



## **Validation Hematological Analyzer for Assay of Erythrogram in Hodeidah City, Yemen**

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### **Authors' contributions**

*This work was carried out in collaboration between all authors. Author SAS revised and supported the study as Former Director – General of National Center of Public Health Laboratories as Reference lab in Yemen. Authors SOT and FB designed, wrote the protocol, and managed the literature searches. Author AD collected the data and analyzed the sample. Authors MAAK performed the statistical analysis, wrote the first draft of the manuscript and managed the analyses of the study. All authors read and approved the final manuscript.*

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### **ABSTRACT**

The hematological indices namely erythrogram is an important parameter for evaluating human's physiological status. It can vary accordingly depending upon the differential traits (Asian, Arabian, European ... etc), sex, age, and health condition. In best of our knowledge, due to lack of researches in absence of constant values in this area this study is aimed to identify the reference values of erythrogram in the adults at Hodeidah, Yemen. The hematological analyzer (Sysmex KX-21) was validated for assessing the linearity, accuracy, precision and quantification with limit of

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different blood samples and was used for sampling in research analysis. Participated volunteers of this study were provided written consent, by following the university of Hodeidah and Office of Health and Public declaration. The erythrogram parameters include hemoglobin (Hb), red blood cells (RBC), packed cell volume (PCV), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), for two investigated groups amongst whom 500 were males and 500 were females. Obtained data were analyzed. The results of validated method were precise to each analyze with percent relative standard deviations (RSD %) of intra-assay and inter – assay (< 5.0%) methods. Furthermore, the accuracy of validated method exhibit well recovery values of  $\pm 5\%$  and the coefficient correlation ( $R^2$ ) value was more than 0.9995 as a good linear method. On the other hand, the results showed the level of Hb, RBC, and PCV to be significantly different from males than that of females ( $p < 0.05$ ). The erythrogram parametric means of this study were constant with the international normal range except for the MCH in both the groups, whereas reduction of Hb is found from the study. These variations might be related to the case of infectious diseases, but low in nutritional values and the geographical location of Hodeidah city, Yemen.

*Keywords: Validation; erythrogram; Hodeidah; Yemen.*

## 1. INTRODUCTION

Blood consists of three types of cells: white blood cells (WBCs), red blood cells (RBCs), and platelets (PLTs). They are produced and mature primarily in the bone marrow [1]. These parameters are available in human body within limiting values that called blood indices and which are known to be important parameters for the evaluation of human physiological status. These changes depend on the human species (Asian, Arabian, European etc), sex, age, the cycle of sexual maturity and health condition etc [2].

The Complete Blood Count (CBC) is a test that evaluates the cells that circulate within blood. A CBC is typically performed using an automated instrument which can measure various parameters. The results of a CBC can provide information about not only the number of cell types but can also give an indication of the physical characteristics of some of the cells. Also, these parameters play an important role in helping doctors to know these values in a true way so that they can come to a sound diagnosis [3].

Many people have gone through CBC performed when they were assessed a routine health examination. Also it may be ordered when a person has any symptoms or signs that may be related to blood disorders. When an individual has fatigue or weakness or has an infection, inflammation, bruising, or bleeding, a doctor can order a CBC to help in the diagnosis of the prior cause to determine the severity. When a person has been diagnosed with a disease which affects the normal blood cells, then CBC will often be

ordered on a regular basis to monitor their ultimate condition. Likewise, if someone is receiving treatment for a blood-related disorder, then a CBC may be performed frequently to determine if the treatment is effective for the patient or not. Some therapies, such as chemotherapy, can affect bone marrow production of a person. A CBC should be ordered on a regular basis to monitor these drug treatments [4].

On the other hand, this study will help us to put programs and true clear values of health inside the office in provision to which it can be referred to and generalize at centers, laboratories and hospitals, and consider as reference values for the area. It can also be used as epidemic and survey studies in the study area.

This study was aimed to identify erythrogram parameters namely the hemoglobin (Hb), packed cell volume (PCV), red blood cells (RBC), mean cell volume (MCV), mean concentration hemoglobin (MCH), mean cell hemoglobin concentration (MCHC) of adults of different sectors in Hodeidah, Yemen to bring up some newer ideas in disease prevention. Another objective of this study is to identify the limit of these parametric ranges in different traits, species, ages, sexes along with geographic variations.

## 2. MATERIALS

### 2.1 Study Design

This is a prospective descriptive cross sectional study about Reference Values for hematological parameters in adults of Hodeidah City, Yemen.

The study was conducted during the period June 2013 to May 2014.

## 2.2 Study Population

The personal data was collected through structured questionnaire, including age, situation condition, sex, Body Mass Index (BMI), heart rate and blood pressure (BP).

## 2.3 Study Area

This study was conducted in Hodeidah city selected Yemen country that is tropical region Hodeidah Governorate borders the Red Sea and is part of the narrow Tihama region. It serves as an important local port city. With a population of 2,687,674 and an area of 17,509 km<sup>2</sup>. It contains 26 districts, three of them in the urban (AL-AL-Hali, Hawak and AL-Meena districts), the remaining districts are in the rural areas. Hodeidah climate is semi tropical (warm and humid in the summer and moderate in winter). The highest temperature reaches 40°C during the summer and the temperature in winter amounts to 24°C.

## 2.4 Sample Technique and Size

Representative sample size was calculated using the computer package Epi info 6 according to the registered number of Hodeidah secondary schools students for the teaching year 2014-2015. The sample was divided equally on the randomly chosen schools and the randomly chosen students were studied as members of study sample.

## 2.5 Inclusion and Exclusion Criteria

### 2.5.1 Inclusion criteria

Healthy volunteers. Age between 18 – 40 year old, accommodation in Hodeidah city, Yemen.

### 2.5.2 Exclusion criteria

Volunteers with chronic diseases (anemia, renal failure, hepatitis, HIV, malaria, bilharzias, heart disease and psychiatric problem).

## 2.6 Sample Collection

Whole blood namely 1000 (500 male and 500 female) samples were collected into EDTA anticoagulated tubes and mixed well with the anticoagulant.

## 2.7 Ethical Issues

The study was integrated within the clinical practice. Volunteers received simple explanation for the aim of the study. If agreed to participate, blood sample was collected and interview was conducted. Volunteers were reassured that this will be treated as usual. Confidentiality of the collected data was achieved by keeping data record in a locked room with limited access to the research team only.

## 3. METHODS

The materials of our study included solutions of hematological analyzer of Sysmex – version 3 (Germany), reagent of hematological analyzer (Diluent CELLPACK), WBC/ RBC lyse reagent (STROMATOLOGYSER – WH), Cell clean detergent, tube with EDTA, microscope (Olympus, Japan), slides, cover slide, platelets count chamber (Brand, Germany), sphygmomanometer, Balance, Meter (China Brand), ethanol 70%, sterile syringes, tourniquet and Gaemsa's stain. After collecting the blood samples, they were immediately examined by using Sysmex hematological analyzer for assaying of Hb, RBC, MCV, MCH, and MCHC [5,6].

### 3.1 Validation of Hematological Analyzer Method

Calibration was performed to compensate for any inaccuracies of the pneumatic, hydraulic, and electric systems which will affect analysis results. This is very important in maintaining the system accuracy, precision and linearity. For calibration, five samples of fresh normal blood were used which meets the following condition: 1) blood of a healthy person who was not taking any medicines, 2) added with EDTA anticoagulant, 3) per – sample whole blood volume to exceed 2 mL, 4) Hb value to exceed 10.0 g/dl, 5) HCT (PCV) value to be within 35.5% - 55.5%.

Reference values of calibration were used as the following: five normal blood samples were prepared for calibration of Hb and HCT and were accurately analyzed three times each in accordance with the reference method. The measurements thus obtained were used as reference values (Hb values: Cyanmethemoglobin and HCT value: Microhematocrit method). The samples were

gently mixed sufficiently and analyzed in the whole blood mode [5].

### 3.2 Assay of Erythrogram Parameters

#### 3.2.1 Principle of hematological analyzer

This instrument works in two modes: whole blood mode for adult and pre-diluted mode for child's blood. Whole blood mode was used in analyzing collected blood samples in the whole blood status. The tube cap was opened and the sample was aspirated through the sample probe one after another. It employs three detectors blocks and two kinds of reagents for blood analysis. The WBC count was measured by the WBC detector block using the direct current (DC) detection method (Data was not used for WBC). The RBC count and platelets took the RBC detector block, also using the DC detection method. The HGB detector block measures the Hb concentration using the non – cyanide hemoglobin method, HCT by Cumulative pulse height detection method [5].

#### 3.2.2 Procedure of hematological analyzer

- Collection and preparing samples: A specified 3 ml amount of venous sample, corresponding to 3 ml of EDTA anticoagulant, was collected
- Selecting whole blood mode: When the line on the side of the system status area on the LCD screen, the pre – diluted (PD) mode was in use for analysis
- Inputting sample number: The sample number was set by the incremented value for each analysis and input the sample number when changing.
- Analyzing samples: The sample was mixed sufficiently, remove the plug while taking care not to allow blood scatter, the tube was set to the sample probe, and in that condition, start switch.

### 3.3 Data Analysis

#### 3.3.1 Validation data analysis

The data obtained were treated and computed using Excel software (2010). A linear regression model was fitted (Equation 1). Accuracy was expressed in terms of % recovery (R) at five concentration levels. On other hand, precision (repeatability) is expressed in terms of percent Relative Standard Deviation (% RSD) at one

concentration levels (100%) of the validation standards.

#### 3.3.2 Routine data analysis

The differences between the females and males groups were analysed by using Excel software 2010 and statistical package social sciences (SPSS) version 15 to calculate the descriptive analysis and Z-test at  $\alpha = 0.05$  that were used to explore the reference erythrogram values in Hodeidah city, Yemen.

## 4. RESULTS

### 4.1 Characteristics of Volunteers

The background information of personal data on the 1000 samples in the adult group in Hodeidah city, Yemen was summarized in Table 1. The personal data namely age, sex, blood pressure (BP), Body Mass Index (BMI), family situation and risk factors namely (Khat chewing, smoking, coffee drinking, tea drinking, chronic disease associated) were recorded and the results showed that the age of the male included in this study between 18 to 40 years with mean of BMI 20 while in the female between 18 –40 years with mean of BMI 22.00. The BP was found to be within normal values. The persons with risk factors namely chronic diseases were excluded. The volunteers with abuse substances namely smoking, coffee drinking, and tea were within normal international range for blood indices.

**Table 1. Mean of personal data**

Parameters	Male (n= 500)	Female (n = 500)
Age (Year)	18 – 40	18 – 40
BMI (Kg/mm)	20	22
BP (mm/Hg)	120/80	120/80

*Normal Range of BP: 120/80 (mm/Hg)*

### 4.2 Validation of Haematological Analyzer Method

#### 4.2.1 Linearity

Linearity was performed for RBC count, Hb (g/dl), and PCV (%), and the coefficient correlation ( $R^2$ ) value was more than 0.9995 as a good linear method for all parameters (Table 2).

#### 4.2.2 Precision

The RSD (%) for repeatability of the blood indices namely RBC count, Hb (g/dl), and PCV (%), was presented in Table 2. The maximum RSD was less than 3% for RBC count in inter and intra – assay, and the minimum RSD was 0.1% for the PCV (%) in intra – assay.

#### 4.2.3 Accuracy

The accuracy (represented by recovery) of hematological analyzer method was determined at the target concentration levels used to construct the hematological profile in Table 2. All recoveries are within acceptable limits ( $\pm 5$ ), indicating that the method was suited for the

analysis of blood indices in adult in Hodeidah, Yemen.

#### 4.3 Erythrogram Parameters

The RBC count, Hb, PCV, MCV, MCH and MCHC levels of adults in Hodeidah, Yemen were determined by hematological analyzer and summarized in Table 3. The results showed that all the means of RBC count and related indices were within normal range of international references. In addition, the results presented showed the level of RBC count, Hb and PCV to be significantly different ( $p < 0.05$ ) between males and females. Also, all RBC count and related indices in male were more than female except MCV and MCHC were similar.

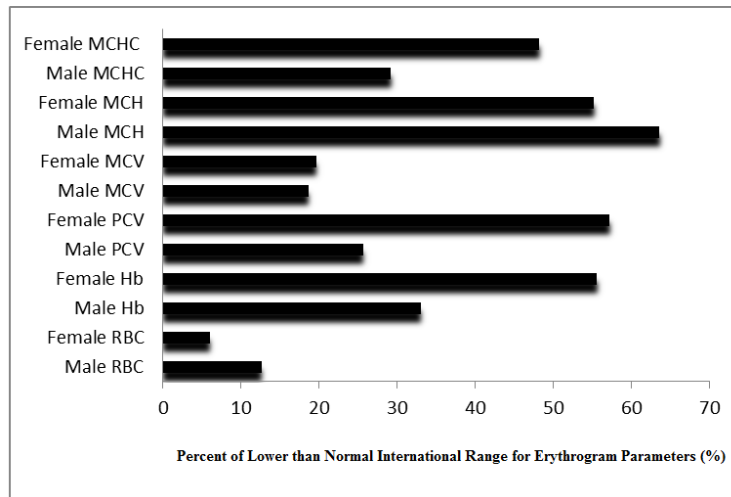
**Table 2. Validation of haematological analyzer method**

Parameters	Linearity		Precision (RSD %)		Accuracy (%)
	Range	R <sup>2</sup>	Intra – assay	Inter – assay	
RBC ( $\times 10^{12}/L$ )	0.3 – 7.0	> 0.995	< 0.3	< 3.0	2
Hb (g/dl)	0.1 – 25	> 0.995	< 0.2	< 2.0	2
PCV (%)	10 – 60	> 0.995	< 0.1	< 3.0	4

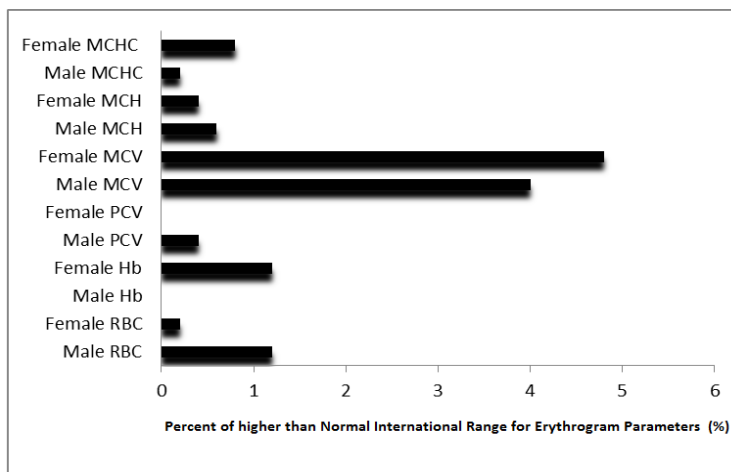
**Table 3. Results of erythrogram parameters**

Parameters	Male (n= 500) Mean $\pm$ SD	Female (n = 500) Mean $\pm$ SD	p value
RBC ( $\times 10^{12}/L$ )	5.1 $\pm$ 0.6	4.4 $\pm$ 0.4	$p > 0.05$
Median	5.1	4.4	
Rang	3.1-7.9	3.2-6.0	
Normal values	5.5 $\pm$ 1.0	4.8 $\pm$ 1.0	$p > 0.05$
Hb(g/dl )	13.5 $\pm$ 1.5	11.5 $\pm$ 1.1	
Median	13.7	11.3	
Rang	09.4 -17.0	08.2-13.9	$p > 0.05$
Normal values	15.5 $\pm$ 2.5	14.0 $\pm$ 2.5	
PCV(%)	42.4 $\pm$ 4.4	36.3 $\pm$ 3.2	
Median	43	36	$p > 0.05$
Rang	27.0 -67.0	28.0-46.0	
Normal values	47 $\pm$ 7	42 $\pm$ 05	
MCH (pg/cell)	26 $\pm$ 3	25.5 $\pm$ 2.8	$p > 0.05$
Median	26	25.9	
Rang	16.0-34.0	17.0-33.2	
Normal values	29.5 $\pm$ 2.5	29.5 $\pm$ 02.5	$p > 0.05$
MCV(fl )	82.3 $\pm$ 8.2	82.5 $\pm$ 7.2	
Median	83	83.1	
Rang	60 -102	62-101	$p > 0.05$
Normal values	85 $\pm$ 8	85 $\pm$ 8	
MCHC (g/dl )	31.4 $\pm$ 01.6	30.7 $\pm$ 01.7	
Median	31.4	31	$p > 0.05$
Rang	26-36	24-46	
Normal values	33 $\pm$ 02	33 $\pm$ 02	

pg: pigogram ; fl: femtoliter; g/dl : gram/deciliter



**Fig. 1. Percent of lower than normal international range for erythrogram parameters(%)**



**Fig. 2. Percent of higher than normal international range for erythrogram parameters (%)**

## 5. DISCUSSION

Erythrogram parameter is very important in diagnosing and following up anemia. This test helps doctors to relate any symptoms, such as weakness, fatigue, or bruising to diagnose certain conditions, such as anemia. Also, it is very important in monitoring of therapy response such as anti – anemia [7].

On the other hand , these values are affected by a number of factors even in apparently healthy populations which include age, sex, ethnic origin, body build, social, genetic disease, nutritional and environmental factors, especially altitude [8-11].

This study was conducted in Hodeidah city that is the fourth-largest city in Yemen, during the period June 2013 to May 2014. The study was

carried out to identify the hemoglobin value in adults of Hodeidah city, Yemen because of the lack of research done in this field and recorded decrease in hemoglobin in our routine work.

Findings of the present study showed that almost all the mean of blood indices of adults sampled from Hodeidah Yemen (collected randomly from healthy volunteers) were found to be within international references. These results were estimated according to direct hematological analyzer that was validated using a classical approach for the assay of hemoglobin. This approach gives enough guarantees for the results that will be generated by this method during blood analysis [12].

In addition, 33.00% and 55.6% showed lower levels in Hb of the males and females, respectively that were not consisted with

international references. These variations might be related to the incidence of infectious diseases and less food security that causes decrease in body nutrition in Hodeidah city in Yemen.

In comparison with previous studies, there are similar results were recorded with comparing with our study. Previous study in Arabic countries Saudi Arabia were carried out, both studies were within normal range of international references [13]. Also, our study was compared with developed countries "the study in United State America (USA) was carried out", the results were within normal range as the same of our study [14].

On the other hand, the mean values for Hb, RBC, PCV, MCV, MCH and MCHC were higher in males than females except PLT of males less than female. These results similar with previous studies from Asian countries such as healthy adult of Western Rajasthan, and Southern India [15,16], and the same results were recorded in Pakistan [17].

In addition, previous study was recorded in African countries such as Togo which similar to our study [18]. Other previous study in Ghana for healthy adults of blood component in the Middle Belt of Ghana were determined, this study, most hematological values in males were higher than females [19].

Also, hematological reference values for healthy adults in Port Harcourt, Nigeria were studied, all parameter of this study similar for our study except MCHC was different namely in female higher than male [20].

Finally, the results of Hematology out of range (OOR) values based on comparison with international values was compared with previous study was carried out in on Healthy adults in the Middle Belt of Ghana [19]. The similarity between both results available in Hb and related indices of both gender.

## 6. CONCLUSION

Hematological analyzer method was validated using a classical approach based on the Sysmex guideline with an aid to international conference harmonization (ICH Q 2) for validation of analytical method to determine the reference values of blood indices of Hodeidah city, Yemen. This approach gives enough assurance of future perception. In addition, the study concluded with

such a result that show most of the means of erythrogram indices were found to be within normal international ranges and the mean values were similar to the ones found in other studies performed in Africa and even in northern countries. On the other hand, erythrogram parameters namely Hb, MCH in more volunteers are out of hematological range that are usually due to some factors influencing hematological values by malnutrition and infectious disease.

## CONSENT

As per international standard or university standard, patient's written consent has been collected and preserved by the author(s).

## ETHICAL APPROVAL

It is not applicable.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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