Development of blood hemoglobin level early detection device based on a noninvasive optical platform

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ARTICLE INFO

Keywords:
Portable device
Hb measurement
Noninvasive
Blood characterization

ABSTRACT

Blood hemoglobin levels are a reliable indicator for anemia screening, which generally uses an invasive system or takes blood using a syringe. Spectrophotometry can work by “substituting” the use of a phlebotomy tube needle with electromagnetic wave radiation or light. This study aims to develop and carry out a noninvasive diagnostic test for measuring hemoglobin levels. There are three main stages in this research: (i) measuring hemoglobin concentration and scanning an incident wavelength on standard hemoglobin solutions and blood controls, (ii) making a prototype variant of a noninvasive blood hemoglobin level measurement device, and (iii) testing the technology unit on the developed prototype. The measured hemoglobin value by the Trax Control Meter for low, middle, and high levels is almost the same as the expected range values, namely, 13.09, 16.8, and 17.81 g/dL, respectively. Three sets of device prototype variants were successfully developed: (i) the noninvasive blood hemoglobin level measuring device based on Raspberry Pi Prototype on Infant Finger and Thigh Probes, (ii) the level measuring prototype noninvasive hemoglobin in blood based on Internet of Things and WebServer, and (iii) the prototype of noninvasive blood hemoglobin level measuring device on in vitro probe with reflectance method. Testing the accuracy of the Biorad Meter Trax Trilevel using a multiformula regression calculation using the ZunZun server shows that the tool has an accuracy ranging from 0.12 to 0.30 g/dL.

1. Introduction

Anemia is common in developing countries and is an ongoing public health challenge. Assessment of hemoglobin (Hb) levels in the blood is a reliable indicator for anemia screening and the deviation of the blood Hb level from its normal value can be used as an indicator for various pathological processes. For example, a decrease in total Hb indicates anemia [1]. According to data obtained from the World Health Organization (WHO), one in four people (24.8%) in the world has anemia [2]. Several methods can be used to determine blood Hb levels. In Indonesia, the cyanmethemoglobin (cyanmeth) method is often used, with the 540 and 546 nm cyanmeth methods being the most popular reference standard methods [3, 4]. The cyanmeth method has also been recognized as the standard method for determining Hb concentration [5]. Other methods, such as the automated analyzer, HemoCue, hemoglobin color scale, Sahli technique, Lovibond Comparator, copper–sulfate

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method, cyanmeth, and Tallqvist method [6], also involve the process of drawing blood. Blood collection, also known as phlebotomy, is a very risky process. The process of taking blood causes pain and discomfort. This process can result in skin bruising, redness, swelling, or inflammation and can cause damage to body structures around the blood draw site. Additionally, some feel dizzy and even faint due to a vasovagal reaction. There are also cases wherein persons undergoing phlebotomy felt weak, nauseous, and even vomited. Furthermore, some feel anxious and afraid during the taking of blood, experiencing psychological trauma. Excessive bleeding, including extravasation, hematomas, petechiae, blood clots or thrombosis, venous fibrosis, and edema, can occur due to the blood collection process [7]. Poor collection handling can increase the risk of spreading disease vectors. The main danger of blood sampling is an infection due to disease vectors and other health problems through blood [7, 8]. Some of the vectors that can spread through the blood include human immuno-deficiency virus, hepatitis B virus, hepatitis C virus, rabies, severe acute respiratory syndrome (Sars), and Escherichia coli [9]. The process of taking blood samples can cause complications such as iatrogenic anemia, myocardial infarction, pneumonia, stroke, and brain damage and can even be fatal [7]. Blood sampling procedures may not be performed for technical reasons, such as difficulty accessing venous blood vessels that are difficult to find or hidden. For example, if the prospective recipient of the measurement is obese. Based on these factors, noninvasive measurement of blood Hb levels, without involving blood collection, is needed. Thus far, there are not many methods of measuring blood Hb levels that are noninvasive.

The spectrophotometric method is the most widely observed noninvasive biomarker measurement method [6, 10]. Spectrophotometry works by “substituting” the use of a phlebotomy tube needle with electromagnetic wave radiation or light. The spectrophotometer principle is based on the absorption of certain wavelengths by Hb in the blood [11]. Researchers have developed absorption spectrophotometry methods [12, 13], occlusion spectroscopy [14], and near infrared spectrophotometry [15] to identify Hb. It has also become more common that measurement tools are portable [16]. Additionally, Chowdhury developed an amplitude modulated ultrasonic method with an infrared technique [6, 17]. The method of measuring Hb by spectrophotometry has included use of several wavelengths, such as 532 nm [18], 542 or 574 nm with 900 nm [19, 20], 545 nm [20], 660 nm [21, 22], 600–750 nm [23, 24], 700 nm [25, 26], 760 nm [20, 26], 850–1000 nm [23, 24], and 940 nm [22, 27]. This method has a high degree of accuracy, is painless (there is no need to injure the patient), has lower variable costs, can be used repeatedly, and has no consumable costs. Most importantly, the spectrophotometric method eliminates the need for blood sampling. This study aimed to develop and carry out a noninvasive diagnostic test for measuring Hb levels.

2. Materials and method

2.1. Characterization of blood hemoglobin samples

2.1.1. First stage hemoglobin characterization

This first stage of Hb characterization aims to measure Hb concentration by wavelength scanning of Hb standard solutions and blood controls (liquid human whole blood-based controls). This stage consists of three steps: (1) creating a standard Hb solution, (2) measuring the Hb standard solutions and blood controls with the Hemoglobin Assay Kit, and (3) measuring the Hb standard solution at a wavelength range of 200–900 nm (spectral scanning).

2.1.1.1. Preparation of the hemoglobin standard solution. The Hb standard solutions used in this study had Hb concentrations of 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20 g/dL. Initial stock was made with a concentration of 20 g/dL by weighing 0.2 g of Hb powder and was dissolved in 1 mL of physiological NaCl. Next, the solution was stirred until the Hb powder was completely dissolved. Then, serial dilutions were created from the 20 g/dL stock to concentrations of 18, 16, 14, 12, 10, 8, 6, 4, and 2 g/dL with physiological NaCl as the solvent (total volume of each concentration was 1 mL). The solution was mixed again until homogeneous at each dilution stage.

2.1.1.2. Measurement of hemoglobin standard solutions and blood controls with the Hemoglobin Assay Kit. Standard measurements of Hb and blood controls (Biorad, Meter Trax Control no cat. 970) were conducted with the Hemoglobin Assay Kit (Sigma, cat no MAK115). This kit can measure serum, plasma, and urine samples directly, but blood samples must be diluted 100× in sterile distilled water. First, 50 μL of distilled water (Blank) and 50 μL of Calibrator were added to the 96-well plate well. Then, 200 μL of distilled water was added to the well containing the Blank and Calibrator. The diluted calibrator is equivalent to a Hb concentration of 100 mg/dL. Next, 50 μL of the sample was introduced into the well and followed by the addition of 200 μL of reagent. The plate was gently tapped to evenly mix the solution. Then, the plate was incubated for 5 min at 20 °C. After incubation, the well containing the sample was measured at a wavelength of 400 nm (A400) three times and the acquired data were stored and exported in MS Excel. The concentration calculation was done using the following:

\[
\text{Hb Concentration} = \frac{A400\text{Calibrator} - A400\text{Blank}}{A400\text{Sample} - A400\text{Blank}} \times 1 \text{g/dL} \times df
\]

where 1 g/dL is the concentration of the diluted calibrator and df is the dilution factor (e.g., for a blood sample the dilution factor is 100).

2.1.1.3. Measurement of hemoglobin standard solution at a wavelength range of 200–900 nm (spectral scanning). The standard Hb solution was placed into a 96-well plate and a Synergy HTX Multi-Mode Reader spectrophotometer was set for scanning spectral readings with a wavelength range of 200–900 nm. The Hb solution absorbance was measured, and the resulting data were saved and exported into MS Excel.

2.1.2. Second-stage hemoglobin characterization

The second phase of Hb characterization aims to create a blood control with a hemoglobin concentration of 7–17 g/dL, conduct spectral scanning of the optimum wavelength in the blood control with a measured concentration, and measure the absorbance of the blood model using a prototype noninvasive Hb measuring device.

2.1.2.1. Measurement of hemoglobin levels in blood controls using the Hemoglobin Assay Kit. Blood controls (Biorad, Meter Trax Control no cat. 970, Lot 9278 and 9288) were measured to determine Hb concentration using the Hemoglobin Assay Kit (Sigma, no cat MAK115). To avoid repeated setups, comparable products are grouped together as a Lot or BATCH during their manufacture. A Lot number is a unique identifier for a specific material number processed on a production line produced by a single manufacturer and without interruption from processing other products. The blood control sample was diluted 100× in sterile physiological NaCl water. Then, 50 μL of physiological NaCl (Blank) and 50 μL of Calibrator were added to the well of the 96-well plate. Next, 200 μL of physiological NaCl was added to the well containing the Blank and Calibrator. The diluted calibrator is equivalent to a Hb concentration of 100 mg/dL; 50 μL of the sample was put into the well, and 200 μL of reagent was then added into the well containing the sample. The plate was gently tapped three times so that the solution was mixed evenly. The wells were incubated for 5 min at 20 °C, and then, the absorbance was measured at a wavelength of 400 nm (A400). The data were stored and exported in MS Excel. Calculation of concentration through Eq. (1) converted the concentration into grams/dL.
2.1.2.2. Creating blood controls with certain concentrations. The blood control with known Hb concentration was mixed using both levels of the mixing equation to obtain the desired concentration. The equation is given by

\[ M_{\text{mixed}} = \frac{V_1 M_1 + V_2 M_2}{V_1 + V_2} \quad (2) \]

so that

\[ V_1 M_1 + V_2 M_2 = V_3 M_3 \quad (3) \]

where \( V \) is the volume of the solution, \( M \) is the concentration of the solution, and \( V_3 = V_1 + V_2 \).

The Hb level of the blood control whose concentration was adjusted (referred to as adjusted) was re-measured using the Hemoglobin Assay Kit for confirmation.

2.2. Simulation prototype of the noninvasive Hb level measurement tool

The simulation prototype of the noninvasive Hb level measurement tool comprises three stages: (1) a noninvasive prototype of a Raspberry Pi-based measuring device for hemoglobin levels in a baby finger and thigh probe; (2) a prototype of a noninvasive blood hemoglobin level measurement device based on the Internet of Things (IoT) and a web server; and (3) a prototype of a noninvasive blood hemoglobin level measurement device on an in vitro probe using the reflectance method.

2.2.1. Prototype of Raspberry Pi-based noninvasive hemoglobin level measuring device in an Infant Finger and thigh probes

This stage aims to find, develop, and validate a Raspberry Pi-based noninvasive Hb level measuring Tool for a baby finger and thigh probes. The model developed in this study is referred to as noninvasive Hb Monitoring.

The designed system, which can be seen in Figure 1, was developed and simulated to measure Hb concentration using a noninvasive technique. The signal from a light-emitting diode (LED) acts as a source (the emitter that emits light). The obtained signal is then processed and prepared to be properly input into the microcontroller. The microcontroller calculates the equivalent Hb concentration according to the detected wave amplitude. The microcontroller processes the signal and calculates the equivalent Hb concentration. Then, the Hb value is matched into one suitable value, and the final value is displayed to the user via a 5 inch liquid crystal display (LCD).

2.2.2. Prototype of noninvasive measurement of hemoglobin levels in blood based on the IoT and a web server

The prototype of a noninvasive IoT and web server-based measuring device for measuring hemoglobin in the blood required the construction of circuits and casings and programming.

2.2.2.1. Circuit manufacturing. The circuit was made by first making a prototype using a breadboard. A breadboard was used to make it easier to construct circuit prototypes so that, if a circuit error occurs, you only need to replace the circuit because there is no soldering process. After the prototype circuit was successfully created, the schematic design process for the LED circuit was made using the EAGLE application. The LEDs in the circuit emitted two different wavelengths: 525 and 555 nm, as obtained according to the previous study reported in Rahmawaty et al. [28].

The cathode of the two LEDs is connected to the ground, whereas the anode is connected to pins gpio0 for the 525 nm LED and gpio2 for 555 nm LEDs. A resistor was added between the LED anode and the gpio pin to reduce the voltage to the LED anode. Pins gpio0 and gpio2 are pulse with modulation (PWM) pins. PWM was used to adjust the LED intensity.

The next circuit constructed was a sensor circuit. The FDS100 sensor has three pins: anode, cathode, and ground. The input voltage is 3.3 V. A noise filter comprising a 1 kΩ resistor and a 0.1 F capacitor was inserted before the cathode pin of the FDS100 sensor. This noise filter reduces signal interference caused by sensor input from a 3.3 V voltage source. The sensor ground pin is connected to the circuit ground, and the anode pin is in series with a grounded resistor. Sensor readings are taken by measuring the output voltage between the anode pin and a 10 kΩ resistor that is then connected to the ADC0 pin. The analog-to-digital converter (ADC) converts analog values into digital values that a microcontroller can read. The ESP8266 has a 10-bit ADC, which means analog readout values range from 0 to 1023.

The next circuit is the battery circuit. The battery circuit consists of integrated circuit (IC) power IP5306 (manufactured by Injonic Technology), resistors, capacitors, inductors, and LED indicators. The IP5306 is an IC that can be used to charge Li-Po 1S batteries. The IP5306 also has a boost converter that can increase the voltage of the Li-Po 1S batteries from 3.7 to 5 V, which can be used as a voltage source for the device.

The next step is the integration of the microcontroller, battery circuit, sensor circuit, organic LED (OLED) display, temperature and humidity sensor, and battery. Figure 2 shows the overall schematic of the device. There are additional OLED displays, aht10, and batteries in the overall device circuit. The OLED display is used to display instrument readings. The temperature and humidity sensor (Aht10) is used to retrieve temperature and humidity data. The batteries are used to power the instrument.

2.2.2.2. Case making. The probe was designed using the Fusion 360 application (education license), as shown in Figure 3a, with a probe depth of approximately 60 mm (Figures 3b and 3c). The sensor and LED are located 50 mm from the front of the probe. The sensor is placed between the 525 and 555 nm LEDs. The position of the LED is at an angle so that the light can focus on one point. The height of the sensor with the focal point of the LED is 8 mm. The first 1 mm is the distance between the sensor and the holder, the next 1 mm is the thickness of the foam installed in the probe, and the next 6 mm is the depth that the LED on the index finger must penetrate with the LED tilt angle of 53.13° (Figure 3b). This angle is the angle with the maximum sensor reading for a combination of two LEDs and a finger inside the probe.

The probe has four main parts: the top cover, the top body, the bottom, and the bottom cover. The top cover of the probe has a place to put the OLED display. The upper body of the probe has a place to put the battery, battery circuit, charger port, and switch. The lower body of the probe has a place to put the microcontroller, LED circuit and sensor, and the aht10. The designs were printed using a three-dimensional (3D) printer. The filament used for 3D printing is PLA+ (polylactic acid). The probe is made using two different colors (black and white).

2.2.2.3. Programming. The C language-based programming stage is divided into four stages: setting the LED intensity, sensor readings, display of the reading results, and communication with the Android application. The data from 150 samples measured by the sensor in long-time interval mode are then calculated by Phyton language-based machine learning and processed to obtain a specific formula of Hb level.

Sensor programming is done to retrieve data that is read by the sensor due to the reflectance of the finger. The sensor is set to read each time the LED has made an intensity change with a 50 m delay. The sensor reading
Figure 2. Schematic of the series of tools.

Figure 3. Probe design (a) as a whole, (b) for the position of the LED and sensor, (c) for placing the index finger.
value is in the form of voltage, which is then converted through the 10-bit ADC so that the microcontroller can read the results. The total time it takes to run one complete process is 38 s.

The next programming stage is programming the display to display the results. Display programming is divided into several sections. The first part is the splash screen, which displays tool information. The second part is a description of the measurement so that the user knows that the tool is measuring. The time required for one measurement process is 38 s, which includes five repetitions. The third part of programming the display involves the display of measurement results so that users can easily see the measurement results of the tool. The last programming stage is communication with the web server.

2.2.3. Prototype of the noninvasive blood hemoglobin level measuring device with in vivo probe with reflectance method

A noninvasive Hb measurement device was designed and developed using a reflectance approach [29, 30]. The working principle of the device is based on LEDs that produce invisible light. The light interacts with the sample molecules and produces characteristic feedback. The amount of feedback is based on the sample matrix, a translucent liquid. The light source in the device is used as a wavelength precision reference to obtain reproducibility and detection accuracy. The whole process is displayed through an LCD with a display that shows the sensor response value [29]. Figure 4 shows a schematic of a series of noninvasive Hb measurement devices.

2.3. Unit testing of the noninvasive Hb level measurement tool

2.3.1. Noninvasive testing of the Raspberry Pi-based hemoglobin level measuring equipment in Infant Finger and thigh probes

Testing the Hb measuring device at this stage consists of nine tests: testing the sensitivity of the Hb sensor to the color of the LED and the color of the reflected field; testing the distance of data transmission, frequency range, and connectivity; testing the integration of the circuit and the mechanics of the tool; testing the display interface of the measurement graph on the LCD; a sensor reading test, data transfer test, and the mechanics of the tool; testing the display interface of the measurement graph on the LCD; a sensor reading test, data transfer test, and tool calibration.

2.3.1.1. Testing data transmission distance. This test was carried out using a function generator as input data and the LED as output at the transmitter. At the receiver, the Hb sensor as a light sensor and an oscilloscope as output are used to determine whether the data are still well-received or not. In the distance measurement, the assumed frequency is 10 kHz with a voltage source for the LED lamp at the transmitter of 4.5 V with a power supply source in the receiver circuit that must equal the maximum amount of voltage that the LED can receive.

2.3.1.2. Frequency range testing. The steps to obtain data from the minimum and maximum frequency measurements are the same as the steps in the distance measurement. The distance between the transmitter and the receiver being tested is half of the maximum distance generated in the testing phase of the data transmission distance. As input data from the function generator, the frequency is set from minimum to maximum frequency. The way to obtain the minimum and maximum frequencies is to rotate the frequency potential on the function generator from 0 Hz to the highest frequency without damaging the signal image in the receiver. The way to determine if the receiver signal is damaged or not is to compare the transmitter input frequency with the receiver output frequency, determining whether or not the frequency can be appropriately channeled.

2.3.1.3. Connectivity testing. The working method of the Hb measuring device is based on wifi. Connectivity testing is carried out to ensure that the Hb and Raspberry pi measuring probes are connected to the same internet network. The internet network used can be sourced from all types of smartphones.

2.3.1.4. Circuit integration and tool mechanics testing. In the circuit integration test, the Hb sensor components, NodeMcu, LEDs, resistors, ADCs, and Rasberry pis must be connected to form a single Hb measuring device. The probe is a medium/container for measuring Hb, wherein a finger is inserted into the already available hole. The probe consists of two parts: (1) the upper part, which is used to place the NodeMcu and ADC, and (2) the bottom, which is used to place two LEDs and one Hb sensor.

The circuit consists of an LED (source) as a light emitter and the Hb sensor that acts as a detector that absorbs or receives light. When the sensor is placed on the finger, the light from the LED will be absorbed by the tissue on the finger. The light that is not absorbed is reflected and received by the Hb sensor detector. The light received by the Hb sensor becomes the light attenuation value, which is converted into an electric current value, and then becomes a voltage in the presence of a load resistor at the anode. The voltage emitted by the sensor depends on the amount of light received, the more light received, the greater the voltage issued by the Hb sensor (value ranges between 0 and 5 V). The 10-bit ADC microcontroller reads the voltage value from the Hb sensor in the

![Figure 4. Schematic of a series of noninvasive hemoglobin measurements.](image-url)
circuit, and this ADC value is converted back into a voltage value. Furthermore, the stress value is entered in a linear equation to convert it into a Hb concentration value.

2.3.1.5. Testing the measurement graph interface display on the LCD. The interface display of a graph/value of the measurement results of the Hb measuring device is displayed on a 5 inch LCD. The program code for displaying the graphic interface on the LCD uses a custom plot widget C++ Source or C++ programming language and takes the source code from previous blood glucose measurement tool [28]. The interface display works by taking analog value data received by the Raspberry Pi and then transforming it with a discrete Fourier transform. The transformation results are processed using the best equation so that the results of Hb readings are obtained in units of g/dL. There are five measurement repetitions in one data collection and the results are averaged to create the output value.

2.3.1.6. Sensor reading test. Testing is done by inserting a finger into the probe. The tool takes five measurements and data read by the sensor is stored on the NodeMcu. The performance of the Hb sensor measurement results on the finger is indicated by the output of the Hb sensor reading on the finger. The reading value can be seen using the termux application, which can be downloaded on the Play Store and AppStore. To display the reading results on the application, the user must enter an IP that matches the IP on the probe.

2.3.1.7. Data transfer test (NodeMcu to Raspberry Pi). The results of the data transfer test (NodeMcu to Raspberry Pi) are shown by the success of the transfer of data content sent via the MQTT protocol to the Raspberry Pi. The content of the data sent via the MQTT protocol is the value of the Hb sensor reading. Sending data from the circuit to the display on the LCD retrieves the analog value data received by the Raspberry Pi and then transforms it with a discrete Fourier transform. The transformation results are processed using the best equation so that the results of Hb readings are obtained in units of g/dL.

2.3.1.8. Tool calibration. Calibration of the tool is done by comparing the readings of the Hb tool that was built with the reading of the "EasyTouch" Hb meter (commercial tool). Measurements using each Hb device were carried out on thirteen volunteers. Measurements using the “EasyTouch” Hb tool were carried out once for each volunteer. The measurement time using the “EasyTouch” Hb device was not too far from the measurement time using the developed wireless Hb tool.

2.3.2. Noninvasive testing of hemoglobin levels in the blood based on the IoT and a web server

The battery circuit was first tested for charging and discharging. This test was carried out to determine the duration of battery charging and the duration of use of the tool. Measurement of battery charging time is done by first emptying the battery. The battery used is a one-cell lithium polymer with a battery capacity of 600 mAh. A battery charging process is then carried out using a 5 V/2 A adapter. The length of time it takes to charge the battery from empty to full is calculated using a stopwatch. The test was carried out five times.

The measurement of the duration of use of the tool was done by charging the battery until it is full. After the battery is fully charged, the tool is set to take measurements continuously until it cannot take measurements, and then, the time required for the length of use of the tool is calculated using a stopwatch. Testing the duration of using the tool was carried out five times. The series of sensors and LEDs were also tested for measurement precision. The following equation:

\[ p = \left(1 - \left(\frac{x_i - \bar{x}}{\bar{x}}\right) \right) \times 100 \]  

(4)
can be used to find the precision (p) of the circuit, where \(\bar{x}\) is the average precision and \(x_i\) is the current precision of the i-th term.

2.3.3. Noninvasive testing of hemoglobin levels in blood on in vitro probes with the reflectance method

The unit testing of the noninvasive blood Hb level measuring device includes preparing a noninvasive probe and device design. The probe is a simulation medium for measuring hemoglobin in vitro. The dimensions of the probe size adjust to the dimensions of the cuvette used. The dimensions of probe 1 are 18.90 mm \(\times\) 18.90 mm \(\times\) 42 mm, the dimensions of probe 2 are 12.00 mm \(\times\) 8.61 mm \(\times\) 35.13 mm, and the dimensions of probe 3 are 18.90 mm \(\times\) 18.90 mm \(\times\) 10.30 mm. Probe 1 serves as a place for placing the cuvette, probe 2 serves as a place for placing detectors and LEDs, whereas probe 3 functions as a cover for probe 1 to minimize noise factors from external light [29, 30].

The position of the detector on probe 2 is between the two LEDs with different wavelengths at a distance of 2 mm to each other. The dimensions of the placement follow the size of each LED and detector. The dimensions of the 1050–1550 nm LED are the same (4.77 mm \(\times\) 6.40 mm) and the dimensions of the FDS100 detector are 8.30 mm \(\times\) 4.20 mm. The distance between probe 2 (LED1-detector-Led2) and probe 1 (cuvette) is 2 mm [30].

The probe design was printed using a 3D printer machine with a 1.75 mm silk-PLA printing filament. Silk-PLA printing followed dataset specifications, namely, an extrusion temperature of 210 °C at a room temperature of 50 °C. Silk-PLA also has a density value of 1.24 g/cm³, a decay index of 190 °C·g/10 mln, an emissivity of 0.96, and a light absorption of 0%. The use of probe filament material is considered in the reflectance method as a reflector material to reflect incident light. Silk filament material can reflect light because it is made of a composite of polyacrylic acid and polyester with a ratio of 10%–20%. Silk filament printing produces gloss, pearlescence, and high-print stability characteristics when compared with other PLA types. The optimal print structure is above 0.1 mm in height or thickness due to the retraction properties of silk.

The noninvasive device requires the following equipment and materials. The electronic devices used include the Raspberry Pi 3 model B+ as a single-board circuit computer device module. LED Variation (IR LED 800 nm, IR LED 750 nm, LED 645 nm, IR LED 600 nm, IR LED 555 nm, and IR LED 525 nm), and FDS100 sensor. Other supporting devices include jumper cables, resistors (330, 1 kΩ, and 100 kΩ), capacitors (0.1 F), IC (LM324N), 5-V/2-A adapter, PCB, breadboard, ADC (ADC-ADS1115), 5 inch waveshare LCD, filament silk-PLA, and a 32 gigabyte secure digital card. Hardware equipment includes 3D printer machines, laptops, and ultra microsized transparent cuvettes and pipettes. Software systems include WiringPi, ALGLIB numerical analysis, Qt creator software development kit, SQLite database management system, RStudio statistical analysis, and ZunZun and TinkerCAD multiformula regression analysis servers [28, 30].

2.4. Testing the simulation model of the noninvasive Hb level measurement tools

2.4.1. Measurement simulation preparation

The material used as the test sample is the Biorad MeterTrax Trilevel. The sample level is divided into low, middle, and high levels. Each level of the sample is given adjustment treatment. The treatment obtained samples with Hb values of 8.4, 9.3, 9.7, 10.4, 11.0, 12.6, 12.7, 15.0, 15.7, 16.0, and 17.7 g/dL [5].

2.4.2. Measurement simulation method

Biorad MeterTrax Trilevel blood hemoglobin samples were inserted into the cuvette using a pipette and placed on the in vitro probe. Measurements listed for blood Hb biomimetics were first carried out to
determine the reflectance value of the sample and to act as a comparison dataset [30]. The measurements used the SynergyTM HTX Multi-Mode Microplate Reader spectrometer from BioTek. The spectrometer has a UV–Vis monochromator optical filter capability with a 200–999 nm wavelength range.

The listed measurements were carried out before the measurement process using a noninvasive biomarker device. The second measurement process included the measurement of biomimetic HB samples that were adjusted. Meanwhile, the measurement using a noninvasive biomarker device was measured five times with the IR LED at wavelengths of 800, 750, 645, 600, 555, and 525 nm, such that the research measurements obtained a total of 330 datasets for each output. The dataset was automatically stored in a data-based output profile file as analysis data (Figure 5) [29,30].

Light sources/LEDs with wavelengths of 800 and 750 nm do not require gain for optimization of sensor responsiveness. A photodiode detects the light from the LED that passes the material. Then, the light is converted into a voltage difference. The actual light remaining is minimal, so it must be amplified with gain. The voltage difference value is converted to a size of 16 bits with a maximum value of 2.5 V. This simple gain mechanism acts as a voltage multiplier to increase the remaining light. Gain values of 2, 4, and 8 were different for each wavelength. The gain selection is based on the average residual run of all wavelengths used. The LED with wavelengths of 645, 600, and 555/525 nm require gains of 2, 4, and 8, respectively, for optimization of sensor response.

### 3. Results and discussion

3.1. Characterization of blood hemoglobin level samples

3.1.1. First stage hemoglobin characterization

Table 1 shows the results of measuring Hb standard solutions using the Hemoglobin Assay Kit (read at 400 nm wavelength) and Synergy HTX spectrophotometry. Based on the measurement of the concentration of the standard Hb solution in Table 1, which are calculated based on Eqs. (2) and (3), it can be seen that there is a difference in expected and measured concentration. This is especially true for standards 12 and 20 g/dL, measured to be 5.1 and 17.8 g/dL, respectively. This could be due to the pipetting error factor at the time of dilution and inhomogeneous dilution. Although the standards are 2, 4, 6, 8, 10, and 16 g/dL, the measured values are close to the expected range values. Standards 14 and 18 g/dL could not be measured due to volume calculation errors making it insufficient for measurements to be carried out.

The results of the measurement of Hb values measured by the Trax Control Meter for low, middle, and high levels in Table 1 show that the measurement results have values that are almost the same as the expected range values, namely, 13.09, 16.8, and 17.81 g/dL. These concentration values are obtained by Eq. (1). Figure 5 shows the measurement results of the Hb standard solution by spectral scanning (read at a wavelength range of 200–900 nm).

Figure 5 also shows that the highest absorption is at wavelengths ranging between 250–260 and 400–410 nm. The graph of the absorbance
value is in the coefficient unit of the absorbance unit, and the tabulation range is less than 10. However, the solution measured in Table 1 is standard Hb dissolved in physiological NaCl. Spectral scanning for standard Hb in blood controls or possibly whole blood needs to be done because components in blood other than Hb could affect wavelength absorption.

### 3.1.2. Second-stage hemoglobin characterization

Table 2 shows the results of the measurement of hemoglobin levels in the Trax Meter Blood Model. An adjustment has been made using human Hb lyophilized powder (Merck KGaA, Germany) with concentration-based modification. Figure 5 shows the results of the absorbance measurement of the blood control at a wavelength of 200–900 nm (spectral scanning). The measurement mechanism at this stage is the same as at the first stage of Hb characterization, although it uses Synergy HTX Multi-Mode Reader spectrophotometry.

### 3.2. Unit test results of the noninvasive Hb level measurement tool

#### 3.2.1. Test results of noninvasive Raspberry Pi-based hemoglobin level measuring device in baby finger and thigh probes

Table 1 provides a comparison of expected and measured hemoglobin standard solution results.

| Sample | A1  | A2  | A3  | Average | Measured concentration (expected concentration) g/dL |
|--------|-----|-----|-----|---------|-----------------------------------------------------|
| Blank  | 0.043| 0.043| 0.043| 0.043   | 0.10 (0.10)                                         |
| Calibrator | 0.562| 0.551| 0.559| 0.557   |                                                     |
| Low | 0.729| 0.715| 0.703| 0.716   | 13.09 (13.2)                                        |
| Middle | 0.825| 1.074| 0.820| 0.906   | 16.80 (16.3)                                        |
| High | 0.965| 0.970| 0.940| 0.958   | 17.61 (20.0)                                        |

#### Table 2. Hemoglobin levels from the Meter Trax Blood Model.

| Blood standard | Sample | A1 | A2 | Mean | Correction | Concentration (g/dL) |
|----------------|--------|----|----|------|------------|----------------------|
| Open 04/03/2021 | Blank | 0.042| 0.043| 0.0425| - | - |
| Low (Lot 92781) | 1 | 0.513| 0.52 | 0.5165| 0.474 | 9.36 |
| Middle (Lot 92782) | 2 | 0.842| 0.835| 0.8385| 0.796 | 15.72 |
| High (Lot 92983) | 3 | 0.923| 0.952| 0.9375| 0.895 | 17.67 |
| Low (Lot 92781) | 4 | 0.47 | 0.468| 0.469 | 0.4265 | 8.42 |
| Middle (Lot 92782) | 5 | 0.69 | 0.67 | 0.68 | 0.6375 | 12.59 |
| High (Lot 92783) | 6 | 0.811| 0.797| 0.804 | 0.7615 | 15.03 |
| Open 6/03/2021 | Blank | 0.043| 0.043| 0.043 | - | - |
| Low (Lot 92781) | L1 | 0.481| 0.482| 0.4815| 0.4385 | 9.18 |
| Low (Lot 92880) | L2 | 0.54 | 0.543| 0.5415| 0.4985 | 10.44 |
| Low (Lot 92880) | L3 | 0.541| 0.54 | 0.5405| 0.4975 | 10.42 |
| Low (Lot 92880) | L4 | 0.539| 0.538| 0.5385| 0.4955 | 10.38 |
| Low (Lot 92880) | L5 | 0.51 | 0.504| 0.507 | 0.464 | 9.72 |
| Low (Lot 92781) | L6 | 0.534| 0.535| 0.5345| 0.4915 | 9.89 |
| Low (Lot 92781) | L7 | 0.503| 0.501| 0.502 | 0.459 | 9.61 |
| Middle (Lot 92782) | M1 | 0.814| 0.795| 0.8045| 0.7615 | 15.95 |
| Middle (Lot 92782) | M2 | 0.822| 0.82 | 0.821 | 0.778 | 16.29 |
| Middle (Lot 92882) | M3 | 0.758| 0.765| 0.7615| 0.7185 | 15.05 |
| Adjusted Result | 11 | 0.574| 0.552| 0.563 | 0.52 | 10.89 |
| Adjusted Result | 13 | 0.643| 0.658| 0.6505| 0.6075 | 12.72 |
| L adjuster (01*) | L8 | 0.422| 0.423| 0.4225| 0.38 | 7.50 |
| M adjuster | M4 | 0.735| 0.726| 0.7305| 0.688 | 13.58 |
| M adjuster +0.005 Hb | M5 | 0.734| 0.726| 0.73 | 0.6875 | 13.57 |
| M adjuster +0.01 Hb | M6 | 0.912| 0.852| 0.882 | 0.8395 | 16.57 |
| H adjuster | H2 | 0.699| 0.696| 0.6975| 0.655 | 12.93 |
| H adjuster +0.015 Hb | H3 | 0.989| 1.023| 1.006 | 0.9635 | 19.02 |
| H adjuster +0.025 Hb | H4 | 1.103| 0.986| 1.0445| 1.002 | 19.78 |

Note: A1 and A2 are sample replications. Lot is a unique identifier for a specific material number produced by a single manufacturer of items processed on a production line without interruptions by processing other products.
LED of 660 nm with sensor responsiveness of 7000, and the lowest sensor response is the LED of 1050 nm with sensor responsiveness of 2000.

In measuring distance, bright light is very influential in the distance of the data sender. The brighter the light, the farther the data transmission distance. The brightness of the LED light is affected by the maximum voltage and the color of the LED lamp. Additionally, the power supply source in the receiver circuit affects the brightness. The power supply source must be equal to the maximum amount of voltage that the LED can accept. If the power supply source in the circuit exceeds the maximum voltage from the LED, the LED will break. Conversely, if the LED gets a voltage less than the power supply source, the LED light will dim such that the LED lighting is less than optimal. From the measurement results obtained at a distance of 1.6 cm, a signal is still obtained with good quality, whereas at a distance greater than 1.6 cm, the received signal is poor.

Testing of the frequency range on the test equipment produced the optimum frequency of 600–45,000 Hz. The signal quality obtained at frequencies below 600 Hz is not much different from the signal quality obtained at a frequency of 600 Hz. However, at frequencies below 600 Hz the light intensity from the LED is unstable (flickering). At frequencies above 45,000 Hz, the LED is bright. However, the resulting signal quality is not good (already indicating the presence of noise). This is because the circuit used is limited (a standard circuit consisting only of buffers, couplings, and bypasses).

Connectivity testing was also carried out to ensure that the Hb and Raspberry pi measuring probes were connected to the same internet network. The internet network used can be sourced from all types of smartphones. The SSID name was set as “markis” with the password “admin123.” Figure 6 shows the display of setting the SSID name and password on the smartphone.

In the circuit integration test, the Hb sensor components, NodeMcu, LEDs, resistors, ADCs, and Raspberry Pi must be connected to each other to form a single Hb measuring device. Figure 7 explains that the circuit consists of an LED (source) as an emitter that emits light and an Hb sensor as a detector that absorbs or receives light.

3.2.2. Test results of noninvasive blood hemoglobin level measuring devices based on the IoT and web server

Table 3 shows the results of noninvasive testing of blood Hb levels based on the IoT and web server. The Series A battery has an average
battery charge time of 43.4 min. The battery circuit B has an average battery charge time of 42.4 min. The measurement of the duration of use of the tool is done by charging the battery until it is full. After the battery is fully charged, the tool is set to take measurements continuously until it cannot take measurements; then, the time required for the length of use of the tool is calculated using a stopwatch. Testing the duration of using the tool was carried out five times. Battery circuit A has an average lifetime of 240.8 min, and Series B batteries have an average run time of 241.4 min. Based on the results obtained, the average difference in battery charging time for Series A and Series B is 1 min. The difference between the average use of the A and B circuits is 0.8 min. Additionally, Table 4 shows the measurement precision of the sensor circuits A and B. The precision value, $p$, is given by Eq. (4).

The sensor reading value is the analog reading value received by the microcontroller when the 525 and 555 nm LEDs are 99% lit. The results show that prototype A has an average precision level for three respondents of 96.84%. Prototype B has an average precision level of

| Battery circuit | Replication | Battery charging time (min) | Tool usage time (min) |
|-----------------|-------------|-----------------------------|-----------------------|
| A               | 1           | 45                          | 243                   |
|                 | 2           | 43                          | 245                   |
|                 | 3           | 43                          | 240                   |
|                 | 4           | 42                          | 238                   |
|                 | 5           | 44                          | 238                   |
| Average         |             | 43.4                        | 240.8                 |
| B               | 1           | 43                          | 240                   |
|                 | 2           | 41                          | 243                   |
|                 | 3           | 43                          | 243                   |
|                 | 4           | 43                          | 241                   |
|                 | 5           | 42                          | 240                   |
| Average         |             | 42.4                        | 241.4                 |

Figure 7. Probe schematic illustrations for baby (a) finger and (b) thighs.
96.46%. These high precision levels indicate that prototype of Hb detection device has good performance.

The measurement results of the probe parameters show that the index finger length of ten respondents had an average of 66.6 mm and the finger length of the respondent who had the shortest index finger is 62 mm. Thus, the probe of the designed tool must have a place to insert a finger that has a depth of less than 62 mm. The result of the index finger thickness of 10 respondents was an average of 11.95 mm. Because the penetration of light entering the finger must hit half of the finger, the light must penetrate at least 6 mm from the finger surface.

4. Conclusion

Three sets of prototype variants for noninvasive blood Hb level measuring instruments have been successfully developed: (i) noninvasive blood hemoglobin level measuring device based on Raspberry Pi Prototype on Infant Finger and Thigh Probes, (ii) level measuring prototype noninvasive hemoglobin in blood based on IoT and web server, and (iii) prototype of noninvasive blood hemoglobin level measuring device on in vitro probe with reflectance method. Additionally, Hb concentration measurements and wavelength scanning were carried out on standard hemoglobin solutions and blood controls. The measurement of the Hb value initially measured by the Trax Control Meter for low, middle, and high levels is almost the same as the expected range values for the prototypes, namely, 13.09, 16.8, and 17.81 g/dL, for prototypes (i), (ii), and (iii), respectively. A series of prototype unit tests as shown in Table 4 indicate good consistency.

| Prototype | Respondent | Replication | Sensor reading value | \( \tau \) | \( p \) | Average precision |
|-----------|------------|-------------|----------------------|------|-----|------------------|
| A 1  | 1 | 273 | 284.67 | 95.90% | 96.88% |
|  | 2 | 298 | | | |
|  | 3 | 283 | | | |
| 2  | 1 | 186 | 180.67 | 97.05% | 98.03% |
|  | 2 | 180 | | | |
|  | 3 | 176 | | | |
| 3  | 1 | 180 | 172.33 | 95.55% | 95.62% |
|  | 2 | 161 | | | |
|  | 3 | 176 | | | |
| B 1  | 1 | 286 | 285.67 | 99.88% | 97.04% |
|  | 2 | 273 | | | |
|  | 3 | 298 | | | |
| 2  | 1 | 286 | 279.4 | 97.64% | 94.32% |
|  | 2 | 257 | | | |
|  | 3 | 298 | | | |
| 3  | 1 | 186 | 180.67 | 97.05% | 98.03% |
|  | 2 | 180 | | | |
|  | 3 | 176 | | | |

Declarations

Author contribution statement

Irzaman: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Yaya Suryana; Sabar Pambudi; Tika Widayanti; Sri Kristiana Rahayu; Naufal Muharram Nurdim: Conceived and designed the experiments.

Renan Prasta Jenie: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Bayu Prastowo; Nazaputul Patonah Har; Vania Rahmawaty; Muhammad Dahrul; Ade Kurniawani; Ridwan Siskandar; Icshan Haridyanto; Johan Iskandar; Arga Ardidarma: Performed the experiments. Aminullah: Analyzed and interpreted the data; Wrote the paper. Husin Alatas: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Funding statement

Irzaman was supported by LPDP [PRJ-78/LPDP/2019].

Data availability statement

Data will be made available on request.

Declaration of interest’s statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

Acknowledgements

We would like to thank “Konsorsium Riset Alat Ukur Hemoglobin, Kadar Gula (Glukosa dan HbA1c) Non-Invasif” between IPB University, National Research and Innovation Agency (BRIN), and PT Tesena Inovindo for their continuous support for this research. This work is funded by grant of fiscal year 2021 from the Ministry of Finance, Republic of Indonesia, through Lembaga Pengelola Dana Pendidikan (LPDP) under contract No. PRJ-78/LPDP/2019.

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12