Using Hematological Methods to evaluate the health status of a 22-year-old non-athletic Asian Male with non-fasting blood sample

By: Jeremy Xu, Shannon Applequist, Nautica Smith, Kendra Tiani, Ezra Mutai

December 18, 2020

Abstract

Hematology is an important lab method that evaluates patients' health status based on the cells in blood regarding their volume, size, and number. The purpose of this experiment is to check whether the subject, a 22-year-old non-athletic Asian male, has any potential risks of diseases based on several blood test results. It is hypothesized that the subject is in health condition since no clinical manifestation was indicated. Non-fasting blood sample was collected 1 hour after breakfast. *Coulter counter* was applied to measure basic indicators of the subject's blood, including hematocrit (Hct), red blood cell (RBC) count, hemoglobin (Hgb); The concentration of hemoglobin was also determined by cyanmethemoglobin method with Drabkin′s reagent; MCV, MCH, and MCHC were either measured by *Coulter counter* or calculated by equations; Blood type and glucose concentration were measured by blood typing method and glucometer respectively. *Immulite 2000* was used to measure the concentration of serum ferritin and Vitamin B_{12}; Serum iron concentration and total iron-binding capacity (TIBC) were determined by spectrophotometric assay; Body iron stores and transferrin saturation were calculated from equations. The value of total cholesterol, HDL, LDL, and triglycerides were measured by fully automatic *Dimension Xpand* or manual blood lipid method. *TITAN GEL* was used to conduct serum electrophoresis, and the fractions of serum proteins were determined by *ImageJ*. The results indicate that the subject's hematocrit and RBC count slightly exceeded the normal range, and MCH and MCHC were a bit below the normal ranges. But it is still concluded that he was in health condition and the subject was not at any risk of cardiovascular diseases based on the fact that most of his hematological parameters were within the normal ranges.

Introduction

Nowadays, cardiovascular diseases have become the primary causes of human death and disability on a global basis, which are typically combined with hypertension, diabetes, hyper-lipidaemia and other diseases.[1] [2]Therefore, early detection is very important to screen the diseases and to reduce the risk of cardiovascular diseases under proper treatment. Hematology, measuring a number of hematological parameters, is an ideal method that can help reflect patients' health status for clinical guidance. Studies have shown that for patients with higher risk of cardiovascular diseases, there are increased values in total cholesterol, triglyceride, LDL, RBC count, hemoglobin, hematocrit, and MCH, but decreased values in HDL and MCV.[3]

Coulter counters, because of its reliability and simplicity, are generally used for measuring concentration and size of biological cells in buffer solution, and thus it can be applied for blood cells analysis.[4] For example, a number of RBC parameters, including RBC count, hemoglobin, hematocrit, MCV, MCH, and MCHC, are measured by Coulter counters, which can reflect abnormalities among patients with diabetes so as to help prognostic treatment.[5] Cyanmethemoglobin method, a manual photometric method for estimating the concentration of hemoglobin, is based on the reaction between hemoglobin and Drabkin's reagent for forming cyanmethemoglobin, which produces color and can be measured by the spectrophotometer at 540nm wavelength. Compared with Coulter counter, cyanmethemoglobin method is more cost effective and feasible for Hemoglobin measurement.[6] MCV, MCH, and MCHC can also be calculated from the value of RBC count, hemoglobin, and hematocrit, based on their corresponding definition where MCV represents the average volume of RBC in blood sample, MCH represents the average amount of hemoglobin in each cell, and MCHC represents the concentration of hemoglobin in RBCs. Abnormally low values of MCV and MCH are found in patients with iron deficiency anemia or anemia of chronic disease.[7] Blood typing test is essential before medical

surgery or treatment such as transfusion and organ transplant, to avoid non-compatible blood types that cause agglutination. The presence or absence of certain antigens on RBCs' surface and antibodies in the plasma leads to the variety of blood types, including type A, type B, type AB, and type O, and the presence or absence of Rh factor classifies each of them into Rh+ and Rh-.[8] Blood glucose concentration can be detected by glucometer. And non-fasting blood glucose level, specifically, can indicate that the patient has diabetes if the value is 200 mg/dL or higher.[9]

Immulite 2000, a fully automatic chemiluminescent immunoassay system, is used to measure the concentration of serum ferritin and Vitamin B_{12}. Serum ferritin concentration is considered as an outstanding indicator to estimate the iron store in humans.[10] Studies have shown that serum ferritin level is positively associated with Type 2 diabetes.[11] And Vitamin B_{12} deficiency is related with macrocytic anemia and identified by hematological abnormalities.[12] Other biomarkers such as serum iron concentration, total iron-binding capacity (TIBC), and transferrin Saturation can also effectively reflect iron stores.

Serum lipids, including total cholesterol, HDL, LDL, and triglycerides are measured by *Dimension Xpand* or manual blood lipid method to identify the potential risk of cardiovascular diseases among patients.[13] A 12-hour fasting is preferred before taking cholesterol test so as to get more accurate results.[14] Serum electrophoresis classifies proteins into different fractions, including albumin, alpha1, alpha2, beta and gamma proteins. Increased or decreased levels of each fraction may result in certain diseases, such as inflammatory disease, hyperlipoproteinemia, malnutrition, chronic liver disease, etc.[15]

The purpose of this experiment is to check whether the subject, a 22-year-old non-athletic Asian male, has any potential risks of diseases based on hematological test results mentioned above. It is expected that the subject will have all the test results within the normal range and at no risk of any cardiovascular diseases.

Methods

Blood Collection

The blood sample of a 22-year-old non-athletic Asian male was drawn by a phlebotomist after 1-hour breakfast eating. The blood sample was collected in one tube containing EDTA to get plasma and the other tube without an anticoagulant to isolate serum.

RBC parameters, blood typing, and glucose level

Primary RBC parameters include RBC count, hemoglobin, hematocrit, MCV, MCH, and MCHC. These values were measured by Beckman-Coulter Coulter counter.

Hemoglobin was also measured by cyanmethemoglobin method, where the Drabkin′s reagent was added to the blood sample to get the hemoglobin compound with color. The sample was duplicated, and a spectrophotometer was used to record twice the absorbance at a wavelength of 540 nm. Average absorbance was thus calculated, and the concentration of hemoglobin was calculated based on the standard curve.

$$Hemoglobin\ Concentration\ (g/100mL) = \frac{Average\ absorbance}{Extinction\ coefficient\ from\ standard\ curve} \div 10$$

Apart from the values from Coulter counter, MCV, MCH, and MCHC were also calculated based on the following equations:

$$MCV\ (fL/cell) = \frac{Hematocrit\ (\%) \times 10}{RBC\ count\ (millions/mm^3)}$$

$$MCH\ (pg/cell) = \frac{Hemoglobin\ (g/dL) \times 10}{RBC\ count\ (millions/mm^3)}$$

$$MCHC\ (g/dL) = \frac{Hemoglobin\ (g/dL) \times 100}{Hematocrit\ (\%)}$$

Blood typing was conducted by mixing the blood samples with Anti-A blood-grouping serum, Anti-B blood-grouping serum, and Anti-Rh serum separately on their corresponding location of the tray. Observed agglutination indicates positive result, and the negative result represents no agglutination.

Blood glucose concentration was measured by glucometer, which calculated the blood glucose level from blood drop on the test strip. Specific steps of measuring RBC parameters, blood typing, and glucose level can be referred to the Laboratory Manual 6a[16].

Serum ferritin concentration, Vitamin B_{12}, and body iron stores

Serum ferritin and Vitamin B_{12} were measured by Immulite 2000, with 150μL of serum sample.

The value of body iron stores was calculated based on the relationship that 1μg/L of serum ferritin equals 10 mg of stored iron[17], which can be written as follows:

$$Body\ iron\ stores\ (mg) = \frac{Serum\ ferritin\ (\mu g/L) \times 10mg}{1\mu g/L}$$

Serum iron concentration, TIBC, and transferrin saturation

Serum iron concentration and TIBC were measured by spectrophotometric assay after a two-part procedure lab method, which were conducted in parallel. Firstly, 1.6mL iron was added to 0.8 mL serum. 350 - 400mg pre-weighed $MgCO_3$ was added and then centrifuged after 30 minutes. Second, 1.0 mL serum, 1.0mL water, 1.0 ml of iron standard, 1.0 mL ascorbic acid, and 1.5 mL 8.8% TCA were added to their designated tubes. Third, 2.0 mL supernatant, 1.0 mL 10% ammonium acetate buffer, and 0.40 mL Ferrozine color reagent were added to their designated tubes. After 5 minutes color development, the reagent blank, standards and samples were sequentially measured by the spectrophotometer at the wavelength of 560nm. More detailed steps can be referred to the Laboratory

Manual 6b[17]. The concentration of Serum iron and TIBC were calculated by their corresponding absorbance values and the following equations:

$$Serum\ iron\ (\mu g/dL) = \frac{Serum\ iron\ absorbance - Correction\ factor\ from\ standard\ curve}{Extinction\ coefficient\ from\ standard\ curve}$$

$$TIBC(\mu g/dL) = \frac{TIBC\ absorbance - Correction\ factor\ from\ standard\ curve}{Extinction\ coefficient\ from\ standard\ curve} \times 3$$

The value of transferrin saturation is calculated based on the result of serum iron and TIBC, with the following equation:

$$Transferrin\ saturation\ (\%) = \frac{Serum\ iron\ (\mu g/dL)}{TIBC(\mu g/dL)} \times 100$$

Serum lipids

A 150μL serum sample was added to the sample cup and measured by *Dimension Xpand* for lipid analysis to get the values of total serum cholesterol, HDL cholesterol, LDL cholesterol, and triglyceride.

Total cholesterol, HDL, and triglyceride were also measured by manual lipid analysis. Whole serum and 200 mg/dL cholesterol standard were used in the total cholesterol assay; 200μL of serum sample was mixed with 20μL HDL precipitating reagent and centrifuged to isolate HDL (supernatant), and thus the supernatant sample and 50 mg/dL cholesterol standard were used in HDL cholesterol assay; whole serum and 200 mg/dL triglyceride standard were used in triglyceride assay. Detailed steps of manual lipid assay can be referred to Laboratory Manual 6c[18]. Their values can be calculated from the following equations:

$$Serum\ Total\ Cholesterol\ (mg/dL) = \frac{A_{sample}}{A_{200\ std}} \times 200 mg/dL$$

$$HDL\ Cholesterol\ (mg/dL) = \frac{A_{supernatant}}{A_{50\ std}} \times 50 mg/dL \times 1.1$$

$$LDL\ Cholesterol\ (mg/dL) = Serum\ Total\ Cholesterol - HDL\ Cholesterol - \frac{Triglycerides}{5}$$

$$Serum\ Triglycerides\ (mg/dL) = \frac{A_{sample}}{A_{200\ TG\ std}} \times 200 mg/dL$$

Serum electrophoresis

The fraction of serum protein was measured by serum electrophoresis on TITAN GEL, where the subject's serum sample was firstly 1:4 diluted with TITAN GEL Serum Protein Buffer. 3.0μL of each sample was then placed on the slits of TITAN GEL SPE Template, and the template was further removed. The gel was electrophoresed at 120 volts for 15 minutes where the agarose was placed side down in the buffer and the application point is on the cathodic (-) side. The gel was placed in the methanol for 5 minutes after electrophoresis was finished, and it was visualized by adding Amino Black stain one-time and then destain solution 3-4 times to the gel until clear bands occurred. The bands were further analyzed by ImageJ to get the serum protein tracing pattern and their distribution percentages. More detailed steps of separation and quantitation of serum proteins can be referred to Laboratory Manual 6d[19].

Results

RBC parameters, blood typing, and glucose level

Basic RBC parameters with their reference ranges[20], are shown in **Table 1**. The data indicate that Hematocrit (%), RBC count are higher than the normal range, while hemoglobin measured from cyanmethemoglobin method, MCH, and MCHC are slightly lower than the normal range.

Hemoglobin standard curve is shown in **Figure 1**. Blood typing is shown in **Table 2**. Blood glucose concentration is **93 mg/dL** measured by glucometer from the non-fasting blood sample, and the reference range of the non-fasting blood sample is **< 200 mg/dL**[20].

Table 1. Chemical blood counts and hematological measurements. Values of RBC parameters were obtained from Coulter counter, cyanmethemoglobin method, or calculated. Reference ranges were based on the criteria of a 22-year-old Asian male.

Test (with units)	Measurement	Reference Range[20]
Hematocrit (%)	53.7 % (Coulter counter)	42% - 50%
RBC count (millions/mm^3)	6.12 (Coulter counter)	4.2 - 5.9
Hemoglobin (g/dL)	16 (Coulter counter)	14 - 18
	13.43 (cyanmethemoglobin method)	
MCV (fL/cell)	87.6 (Coulter counter)	80 - 98
	87.75 (calculated)	
MCH (pg/cell)	26.2 (Coulter counter)	28 - 32
	26.14 (calculated)	
MCHC (g/dL)	29.9 (Coulter counter)	33 - 36
	29.8 (calculated)	

Figure 1. Hemoglobin standard curve using Drabkin's reagent. Reagent blank, standards and samples were measured in duplicate using spectrophotometer at the wavelength of 540nm. The linear regression equation is y=0.0035x, R^2=0.9996.

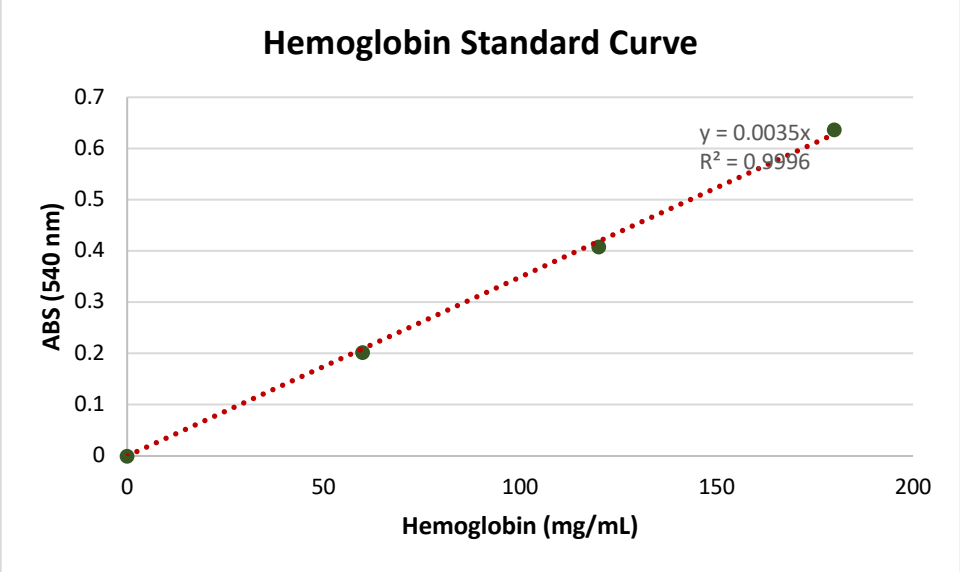


Table 2. Blood Typing using Anti-A, Anti-B, and Anti-Rh blood-grouping serum. Observe for agglutination of the red blood cells. (+) = agglutination observed; (-) = agglutination not observed.

Antibody	Agglutination	Antigen Present (blood type)
A	-	No
B	+	Yes (B Antigen)
Rh_0 (anti-D)	+	Yes (Rh Antigen)
BLOOD TYPE		B+

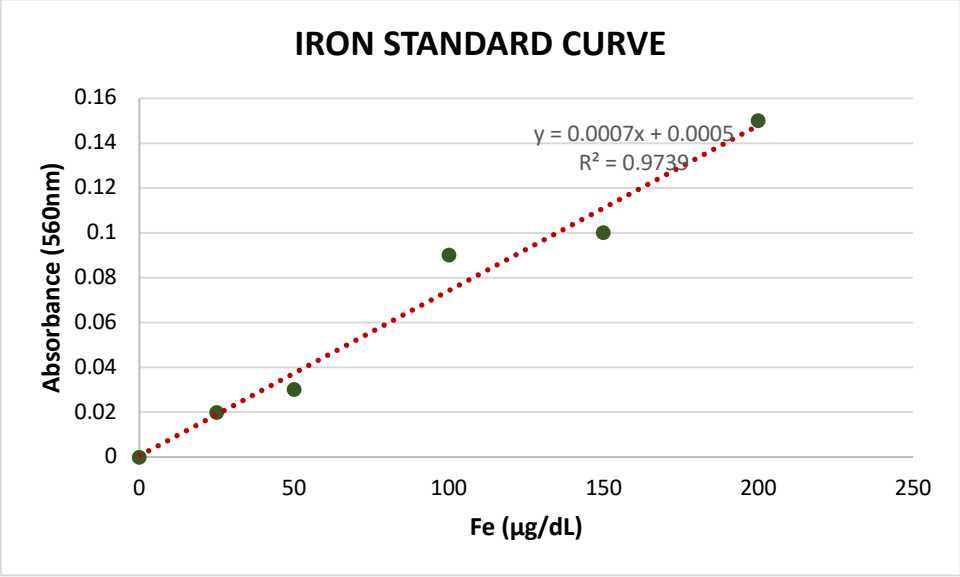
Iron Status and Vitamin B_{12}

Serum Iron Status and Vitamin B_{12} Indicators are shown in **Table 3**. Iron Standard Curve is shown in **Figure 2**.

Table 3. Serum Iron Status and Vitamin B_{12} Indicators. Serum ferritin and Vitamin B_{12} were measured by Immulite 2000. Body iron stores were calculated based on the relationship that 1µg/L of serum ferritin equals 10mg of stored iron. Serum iron and TIBC were measured by spectrophotometric assay. Transferrin saturation is calculated based on the result of serum iron and TIBC.

Biomarker (with units)	Measurement	Reference Range[20]
Serum ferritin (ng/mL)	278	24 - 336
Body iron stores (mg)	2780	240 - 3360
Vitamin B_{12} (pg/mL)	517	200 - 800
Serum iron (µg/dL)	70.71	50 - 150
TIBC (µg/dL)	255	250 - 310
Transferrin saturation (%)	27.73%	20% - 50%

Figure 2. Iron Standard Curve. The reagent blank, standards and samples were sequentially measured by the spectrophotometer at the wavelength of 560nm. The absorbance of Serum iron and TIBC are 0.05 and 0.06 respectively.



Serum lipids

Values of total cholesterol (mg/dL), HDL (mg/dL), LDL (mg/dL), and triglycerides (mg/dL) are shown in **Table 4**.

Table 4. Serum lipid measurements. Fully automated Dimension Xpand and manual blood lipid method were used to measure serum lipids.

Biomarker (with units)	Dimension Xpand	Manual measurement	Reference Range[20]
Total cholesterol (mg/dL)	117.7	248	< 200
HDL (mg/dL)	48	132	40 - 200
LDL (mg/dL)	54.7	82.6	< 129
Triglycerides (mg/dL)	112.4	167	< 150

Serum electrophoresis

Serum protein bands on gel and their corresponding tracing patterns are shown in **Figure 3**.

Serum protein distribution is shown in **Table 5**.

Figure 3. Image of protein bands on gel (Column 9 and 10) with tracing patterns by ImageJ. *TITAN GEL* was used to conduct serum electrophoresis. ImageJ was used to detect the tracing patterns from two protein bands.

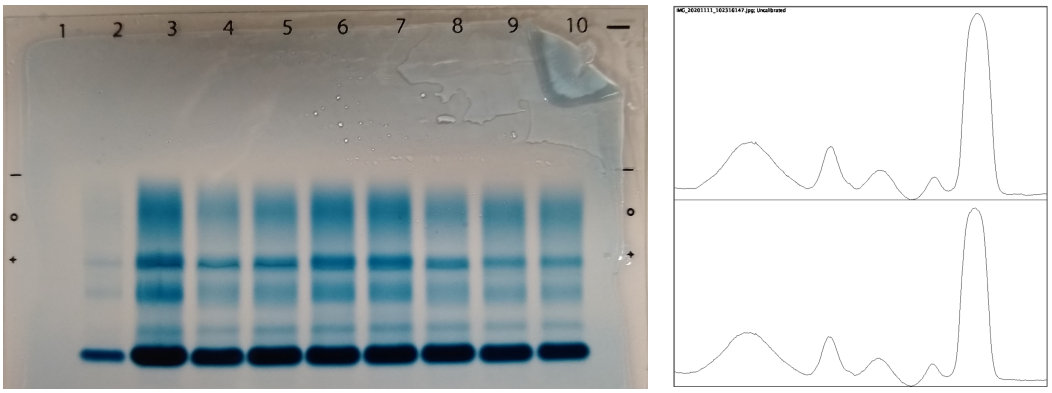


Table 5. Serum protein distribution. The fractions of serum proteins (albumin, alpha1, alpha2, beta and gamma proteins) were determined by *ImageJ* based on their corresponding areas.

Protein fraction	Average distribution percentage (%)	Reference range (%)[21]
Albumin	53.94	60 - 75
Alpha 1	2.93	1.7 - 5
Alpha 2	6.80	6.7 - 12.5
Beta	8.73	8.3 - 16.3
Gamma	27.60	10.7 - 20

Discussion

The subject's blood type is B+, meaning that he can donate blood to others with B+ or AB+ blood types, and can receive B+, B-, O+, O- types of blood from others.

In order to see whether the subject has any risks of cardiovascular diseases, it is necessary to understand how these hematology results reflect his health status. This hematological lab experiment demonstrates various dimensions of evaluation indices, including chemical blood counts, glucose level, iron status, vitamin B_{12}, serum lipids, and serum protein distribution.

Chemical blood counts and hematological measurements provide much detailed information about red blood cells. In this experiment, the Hematocrit and RBC count were determined by Coulter counter, which was 53.7 % and 6.12 millions/mm^3 respectively. The values were higher than the reference ranges, indicating that the subject may have risk of hypertension and cardiovascular diseases based on the previous statements.[3] However, there was a decreased level of MCH (26.2 pg/cell by Coulter counter; 26.14 pg/cell by calculated), which should be increased if the subject has hypertension risks. Therefore, there was a contradiction between the two statements. Since all of these values didn't exceed far beyond the reference range, it is assumed that the subject was in good health condition, though further analysis is needed to evaluate the result once again. Cyanmethemoglobin method was used to measure the hemoglobin concentration of the subject, in which the value (13.43 g/dL) was lower than the reference range (14 - 18g/dL), while the hemoglobin level measured from Coulter counter reflected the result (16g/dL) within the normal range. The glucose level measured by glucometer from the non-fasting blood sample was regarded as the random blood sugar test[9], and the value (93 mg/dL) was lower than reference range (< 200 mg/dL), showing that the subject doesn't have risk of diabetes.

Serum ferritin and Vitamin B_{12} measured by Immulite 2000 were good indicators of whether the subject was at risk of iron deficiency, Type 2 diabetes, or macrocytic anemia. [101112] The test showed that

all the values regarding serum iron status and Vitamin B_{12} were in the normal range, indicating that the subject had sufficient iron stores and was at no risk of these diseases.

Serum lipid measurement is an important method to investigate whether the subject is at risk of cardiovascular diseases. Both the *Dimension Xpand* and manual blood lipid method were used to measure serum lipids in this experiment, and some abnormal results of total cholesterol (248 mg/dL) and triglycerides (167 mg/dL) in the manual measurement were observed, compared with the reference range of < 200 mg/dL and < 150 mg/dL respectively. However, all the values measured by *Dimension Xpand* were within the normal range. On condition that the subject is in health condition, it is expected that he should have all his serum lipids within the normal range, and some errors may occur during the manual measurement. For example, long-time room temperature exposure may falsely elevate triglycerides values. In addition, since the blood was collected 1 hour after the subject ate breakfast (non-fasting blood), the value may not reflect the accurate results of the lipid status, and thus further analysis is needed to know whether the subject is at risk of cardiovascular diseases.

Serum electrophoresis helps to separate different serum proteins and identify the distribution of these sections. In this experiment, Albumin (53.94%), Alpha1 (2.93%), Alpha2 (6.80%), Beta (8.73%), and Gamma (27.60%) proteins were separated through the serum electrophoresis, and all their distributions were within the normal range. However, the quantification of these bands using ImageJ was subjective when calculating different areas of distribution.

Conclusion

The experiment was conducted to check whether the subject was in good health condition or at any risks of cardiovascular diseases by using a series of hematological methods based on the non-fasting blood sample from a 22-year-old non-athletic Asian male. The study found that the subject's hematocrit

and RBC count slightly exceeded the normal range, and MCH and MCHC were a bit below the normal ranges. But it is still concluded that he was in health condition and the subject was not at any risk of cardiovascular diseases based on the fact that most of his hematological parameters were within the normal ranges. Further analysis may be needed to evaluate the subject's serum lipids by collecting his 12-hour fasting blood sample so as to achieve a more accurate result.

References

1. Cardiovascular diseases (CVDs). (2017, May 17). World Health Organization. https://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds).
2. Cardiovascular Diseases | Division of Global Health Protection | Global Health | CDC. (2020). Centers for Disease Control and Prevention. https://www.cdc.gov/globalhealth/healthprotection/ncd/cardiovascular-diseases.html.
3. Emamian, M. *et al.* Association of hematocrit with blood pressure and hypertension. *J. Clin. Lab. Anal.* **31**, e22124 (2017).
4. Zhang, W. *et al.* Microfluidic multiple cross-correlated Coulter counter for improved particle size analysis. *Sens. Actuators B Chem.* **296**, 126615 (2019).
5. Adane, T., Getaneh, Z. & Asrie, F. Red Blood Cell Parameters and Their Correlation with Renal Function Tests Among Diabetes Mellitus Patients: A Comparative Cross-Sectional Study. *Diabetes Metab. Syndr. Obes. Targets Ther.* **Volume 13**, 3937–3946 (2020).
6. Kumar Jain, D. & Pathology. Comparison of automated method and photometric cyanmethemoglobin method for haemoglobin estimation. *IP J. Diagn. Pathol. Oncol.* **5**, 273–276 (2020).
7. Archer, N. M. & Brugnara, C. Diagnosis of iron-deficient states. *Crit. Rev. Clin. Lab. Sci.* **52**, 256–272 (2015).
8. Karimi, S., Mehrdel, P., Farré-Lladós, J. & Casals-Terré, J. A passive portable microfluidic blood–plasma separator for simultaneous determination of direct and indirect ABO/Rh blood typing. *Lab. Chip* **19**, 3249–3260 (2019).
9. Diabetes Testing. (2019, May 15). Centers for Disease Control and Prevention. https://www.cdc.gov/diabetes/basics/getting-tested.html.
10. Asif, N., Ijaz, A., Rafi, T., Haroon, Z. H., Bashir, S., & Ayyub, M. (2016). Diagnostic accuracy of serum iron and total iron binding capacity (TIBC) in iron deficiency state. Journal of the College of Physicians and Surgeons Pakistan, 26(12), 958+. https://link.gale.com/apps/doc/A477641638/HRCA?u=cornell&sid=HRCA&xid=f1a99162.
11. Chen, L. *et al.* Elevated serum ferritin concentration is associated with incident type 2 diabetes mellitus in a Chinese population: A prospective cohort study. *Diabetes Res. Clin. Pract.* **139**, 155–162 (2018).
12. Shipton, M. J., & Thachil, J. (2015). Vitamin B12 deficiency - A 21st century perspective . Clinical medicine (London, England), 15(2), 145–150. https://doi-org.proxy.library.cornell.edu/10.7861/clinmedicine.15-2-145.

13. Wen, J., Huang, Y., Lu, Y. & Yuan, H. Associations of non-high-density lipoprotein cholesterol, triglycerides and the total cholesterol/HDL-c ratio with arterial stiffness independent of low-density lipoprotein cholesterol in a Chinese population. *Hypertens. Res.* **42**, 1223–1230 (2019).
14. Cholesterol test - Mayo Clinic. (2019, July 12). Mayo Clinic. https://www.mayoclinic.org/tests-procedures/cholesterol-test/about/pac-20384601.
15. Protein electrophoresis - serum. (2020). Mount Sinai Health System. https://www.mountsinai.org/health-library/tests/protein-electrophoresis-serum.
16. Mutai, E. and Field, M. (2020). Lab 6a. Hematology. Blood panel, blood typing, hemoglobin, glucose measurements.
17. Mutai, E. and Field, M. (2020). Lab 6b. Hematology. Determination of Serum Iron, Total Iron Binding Capacity, Percent Transferrin Saturation, and Vitamin B12.
18. Mutai, E. and Field, M. (2020). Lab 6c. Hematology. Enzymatic Determination of Serum Lipids.
19. Mutai, E. and Field, M. (2020). Lab 6d. Hematology. Serum electrophoresis.
20. American Board of Internal Medicine. (2020, January). ABIM Laboratory Test Reference Ranges. https://www.abim.org/~/media/ABIM%20Public/Files/pdf/exam/laboratory-reference-ranges.pdf.
21. Protein Electrophoresis (Blood) - Health Encyclopedia - University of Rochester Medical Center. (2020). University of Rochester Medical Center. https://www.urmc.rochester.edu/encyclopedia/content.aspx?contenttypeid=167&contentid=protein_electrophoresis_serum.