole blood processing microchips can further increase the platform usability, especially in middle- or low-resource settings [142, 143].

Paper-based microfluidics have also been implemented for anemia detection. Berry et al. developed a paper-based microfluidic device with selected paper properties and customized device treatment and geometry to enable controlled transport of RBCs. The traveled distance of RBCs is then used to determine hematocrit of whole blood based on the in a thermometer-styled channel (Fig. 4C) [144]. Our group has developed a paper-based microchip electrophoresis platform that allows hemoglobin variant identification [145] and a two-step hemoglobin separation [146, 147]. The first step separates total Hb and a standard calibrator based on the major difference in electrophoretic mobility. The Hb level is determined by measuring the relative abundancy of Hb band and standard calibrator band to allow anemia detection. The second step further separates Hb variants due to their finer mobility differences to allow identification of abnormal Hb variants, including sickle hemoglobin, hemoglobin C, and hemoglobin E (Fig. 4D) [145–152].

5.2. Emerging Non-Invasive Anemia Detection

In certain cases, utilization of minimally invasive methods for anemia detection may be challenging, for example for infants, elderly, pregnant women, as well as for patients who may need continuous Hb level monitoring [61, 153]. Frequent blood sampling can create discomfort to the patient and can be relatively expensive, which is specifically challenging in low- and middle-resource settings. As a result, there is great interest of developing methods and tools that enables Hb level measurement non-invasively, with reduced costs, both in the lab and at the point-of-care [61, 153].

In clinical the environment, rapid non-invasive screen for anemia at the primary care level are conducted by clinicians by examining palmar, nailbed, and conjunctivae for pallor [154, 155]. Although these initial subjective screenings can be useful, especially for identifying severe anemia [154, 156], this method overall demonstrates low sensitivity and specificity for anemia detection and suffers from poor inter-observer agreement [154, 157–159]. The commercially available non-invasive technologies for Hb measurement (Section 3.2) are mainly based on photospectroscopy and photoplethysmography analysis on these body sites [56, 102, 106, 108, 160–164]. However, most of these technologies suffer from one or more of the following limitations: challenging data collection methods; complex data analysis and feature extraction processes; affordability and portability; and relatively low accuracy [165].

5.2.1. Near Infrared (NIR) Spectroscopy Based Technologies—NIR-based Hb level measurement can be traced back to early 2000 [55, 166–169]. However, most of those systems suffered from low detection accuracy. Ongoing research efforts in NIR spectroscopy-based anemia detection have focused on addressing the insufficient signal-to-noise ratio (SNR) of measuring instruments and influences of individual differences remain a challenge [170]. Emerging approaches and technologies have been developed to improve measurement accuracy from the perspectives of light sources (i.e., LED, tungsten halogen light), focus lens, optical collector, beam-splitting system, data collection system, and data analysis algorithm [160, 164, 171–176]. Tian et al. used Equidistant Combination Multiple Linear Regression method and Partner wavelength method to select wavelengths for Hb level measurement in whole blood and concluded that using only two wavelengths combination (1143 nm and 1298 nm) as performed as fine as full-spectrum model [177]. Yuan et al. implemented indium gallium arsenide array detector to avoid the influence from blood flowing, as well as incorporated multiple independent amplifier circuit to obtain higher SNR and sampling rate [164]. Ding et al. have used nine-wavelength broadband light source and silicon photodiode array and developed a high-performance spectrophotometry detectors, with implemented back propagation artificial neural network to analyze the principal components of the spectra extracted [160]. In a most recently published work, Askari et al. implemented novel periodic metamaterial structure that perfectly absorbs the electromagnetic wave in a narrow frequency band in NIR region for Hb level measurement [173]. Readers should refer to recently published review articles for additional information on NIR based sensors [174–176].

5.2.2. Photoplethysmography (PPG) Based Technologies—Numerous commercially available and emerging PPG-based technologies have been developed during the past decade and have demonstrated significant progress. However, the non-invasive Hb level measurement demonstrated inconsistent or compromised accuracy during various tests as summarized by us and others. Ongoing research efforts in PPG-based anemia detection have focused on selection of light sources and wavelengths (i.e., LED, lasers, or lights of different spectra [178–181]), reducing the power consumption, design of photodetectors, and data processing methods. Timm et al. implemented three wavelengths (670, 810, and 1300 nm) to measure Hb level, pulse, and oxygenation, and a fourth wavelength at 1150 nm to measure water absorption to facilitate the calculation of Hb level [182]. Doshi et al. and Nirupa et al. independently developed non-contact sensor using combinations of red

and infrared light wavelengths at 660, 940 nm [183] and 624, 850 nm [184] for Hb level measurement. Liu et al. implemented eight wavelength LEDs with broadband light source combined with temporally constrained independent component analysis and adaptive filters to derive high-quality PPG signals [185]. To reduce the device power consumption, Glaros et al. developed a fully integrated pulse oximeter front-end with sub-mW power consumption [186]. Various of photodetectors were developed to improve signal detection. Schidl et al. implemented standard CMOS process to build multi-junction device [187]. Suzaki et al. implemented silicon photodiode combined with indium gallium arsenide [188]. Costa et al. implemented vertically stacked double junction photodiodes [189]. Yuan et al. developed a multi-sensor approach combining the PPG probe, an accelerometer, and a wearable chest respiration monitor to minimize signal noise including motion artefact, blood pressure and respiration variations [190].

Recent advances in digital image processing and machine learning methods can potentially offer solutions to improve these non-invasive anemia screening methods, as summarized here. For example, Golap et al. utilized symbolic regression of multigene genetic programming to facilitate data analysis [191]. Liu et al. developed models based on partial least squares regression and backpropagation artificial neural network to process PPG signals [185]. Kavsaoğlu used eight regression machine learning algorithms to recognize the characteristic features of PPG signals [192]. Acharya et al. implemented Stacked Regressor Model machine learning model comprising of a two-layer stack of regressors including Least Absolute Shrinkage and Selection Operator, Ridge, Elastic Net, Adaptive Boost and Support Vector Regressors [193]. The readers can refer to recently published reviews for ongoing research efforts on PPG-based Hb measurement and other applications [176, 194–196].

5.2.3. Smartphone Based Technologies—With recent advances in high performance photographic sensors in smartphones, smartphone-based tests have demonstrated significant advantages of being easily accessible and making anemia screening low cost [61, 153]. However, the variety of photographic sensors installed in smartphones poses challenges in the standardization of methods and in the development of universal tools for image acquisition and data analysis. The majority of smartphone-based technologies analyze tissue colors using captured images from conjunctiva [197–209], fingertip [210–215], nailbed [216], or retinal fundus [217]. Collings et al. determined erythema index calculated from smartphone images of palpebral conjunctiva taken under ambient light conditions can be used to measure Hb levels (Fig. 5A) [203]. Dimauro et al. developed an accessory that consists of a special spacer and a macro lens to acquire images using smartphone camera without impact from ambient light (Fig. 5B) [201]. Park et al. leveraged spectral super-resolution spectroscopy to mathematically reconstruct high-resolution spectra of blood Hb acquired by the built-in camera of a smartphone (Fig. 5C) [218]. Wang et al. developed HemaApp involved white and IR LED for anemia detecting over fingertip [213], which has been improved to require only white LED through hardware configuration [212]. Mannino et al. developed machine learning based smartphone app to measure Hb levels from images of the fingernail bed (Fig. 5D) [216]. Mitani et al. developed deep learning algorithm to measure Hb levels using images of retinal fundus, which can be captured in teleretinal

disease screening (Fig. 5E) [217]. This method can be valuable for studying the association of anemia and ocular disease, for which fundus images are already available [217].

In a most recently published review article, Hasan et al. recommended to use fingertip as data collection site due to easy access, use of three different NIR lighting sources, specific signal processing techniques and feature selection methods, and region of interest selection methods, for the optimal development of an accurate Hb measurement model [61]. In addition to these, Dimauro et al. also recommended to increase data sharing and data availability [153]. The readers may refer to these articles for further details regarding smartphone-based technologies [61, 153, 176].

6. Discussion and Future Perspectives

The WHO has declared anemia as a public health priority and has listed anemia testing in the essential list of in vitro diagnostics [29]. Recently developed novel micro-technologies offer simple, rapid, and affordable screening and diagnosis of anemia in minimally invasive and non-invasive manner. These technologies have the potential to facilitate universal diagnosis and screening in low-resource settings. Smart and cautious utilization and wide-spread application of these technologies may revolutionize anemia diagnosis and screening in resource-limited settings and dramatically decrease early mortality due to anemia.

Minimally invasive technologies overall demonstrate relatively higher reproducibility and accuracy in Hb level measurement and have demonstrated the potential to be integrated with additional tests for identifying anemia etiology. Current and future improvement considerations on minimally invasive anemia detection technologies include whole blood processing, reagent mixing, selection of detection wavelength, and assay multiplexing. Integration with blood pre-processing modules or microchips may facilitate efficient sample handling for Hb level measurement as demonstrated by Berkel et al. [219]. Review articles are available on microfluidic-based plasma separation [220] and blood cell sorting [221] technologies that could potentially be available for integration. Potential challenges when integrating these technologies may arise from device cost, usability, portability and the relatively strict requirements for operating environment [219].

Non-invasive technologies have obvious advantage of avoiding discomfort associated with blood sampling, eliminating biohazardous specimen handling, and enabling real-time Hb level monitoring. There have been more than one hundred articles published in recent years reporting various technologies for non-invasive anemia detection. Current research interests are oriented towards the combination of smartphone-based data collection and machine learning algorithm-based image analyses. Current and future improvement considerations on non-invasive anemia detection technologies include selection of body sites for signal acquisition, the type of light source, image sensors, additional accessories, data analysis algorithms, and data availability [61, 153, 176].

Reproducible and accurate Hb level measurement is necessary for clinicians to establish efficient treatment plana for individual patients. To address the need for convenience and accuracy, we envision that one option for future POC anemia detection technologies is

the integrated use of non-invasive and minimally invasive tests (Fig. 6). Such an approach would allow rapid and easy to use non-invasive screening with high sensitivity, followed by minimally invasive diagnosis with high specificity to provide accurate confirmation and anemia severity determination to facilitate accurate clinical decisions.

Upon detection, timely and efficient treatment of anemia require identification of specific disease etiology [6, 222] thus integrating tests for the root cause would be helpful (Fig. 6). For example, policy in Kenya states that women should be tested for multiple diseases at first antenatal care including anemia, malaria, HIV and syphilis [223]. Iron deficiency, hemoglobinopathy (such as SCD), and malaria are the three major causes for anemia and are typically most prevalent in the same areas [222]. These diseases are also listed by the WHO as essential items for in vitro diagnostics and are suggested to be assessed with anemia testing [29]. For example, the WHO recommends that iron supplements should be used only where malaria prevention and control systems are available [20]. Also, the United States Agency for International Development recognized the need for pairing of malaria testing with complementary anemia testing [224, 225]. As a result, there is a significant need for affordable, easy-to-use POC platforms that can perform integrated detection of anemia, iron deficiency, malaria, and sickle cell disease. Current detection of these diseases, however, would require involvement of multiple single-purpose technologies. For example, Smart et al. in 2018 conducted a study for screening anemia and sickle cell disease in Tanzania using two POC technologies [226], while Young et al. in 2018 conducted a study for screening anemia and malaria in western Kenya implementing two separate POC methods [227]. Recent collaborative efforts from the U.S. National Institute of Biomedical Imaging and Bioengineering of National Institute of Health, and Bill & Melinda Gates Foundation have emphasized the need for developing non-invasive POC technologies for integrated testing of anemia, sickle cell disease, and malaria. Such integrated platforms can be used to rapidly screen large populations as well as provide physicians with a practical tool for optimizing therapy in individual patients. In a few of the emerging technologies reviewed in this article have already demonstrated the potential to perform integrated test for anemia and anemia etiology as well as other diseases such as megaloblastic anemia and WBC count [142, 143], microcytic anemia [131], ocular disease [217], and SCD [146, 147], and iron deficiency [33]. Comprehensive review articles on POC detection of iron deficiency [31–33], malaria [34–36], and sickle cell disease [37–39] are available for investigators who are interested in integrating these test capabilities to provide a diagnostic platform for anemia surveillance.

Finally, we would like to emphasize the importance of testing and publishing on new technologies following standard guidelines. We have noticed that the studies conducted for evaluating the performance of these technologies lack consistency in data reporting. Conducting and reporting diagnostic accuracy studies following published guidelines would enable researchers or clinicians to more easily compare and contrast different technologies. Guidelines for clinical study design, study participants, sample size calculations, details on test methods, as well as data reporting are available, such as the Reference and Selected Procedures for the Quantitative Determination of Hemoglobin in Blood [40], Evaluation of Precision of Quantitative Measurement Procedures [133], Evaluation of Stability of In Vitro Diagnostic Reagents [134] published by National Committee for Clinical Laboratory Standards, Standards for Reporting Diagnostic Accuracy [228], and Guidance Document on

Management of Point-of-Care Testing published by the American Association for Clinical Chemistry [229].

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Significance

In this critical review, emerging non-invasive and minimally invasive point-of-care anemia detection technologies are discussed, highlighting the future directions and the need for multiplexed approaches and integrated disease etiology identification.

Figure 1. Minimally invasive point-of-care technologies for Hb level measurement and anemia testing.

(A) The WHO Haemoglobin Colour Scale (Reproduced with permission from [230]). (B) HemoCue® Hb 301(Reproduced with permission from Hemocue, Inc, HemoCue America), (C) DiaSpect™ (Reproduced with permission from EKF Diagnostics, Inc), (D) Mission® Hemoglobin Plus Hb (Reproduced with permission from *ACON* Laboratories, Inc), and (E) AnemoCheck™ (Reproduced from with permission from [98]).

Figure 2. Non-invasive point-of-care Hb level measurement technologies.

(A) Pronto-7® (Reproduced from online resource [231], Masimo, Inc), **(B)** Haemospect® (Reproduced from online resource [232]), and **(C)** NBM-200 (Reproduced with permission from OrSense U.S., LLC)

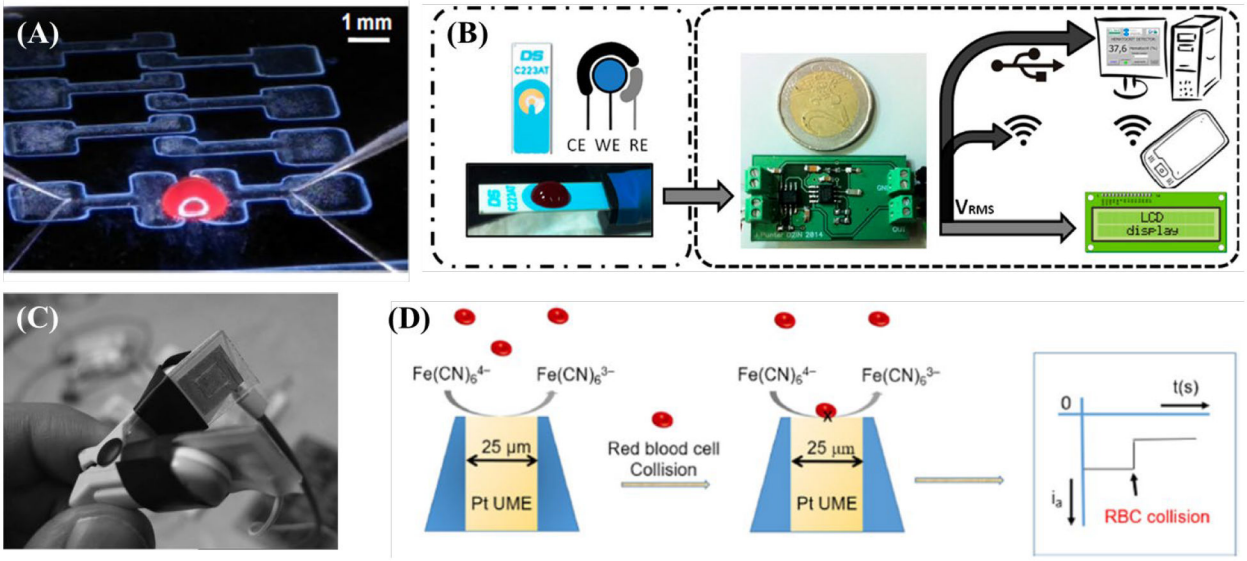

Figure 3. Electrical and electrochemical based Hb level measurement methods.

(A) Silicon based two electrodes microfluidic system for Hb level measurement (Reproduced with permission from [127]). **(B)** Three-electrodes system for electrochemical impedance spectroscopy (EIS) based Hb level measurement (Reproduced with permission from [129]). **(C)** Customized capacitance sensor for measuring Hb levels non-invasively (Reproduced with permission from [130]). **(D)** Measurement of red blood cell count and mean corpuscular volume using electrochemical collision events (Reproduced with permission from [131]).

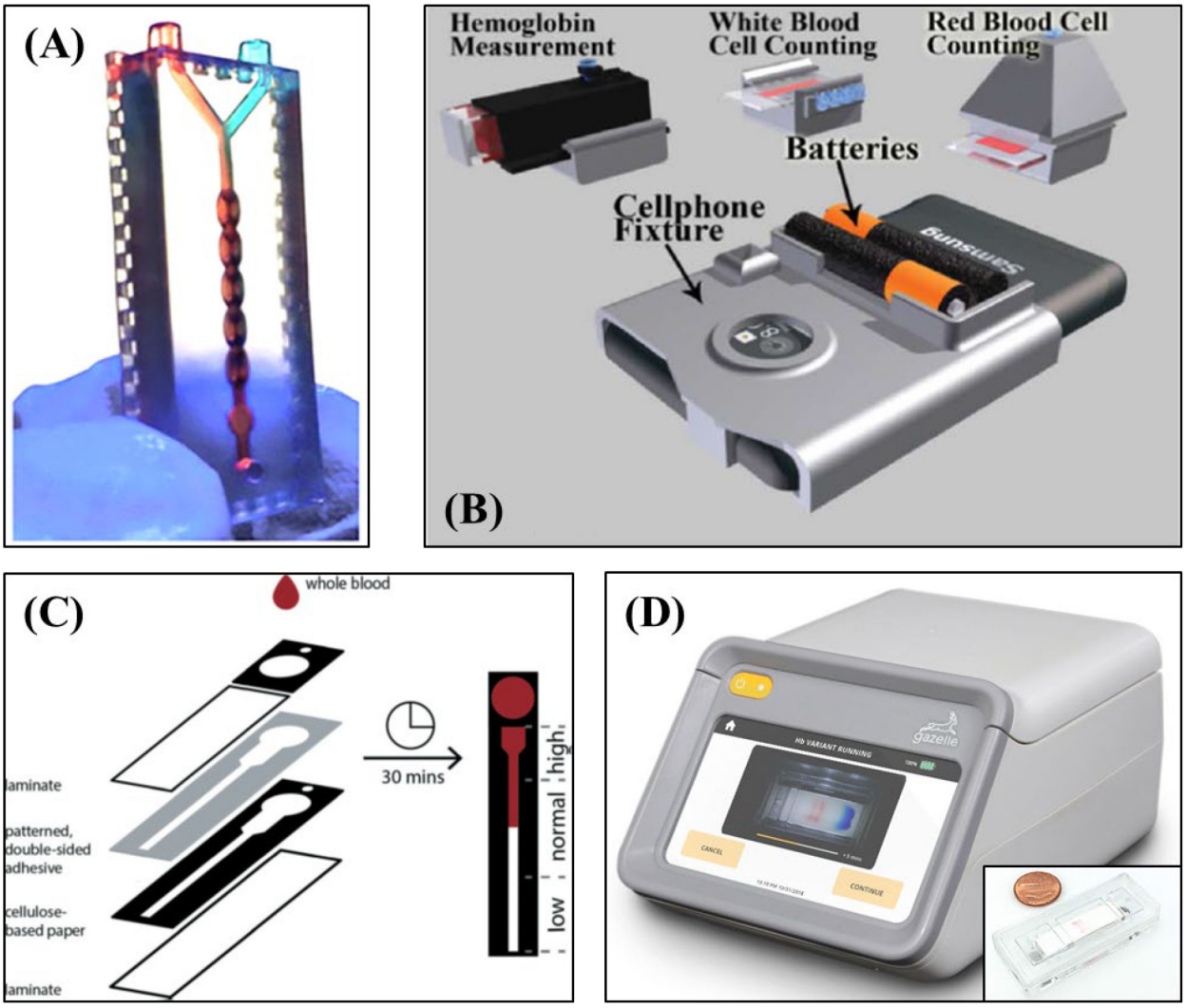

Figure 4.

Emerging optical sensing based minimally invasive Hb level measurement methods. **(A)** iPOC^{3D} automatic blood mixer (Reproduced with permission from [233]). **(B)** Microfluidic cartridges for blood count (Reproduced with permission from [142]). **(C)** Paper-based thermometer-style channel for determining hematocrit (Reproduced with permission from [144]). **(D)** Gazelle Hb Variant/Anemia paper-based microchip electrophoresis for integrated anemia detection and hemoglobin variant identification (Hemex Health Inc, Portland, Oregon) [145–147].

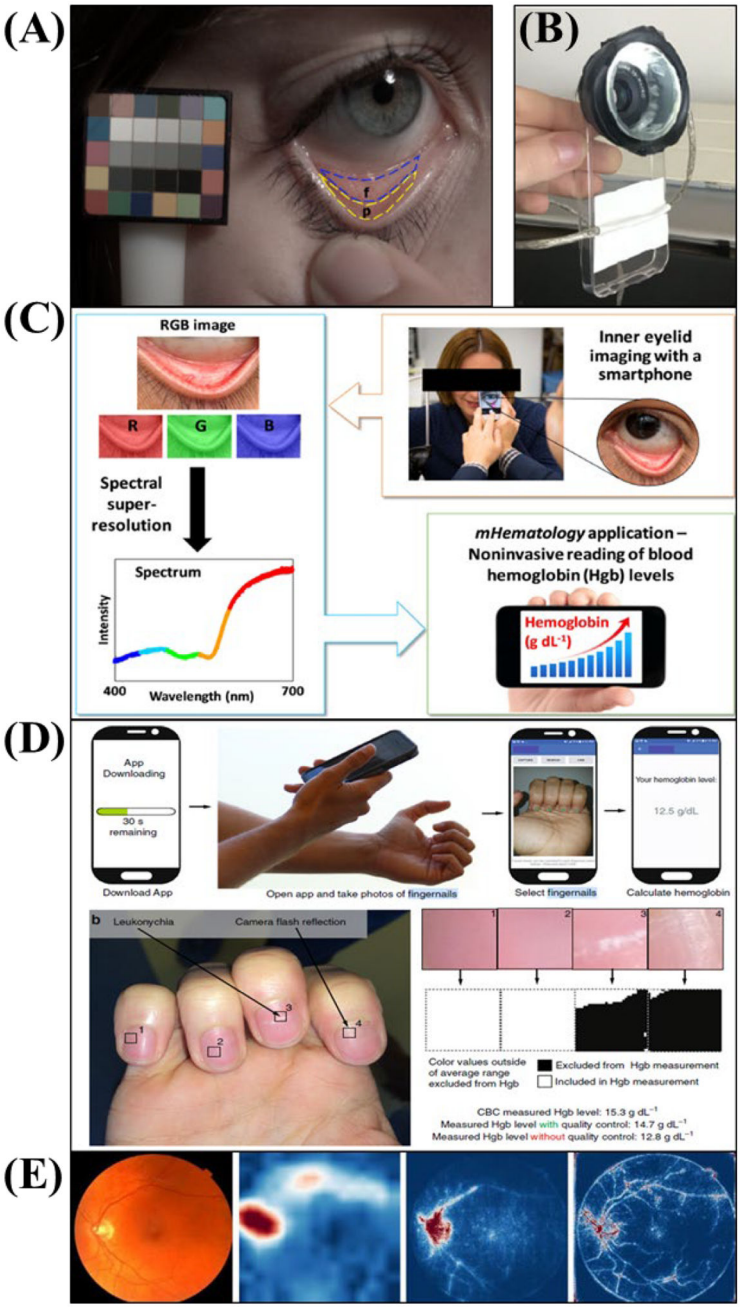

Figure 5.

Emerging smartphone based non-invasive anemia detection. **(A)** Erythema index measurement for determining Hb level from the palpebral and forniceal portions of the conjunctiva, with in-frame color calibration target (Reproduced with permission from [203]). **(B)** Accessory to eliminate impact from ambient light during non-invasive Hb level test (Reproduced with permission from [201]). **(C)** Spectral super-resolution spectroscopy (SSR) based Hb level measurement using inner eyelid as accessible sensing site. The smartphone application collects red, green and blue color information and applies SSR to construct spectra used for determining Hb levels (Reproduced with permission from [218]). **(D)**

Fingernail bed based Hb level measurement (Reproduced with permission from [216]). **(D)** Retinal fundus based Hb level measurement using red and white areas in each saliency map. This technology has the potentially to determine the association of anemia and ocular disease (Reproduced with permission from [217]).

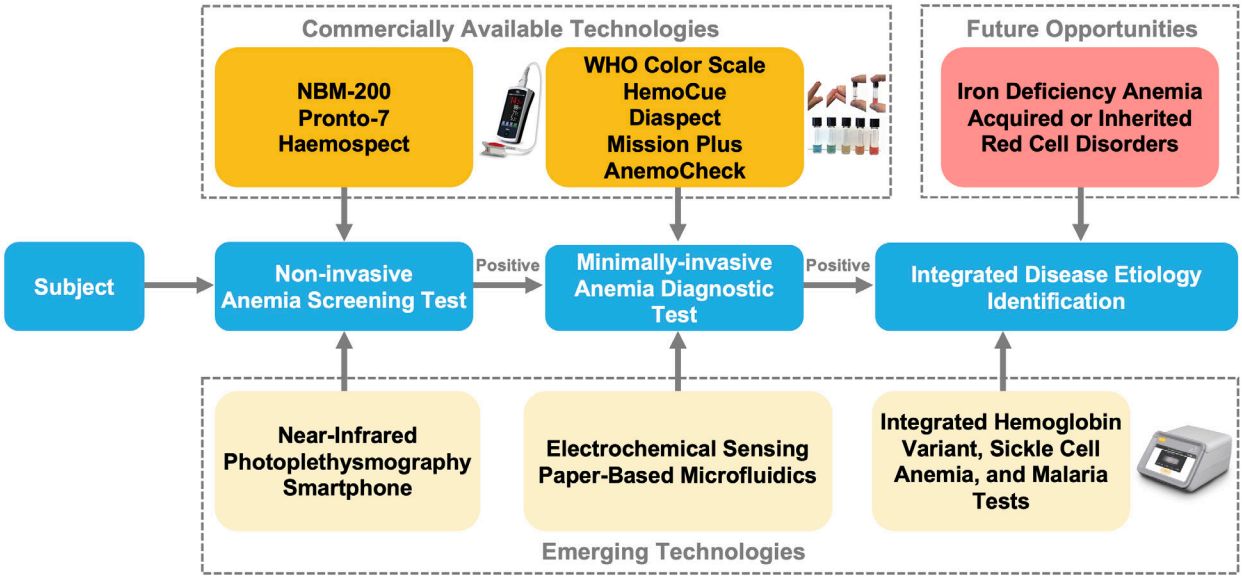

Figure 6.

There is a need for multiplexed approaches and integrated disease etiology identification in point-of-care anemia testing. Future efforts should focus on cascading non-invasive and minimally invasive diagnostic methods to enhance widespread use and diagnostic accuracy, as well as multiplexing additional test capabilities to identify the root-cause of anemia, such as integrated tests for red cell disorders, iron deficiency, and malaria.

Table 1.

Hemoglobin thresholds (g/dL) by age, sex, and pregnancy status used in classifying anemia cases as mild, moderate, and severe in Global Burden of Disease 2013

Severity of Anemia	Severity of Anemia		
Variable	**Mild**	**Moderate**	**Severe**
Age < 1 mo			
Males	13.0 – 14.9	9.0 – 12.9	< 9.0
Females	13.0 – 14.9	9.0 – 12.9	< 9.0
Age 1 mo – 5 y			
Males	10.0 – 10.9	7.0 – 9.9	< 7.0
Females	10.0 – 10.9	7.0 – 9.9	< 7.0
Age 5 – 14 y			
Males	11.0 – 11.4	8.0 – 10.9	< 8.0
Females	11.0 – 11.4	8.0 – 10.9	< 8.0
Age ≥ 5 y			
Males	11.0 – 12.9	8.0 – 10.9	< 8.0
Females, non-pregnant	11.0 – 11.9	8.0 – 10.9	< 8.0
Females, pregnant	10.0 – 10.9	7.0 – 9.9	< 7.0
Disability weights			
All cases	0.005 (0.002–0.011)	0.058 (0.038–0.086)	0.164 (0.112–0.228)

Table adopted and modified from The World Health Organization (WHO) reference [1]

Table 2.

Major Causes for Anemia

Causes for which allocation of the anemia envelope was based on prevalence results from GBD 2010*
Malaria
Hookworm
Schistosomiasis
Peptic ulcer disease
Gastritis and duodenitis
Maternal hemorrhage
Sickle cell disorders
Homozygous sickle cell disease (HbS-HbS or Hb SS)
Heterozygous sickle cell disease (HbS-HbA or Hb SA)
Compound heterozygous sickle cell disease
Compound heterozygous sickle cell disease-thalassemia (HbS-Hbβ or Hb Sβ)
G6PD deficiency
Homozygous class I
Homozygous class II
Heterozygous (female only)
Thalassemias
β thalassemia major (Hbβ-Hbβ)
Heterozygous β thalassemia (Hbβ-HbA)
Compound heterozygous “E-β” thalassemia I (HbE-Hbβ)
Heterozygous hemoglobin E (HbE-normal)
Hemoglobin H disease (genotype = [− −/−alpha])
CKD (Chronic Kidney Disease)
Due to diabetes mellitus
Due to hypertension
Other and unspecified
Causes for which allocation of anemia envelope was based on systematic redistribution methods*
IDA (Iron-deficiency anemia)
Other infectious disease
Other neglected tropical diseases
Other hemoglobinopathies and hemolytic anemias
Uterine fibroids
Other gynecologic disorders
Other endocrine, nutrition, blood and immune disorders

Table adopted and modified from reference by Kassebaum et. al. 2014 [13].

Table 3.

Performance of commercially available technologies for anemia detection

Technology	Lowest Sensitivity Reported			
	Reported Sensitivity at (Indicated Hb cutoff level)	**Test Population**	**Reference Method**	**Reference**
WHO Hemoglobin Color Scale	70.9% (11.0g/dL)	Pregnant women (194 subjects)	Hematology analyzer (Advia 2120i)	[69]
HemoCue® 201	56.00% (12.0g/dL)	Blood donor (969 subjects)	Hematology analyzer (ABX Pentra 60)	[80]
HemoCue® 301	25.0% (Male: 13.5 g/dL), 22.6% (Female: 12.5 g/dL)	Blood donor (553 subjects)	Hematology analyzer (Advia 2120i)	[81]
Pronto-7®	54.5% (Male: 13.5 g/dL), 65.2% (Female: 12.5 g/dL)	Blood donor (553 subjects)	Hematology analyzer (Advia 2120i)	[81]
NBM-200	33.70% (11.0g/dL)	Pregnant women (269 subjects)	Hematology analyzer (Sysmex XP-100)	[122]
Haemospect	66.7% (Male: 13.5 g/dL and Female: 12.5 g/dL)	Blood donor (353 subjects)	Hematology analyzer (Sysmex XE-2100)	[234]
Technology	**Highest Sensitivity Reported**			
	Reported Sensitivity at (Indicated Hb cutoff level)	**Test Population**	**Reference Method**	**Reference**
WHO Hemoglobin Color Scale	99.3% (12.0g/dL)	Patients attending the general outpatient department (200 subjects)	Cyanmethemoglobin method	[65]
HemoCue® 201	93% (11.0g/dL)	Pregnant women (102 subjects)	Hematology analyzer (Sysmex XS 1000i)	[83]
HemoCue® 301	90% (11.0g/dL)	Pregnant women (102 subjects)	Hematology analyzer (Sysmex XS 1000i)	[83]
Pronto-7®	93% (Male: 13.5 g/dL and Female: 12.5 g/dL)	Blood donor (445 subjects)	Cell Counter (Beckman Coulter)	[105]
NBM-200	96.36% (12.5g/dL)	Blood donor (445 subjects)	Hematology analyzer (Beckman LH500)	[77]
Haemospect	94.60% (12.5g/dL)	Blood donor (161 subjects)	Hematology analyzer (Advia 2120i)	[115]
Technology	**Lowest Specificity Reported**			
	Reported Specificity at (Indicated Hb cutoff level)	**Test Population**	**Reference Method**	**Reference**
WHO Hemoglobin Color Scale	27.69% (12.0 g/dL)	Patients attending the general outpatient department (200 subjects)	Cyanmethemoglobin method	[65]
HemoCue® 201	76% (11.0 g/dL)	Pregnant women (102 subjects)	Hematology analyzer (Sysmex XS 1000i)	[83]
HemoCue® 301	80% (11.0 g/dL)	Pregnant women (102 subjects)	Hematology analyzer (Sysmex XS 1000i)	[83]
Pronto-7®	75.4% (Male: 13.8 g/dL and Female: 11.6 g/dL)	Convenience sample of patients in the emergency department (350 subjects)	Hematology analyzer (Tosoh G8)	[101]
NBM-200	91.43% (12.5 g/dL)	Blood donor (506 subjects)	Hematology analyzer (Beckman LH500)	[77]
Haemospect	0.00% (12.5 g/dL)	Blood donor (161 subjects)	Hematology analyzer (Advia 2120i)	[115]

Technology	Highest Specificity Reported			
	Reported Specificity at (Indicated Hb cutoff level)	**Test Population**	**Reference Method**	**Reference**
WHO Hemoglobin Color Scale	49.1% (11.0 g/dL)	Pregnant women (643 subjects)	Hematology analyzer (Advia 2120i)	[69]
HemoCue® 201	99.80%(12.0 g/dL)	Blood donor (969 subjects)	Hematology analyzer (ABX Pentra 60)	[80]
HemoCue® 301	99.3% (Male: 13.5 g/dL), 98.9% (Female: 12.5 g/dL)	Blood donor (553 subjects)	Hematology analyzer (Advia 2120i)	[81]
Pronto-7®	95% (11.5 g/dL)	Healthy children (110 subjects)	Hematology analyzer (Coulter LH780)	[109]
NBM-200	99.20% (12.5 g/dL)	Blood donor (993 subjects)	Hematology analyzer (Sysmex KX-21N)	[110]
Haemospect	77.10% (12.5 g/dL)	Blood donor (353 subjects)	Hematology analyzer (Sysmex XE-2100)	[234]
Technology	**Lowest Bias±SDD Reported**			
	Reported Bias±SDD at (Indicated Hb cutoff level)	**Test Population**	**Reference Method**	**Reference**
WHO Hemoglobin Color Scale	0.2±1.96 (13.1 g/dL)	Patients in secondary care hospital (100 subjects)	Cyanmethemoglobin method	[71]
HemoCue® 201	0.56±0.099 (12.0 g/dL)	Pregnant women (969 subjects)	Hematology analyzer (ABX Pentra 60)	[80]
HemoCue® 301	− 0.25±0.85 (11.0 g/dL)	Pregnant women (102 subjects)	Hematology analyzer (Sysmex XS 1000i)	[83]
Pronto-7®	0.1±0.7 (12.5g/dL)	Blood donor (993 subjects)	Hematology analyzer (KX-21N)	[110]
NBM-200	0.18±0.09 (11.0 g/dL)	Blood donor (110 subjects)	Hematology analyzer (KX-21)	[90]
Haemospect	− 0.23±1.21 (Male: 13.5 g/dL and Female: 12.5 g/dL)	Blood donor (1483 subjects)	Hematology analyzer (Tosoh G8)	[114]
Technology	**Highest Bias±SDD Reported**			
	Reported Bias±SDD at (Indicated Hb cutoff level)	**Test Population**	**Reference Method**	**Reference**
WHO Hemoglobin Color Scale	1.121±1.87 (12.0 g/dL)	Patients attending the general outpatient department (200 subjects)	Cyanmethemoglobin method	[65]
HemoCue® 201	− 0.53 ±1.01(11.0 g/dL)	Pregnant women (102 subjects)	Hematology analyzer (Sysmex XS 1000i)	[83]
HemoCue® 301	0.43±1.243 (Male: 13.5 g/dL and Female: 12.5 g/dL)	Blood donor (553 subjects)	Hematology analyzer (Advia 2120i)	[81]
Pronto-7 ®	− 0.59±1.98 (Male: 13.5 g/dL and Female: 12.5 g/dL)	Blood donor (553 subjects)	Hematology analyzer (Advia 2120i)	[81]
NBM-200	0.7±0.8 (12.5 g/dL)	Blood donor (993 subjects)	Hematology analyzer (KX-21N)	[110]
Haemospect	− 0.6±1.96 (12.5 g/dL)	Blood donor (353 subjects)	Hematology analyzer (Sysmex XE-2100)	[234]

Table 4.

Performance analysis of emerging anemia detection technologies

Technology	Sensitivity	Specificity	R^2, r and p value	Reference method	Threshold value	Test subjects	Reference
Computer vision and deep learning assisted electrophoresis	100%	92.30%	$p < 0.05$	Complete blood count (Not Specific)	11.0 g/dL	Clinical and healthy patient samples (46 subjects)	[147]
Multi-Model Stacking Regressor	N/A	N/A	r=0.81, p<0.01	N/A	N/A	Blood donors (1583 subjects)	[193]
Physical examination	64.30%	83.60%	$p = 0.04$	Automated cell counter (Not Specific)	11 g/dL	Physicians, residents and students in hospital (10Physicians, 18 residents and 16 students)	[159]
Simple Paper-based Assay	87.50%	100%	$r = 0.909$	N/A	11 g/dL	Patients covering a wide spectrum of anemia (32 subjects)	[235]
Computer vision techniques	100%	82.40%	N/A	N/A	13 g/dl	Recruited random participants (77 subjects)	[198]
Digital Photographs of the Conjunctiva	74% (Apple iPhone 5S), 57% (Panasonic DMC-LX5)	71% (Apple iPhone 5S), 83% (Panasonic DMC-LX5)	R^2=0.397, p< 0.0001 (Panasonic DMC LX5); p< 0.0001 (Apple iPhone 5S);	Hematology analyzer (Sysmex XE-2100)	11.0 g/dL	Patients in Wellington Blood and Cancer Centre (47 Subjects)	[203]
A Kalman Filtering and Nonlinear Penalty Regression Approach	76.19%	80.85%	N/A	N/A	11 g/dL	N/A	[200]
Digital Camera-Based Spectrometry	N/A	N/A	r = 0.96, P<0.0001	Hematology analyzer (KX-21NTM)	N/A	Patient samples from the outpatient department in Nil Ratan Sircar Medical College and Hospital, Kolkata (300 subjects)	[141]
Medical Imaging, m-Health and Emerging Communication Systems	83%	82%	N/A	N/A	12 g/dL	Blood donor (33 subjects)	[236]
Combined reflectance spectroscopy and stochastic modeling approach	86%	N/A	N/A	N/A	N/A	Patients were enrolled at the Emergency Department (32 subjects)	[237]
Machine learning techniques with the PPG signal's characteristics features	N/A	N/A	r=0.98918 (Classification and regression trees), r=0.96729 (Support vector regression), p < 0.01	Hemocue Hb-201TM	N/A	Blood donor (33 subjects)	[192].
Spectrophotometry	N/A	N/A	r=0.77, P<0.001	Hematology analyzer (SP 1000i®)	N/A	Adult patients scheduled for potentially hemorrhagic major urologic surgery (44 subjects)	[238]

Technology	Sensitivity	Specificity	R^2, r and p value	Reference method	Threshold value	Test subjects	Reference
Smartphone app with Artificial Neural Network	94%	96%	R2 = 0.93	N/A	N/A	Patients with an age range from 20 to 56 years (75 subjects)	[210]
Dynamic Spectrum	N/A	N/A	R^2=0.8399 (Experimental group), R^2=0.6686 (Control group)	N/A	N/A	Volunteers at the Physical Examination Center of Tianjin People's Hospital (187 subjects)	[180]
Photoplethysmography System	N/A	N/A	R^2=0.43, r=0.61, p< 0.001 (partial least squares); R^2=0.42, r=0.62, p< 0.001 (backpropagation artificial neural network);	Hematology analyzer (Sysmex XS-1000i)	12g/dL	Blood donor (238 subjects)	[185]
Smartphone app with patient-sourced photos	97%	76%	r =0.82	Hematology analyzer (Advia 2120i)	12.5 g/dL	Patients with various anemia etiologies (265 subjects)	[239]
Micro-Hct using HemataSTAT II	39.50%	93.20%	r = 0.533, p < 0.001	Hematology analyzer (ABX Pentra 60)	12.0 g/dL	Unselected potential female donors (969 subjects)	[80]
3D printed auto-mixing chip	81.2% (Severe anemia),100% (Mild anemia)	100% (Severe anemia),83.3% (Mild anemia)	r=0.808, p<0.01	N/A	12g/dL (Mild anemia), 8g/dL (Severe anemia)	Patient and healthy donors (22 subjects)	[233]
Retinal fundus photos	96.90%	91.70%	p=0.019	Image Structure Clustering (Not Specific)	N/A	N/A	[240]
Finger probe with five PPG signals	N/A	N/A	R2 = 0.964 (five wavelengths PPG system), R2 = 0.688 (three wavelengths PPG system)	N/A	N/A	N/A	[241]
Visual and color-based point-of-care anemia self-testing	90.20%	83.70%	r = 0.864	Hematology analyzer (Advia 2120i)	11 g/dL	Patients with various anemia etiologies (238 subjects)	[242]
Microfluidic approach	N/A	N/A	N/A	Complete blood count (Not Specific)	8.0 g/dL (severe anemia)	N/A	[140]
Novel multi wavelength sensor	N/A	N/A	R^2=0.99 (Cold hands), R^2=0.82 (previous heat)	HemoCue® and a blood gas analyzer (Radiometer ABL 80)	13.5 g/dL (male), 12.5 g/dL (female)	Blood donor (1008 subjects)	[243]
HemaApp with smartphone cameras	85.70%	76.50%	r = 0.69 (White+970nm LEDS), r = 0.74 (White+ 970nm LEDS+Incandescent), r = 0.82 (White+880nm or 970nm LEDS+Incandescent)	Complete blood count (Not Specific)	N/A	Patients ranging from 6 – 77 years of age (31 subjects)	[213]

Technology	Sensitivity	Specificity	R^2, r and p value	Reference method	Threshold value	Test subjects	Reference
Near-infrared spectrophotometric system with InGaAs detector array and plane grating spectrometer	N/A	N/A	(r=0.74, r=0.81 in predicting test 1), (r=0.60, r=0.81 in predicting test 2)	N/A	N/A	Blood donor (91 subjects)	[164]
Imaging cytometry platform	N/A	N/A	r=0.98	Hematology analyzer (Sysmex KN21)	N/A	Blood sample from UCLA Blood and Platelet Center (No subject report)	[142]

Table 5.

Time and cost analysis of commercially available anemia detection technologies

Technology	Test Time	Cost
WHO Haemoglobin Colour Scale	30 seconds [65]	$0.01/test
HemoCue® 201	60 seconds [244]	Device: $698 Consumable: $1.5/test
Diaspect ™	within 2 seconds [90]	Device: $225 Consumable:
HemoControl	25 seconds [245]	Device: $425 Consumable: $1.14/test
URIT-12	10 to 15 seconds [246]	Device: $159 Consumable: $0.7/test
Anemo Check	60 to 120 seconds [234]	$1/test
Pronto-7®	30 to 45 seconds [101]	$629