Study of RBC Histograms and Its Correlation with Etiopathogenesis and Other Parameters in Various Anemias

A. P. Gokula Kannan¹, R. Govindarajan¹ and J. Thanka¹*

¹Department of Pathology, Sree Balaji Medical College and Hospital (Affiliated to Bharath Institute of Higher Education and Research), Chennai, Tamil Nadu, India.

Authors' contributions
This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information
DOI: 10.9734/JPRI/2021/v33i21A31369
(1) Dr. R. Deveswaran, M.S.Ramaiah University of Applied Sciences, India.
(2) Karen Cordovil, Fiocruz, Brazil.
B.Puvarajan, Veterinary College and Research Institute Orathanadu, India.
Complete Peer review History: http://www.sriarticle.com/review-history/66768

Received 25 January 2021
Accepted 31 March 2021
Published 07 April 2021

ABSTRACT

The pluripotent stem cells which are present in the bone marrow renew by its own and differentiate into mature cells. These stem cells undergo division by the presence of erythropoietin, where the nucleus is extruded out from the cell during the end of differentiation, thereby retaining cytoplasmic RNA to form a reticulocyte. The reticulocyte is a precursor to red blood cell and on losing the RNA it matures into a Red Blood Cell. The present study aimed to analyze the correlation between the automated histogram patterns along with morphological features of RBC’s prepared from peripheral smear examination in different types of anemia, viz., MCV, MCH, MCHC & RWD- CV.

Keywords: Histograms; etiopathogenesis; MCV; MCH; MCHC.

1. INTRODUCTION

Anemia is a condition in which the physiological function have neo been supplied with adequate oxygen due to the reduced number of blood red blood cells (RBC) or any hindrances in their oxygen carrying capacity. It may vary with a number of factors including age, sex, altitude,
smoking, and pregnancy status. Iron deficiency is the predominant cause of anemia occurring globally besides other important conditions particularly deficiencies in folate, vitamin A and B12 synthesis. Apart from these, chronic inflammation, parasitic infections, and inherited disorders can also cause severe anemia in people. Complete blood count (CBC) using automated analyser and microscopic peripheral smear examination are the main diagnosis methods. Complete blood count (CBC) is a routine test conducted now days to evaluate the concentration & count of various cellular components of blood such as RBC count, WBC count, platelet count, Hb, haematocrit, red cell indices, differential count( WBC). Mean platelet volume, histograms of RBC, WBC, platelets & red cell distribution width. In recent days, many of the laboratories have replaced the traditional methods in haematology by automated analysers & automated data [1]. As there is advancement in the technology of automated analyzers with increase in its precision, the rate of manual peripheral smear review have been declining [2]. RBC histogram is a symmetrical bell-shaped curve, a diagrammatic representation for better understanding & interpretation of various anemias. But it is still limited in day to day use as the technologists are unaware & only a few have understanding in correlating & interpreting [1]. Width of red blood cells, haemoglobin distribution and its width and reticulocyte cell count are the parameters that have gained popularity as they provide useful information & a less importance is given to scatter plots & histograms [3,4,5]. In spite of the latest sophisticated instruments present today, there are certain manual techniques that we still rely upon. Our study was planned & conducted with an aim to observe the relationship between BC-5380 Mindray Auto Haematology Analyzer and peripheral smear analysis, using human blood samples in the department of Pathology, Sree Balaji Medical College & hospital, Chennai.

2. MATERIALS AND METHODS

This is a prospective study conducted in the department of pathology, Sree Balaji Medical College and Hospital after getting Institutional Ethical Committee approval. Patients were sourced from the clinical departments. A total of 500 patients with anemia were studied for 24 months since October 2015 to September 2017.

2.1 Inclusion Criteria

Patients presenting with anemia with hemoglobin levels, I less than 11.5 gm%, were included for the study irrespective of patients age group.

2.2 Exclusion Criteria

Patients with a recent history of blood transfusion were excluded from the study. Samples with inadequate e quantity (< 3 ml) for analysis and study were excluded.

2.3 Method of Sample Collection

A 3 ml of venous blood sample is collected in a EDTA BD Vacationer, from all the patients that were included in the case, & was used for Automated analysis & peripheral smear. Study. The automated analysis was done using BC-5380 Mindray Auto Hematology Analyzer. It is a fully automatic 5 – part differentiation of WBC with 27 parameters, 3 histograms & 1 scatter gram. It uses laser scatter, advanced flow cytometry & chemic al dye methods. Peripheral smear study was done in all the cases simultaneously. The peripheral smear reporting was done without privy to the histogram pattern of the concerned sample.

2.4 Staining of Thin Blood Smear

Leishmans stain was used in this study for the staining of blood smear. Leishmans stain is added drop by drop to the slide and it is left to wait over a period of two minutes, which helps in fixation of the blood smear. Double the quantity of buffered / distilled water is added to the slide and mixed gently for 8 minutes. It is washed in a slow stream of running tap water and left to dry. After drying, the slide is studied under oil emersion lens of the microscope [6-9].

2.5 Statistical Data Analysis

The data collected in the study were assorted using Microsoft excel and analysis of the data was done using IBMSPSS statistics software, Pearson’ s Chi square test was used wherever appropriate.

3. RESULTS

This present prospective study was carried out in the department of Pathology, Sree Balaji Medical College & Hospital for 24 months duration started
from October 2015 to September 2017. A total of 500 cases of anemia that belonged to the inclusion criteria were studied.

### 3.1 Age of the Study Group

The study comprised of a population with the age group ranging from 1 to 93 years. Major cases of patients were between 41 to 50 years of age.

### 3.2 Sex Distribution

The majority of the study population were females which contributed 61% of the total.

### 3.3 Classification of Anaemia Based on Haemoglobin

Based on the levels of haemoglobin, anaemia is classified as Mild, Moderate & Severe.

- Mild > 10.0 gm/ dl
- Moderate, between the values of 7.1 and 9.9 gm/ dl
- Severe, < 7 gm/ dl

### 3.3.1 Distribution of cases based on peripheral smear study

Among 500 cases which comprise the whole of the study population, peripheral smear study was done for all the cases and based on the interpretation; the distribution of various anaemia was analyzed. Diagnosis based on morphology alone was carried. Cases that had predominantly microcytic hypochromic RBCs were reported as Microcytic Hypochromic anaemia, & those with macrocytic RBCs were reported as Macrocytic anaemia. Cases which had multiple populations of cells, like microcytic hypochromic RBCs with Macrocytic RBCs & Normocytic Normochromic RBCs were reported as dimorphic anaemia. Some cases had a major population of normocytic normochromic RBCs with or without microcytes or macrocytes; they were reported as Normocytic Normochromic anaemia. Out of 500 cases 361 (72.2%) cases was Microcytic Hypochromic anaemia with a majority of 233 female cases. 23 cases were reported as Macrocytic anaemia. Dimorphic anemia was interpreted in 97 cases & Normocytic Normochromic Anaemia was reported in 19 cases [10-24].

### 3.3.2 Anemia based on RBC histogram pattern

Histogram patterns of all 500 cases were studied and among this

| Peripheral Smear                     | Histogram Pattern |
|-------------------------------------|-------------------|
| Microcytic Hypochromic Anaemia      | BB 8 BM 12 LS 317 NC 24 RS 0 Total 361 |
| Macrocytic Anaemia                 | 20 0 0 0 3 23     |
| Dimorphic Anaemia                  | 36 16 24 21 0 97  |
| Normocytic                         | 0 0 3 16 0 19     |
| Normochromic                       | 64 28 344 61 3 500 |

| Table 1. Age variation of the study population |
|-----------------------------------------------|
| **Age Groups** | **Frequency** | **Percent** | **Valid Percent** | **Cumulative Percent** |
|----------------|---------------|-------------|-------------------|------------------------|
| Valid 1 - 10   | 64            | 12.8        | 12.8              | 12.8                   |
| 11 - 20        | 30            | 6.0         | 6.0               | 18.8                   |
| 21 - 30        | 71            | 14.2        | 14.2              | 33.0                   |
| 31 - 40        | 60            | 12.0        | 12.0              | 45.0                   |
| 41 - 50        | 96            | 19.2        | 19.2              | 64.2                   |
| 51 - 60        | 80            | 16.0        | 16.0              | 80.2                   |
| 61 - 70        | 62            | 12.4        | 12.4              | 92.6                   |
| 71 - 80        | 24            | 4.8         | 4.8               | 97.4                   |
| 81 and Above   | 13            | 2.6         | 2.6               | 100.0                  |
| Total          | 500           | 100.0       | 100.0             |                        |
Graph 1. Distribution of age among the study population
344 (68.8%) cases had Leftshift curve, 3 (0.6%) had Right shift curve, 64 (12.8%) cases had Broad base curve, 28 (5.6%) cases had bimodal curve, 61 (12%) had normal curve.

3.3.3 Correlation of RBC histogram patterns with peripheral smear study

The histogram patterns obtained for each case is correlated with the peripheral smear and tabulated for statistical analysis.

3.3.4 Microcytic hypochromic anaemia

Among the cases which were reported as Microcytic Hypochromic Anemia 87.8% cases showed Left shift in the histogram pattern & 6.6% showed normal curve.

3.3.5 Macrocytic anaemia

Among the cases which were reported as Macrocytic Anaemia 86.9% cases showed Broad Base curve in the histogram pattern & 13.1% showed Right shift curve.

3.3.6 Dimorphic anaemia

Among the cases which were reported as Dimorphic Anaemia 37.2% cases showed Broad Base curve in the histogram pattern & 24.7% showed Left shift curve.

3.3.7 Normocytic normochromic anaemia

Among the cases which were reported as Normocytic Normochromic Anaemia 84.3% cases showed Normal curve in the histogram pattern & 15.7% showed Left shift curve.

3.3.8 Prevalence of anemia based on Mean Corpuscular Volume (MCV)

In all the 500 samples the MCV values were recorded, & based on the values 57.6% cases showed an MCV value less than 80 f L, 36.6% showed a normal MCV range & only 5.8% case showed MCV > 100 f L.

On applying Pearson correlation between MCH of various anaemia & other parameters it shows the following interpretation. MCH in samples categorized under Microcytic Hypochromic anaemia is showing a highly significant correlation (p<0.005) to all the parameters except hematocrit. RDW- CV alone is showing a significant difference in correlation to MCH of cases in Macrocytic Anaemia. MCH in Dimorphic anaemia on correlation is showing a significant difference to haemoglobin & haematocrit. MCH of cases with Normocytic Normochromic Anaemia show a significant correlation to RBC count, haematocrit & MCV [25-41].

Chart 1. Distribution of sex among the study population
Graph 2. Age and gender wise distribution

Distribution of Anemia based on Mean Corpuscular Haemoglobin Concentration (MCHC): In all the 500 samples the MCHC values were recorded, & based on the values 60.8% cases showed an MCHC value less than 31 gm/ dl, 38.6% showed a normal MCHC range & only 30.6% case showed MCHC > 36 pg.
Chart 2. Distribution based on Haemoglobin

Graph 3. Distribution based on peripheral smear Study (gender wise)
Chart 3. Distribution of anemia based on peripheral smear study

Graph 4. RBC Histogram Pattern wise distribution
Chart 4. Distribution of histogram patterns in microcytic hypochromic anaemia

Chart 5. Distribution of histogram patterns in macrocytic anaemia

Chart 6. Distribution of histogram patterns in dimorphic anaemia
Chart 7. Distribution of histogram patterns in normocytic normochromic anaemia

Graph 5. Distribution of anemia based on MCV
Graph 6. Distribution of anemia based on MCH

Graph 7. Distribution of Anemia based on MCHC

Distribution of Anemia based on Mean Corpuscular Haemoglobin Concentration (MCHC): In all the 500 samples the MCHC values were recorded, & based on the values 60.8% cases showed an MCHC value less than 31 gm/ dl, 38.6% showed a normal MCHC range & only 30.6% case showed MCHC > 36 pg.

In dimorphic anemia the peripheral smear usually shows a dual population of microcytes and normocytes; macrocytes and normocytes; sometimes a mixture microcytes, normocytes and macrocytes. The histogram pattern varies showing a change in the centre of the curve and the width of the curve. The red cell indices may
or may not be normal. Hence diagnosis purely based on automated parameters will be sometimes misleading in this condition. Thus peripheral smear study of the morphology of red cells in correlation will help in interpreting the appropriate diagnosis. Earlier a similar finding was done where they have stated that dual population of RBCs can be identified by histograms in most cases.

### 3.3.9 Comparative analysis of various anemia’s represented by peripheral smear with red cell indices

In all the 500 cases of our study, red cell indices (MCV, MCH, MCHC) was recorded from the automated analyser BC- 5380 Mind ray which also provided the histogram. The red cell parameters were compared and correlated with the peripheral smear findings of each sample. The values of MCV were categorized as microcytic, normocytic/ dimorphic, macrocytic. Cases with the MCV value < 80 fl were labeled as microcytic. Cases that were in the normal range of MCV (8 to 100 fl) were labeled as normocytic or dimorphic. Other cases which had an MCV above 100 fl were labeled as macrocytic. A similar method of labeling was applied for the MCH values of all the cases. Microcytic - < 26 pg; normocytic/ dimorphic - 26 to 34 pg; macrocytic - > 34 pg.

On applying statistical analysis and correlation with two variables one was anemia based on peripheral smear and the other was anemia based on red cell indices. We obtained P values in the correlations in which some showed significant correlation and a few showed a significant difference. Made a study in which they stated that the MCV level was normal in 61% of anaemic patients among their study population. In our study 36.6% of the total 500 anemia cases had a normal MCV. This shows that MCV alone cannot be used as an independent sensitive parameter to classify anemia. This was studied and they proposed few probable reasons stating that MCV is a mean value and it does not represent the various red cell population present in the blood sample. MCV is insensitive when the microcytes and macrocytes are very few in number. MCH and MCHC also gives a very little information.

This concludes that all cases with decreased haemoglobin levels require a peripheral smear examination for diagnosis. In 2005 Barbara J. Bain [42] stated that in the current age of automation and even during the age of molecular analysis peripheral smear examination will remain as the most important diagnostic method and along with the latest modern investigative methods peripheral smear examination will also be in light. In comparison to peripheral blood smear examination visual examination of RBC histograms is usually more sensitive and objective in identifying the presence of group of cells that are few in population and morphologically varied in size.

![Fig. 1. Normal curve - normocytic normochromic anaemia](image-url)
Fig. 2. Peripheral smear- normocytic normochromic anaemia (Leishman stain – 1000X)

Fig. 3. Right shift histogram - Macrocytic anaemia

Fig. 4. Peripheral Smear - Macrocytic anaemia (leishman stain – 1000x)
Fig. 5. Bimodal histogram - Dimorphic anaemia

Fig. 6. Peripheral smear - Dimorphic anaemia (Leishman stain – 1000X)

Fig. 7. Left shift histogram - Microcytic anaemia
3.3.10 Red cell distribution width - a parameter for interpretation in anemia

RDW is an additional parameter provided by the automated haematology analysers nowadays. In our study majority of the cases were microcytic hypochromic anaemias by peripheral smear study. Among these cases majority had a high RDW. Anisocytosis usually gives a high RDW value. Thus RDW helps in identifying anisocytosis in conditions where the MCV is not in abnormal range, like Early Iron Deficiency Anaemia where diagnosis is difficult. In this study, previously we have discussed that cases interpreted as dimorphic anaemia had a majority of broad base histograms which explained the presence of multiple population of RBC and this is described as a high rate of anisocytosis which will automatically reflect as an increase in RDW. showed that the RDW was increased in case of microcytosis; stated that when there is a higher degree of anisopoiikilocytosis there is an increase in RDW. These studies are in concordance with our study.

4. CONCLUSION

The RBC histogram obtained from the automated haematology analyser provides valuable information in view to the diagnosis of various anemias. Only a few studies have been done on RBC histograms to reveal its importance whereas, much more importance was given to WBC histograms to bring it to light for the diagnosis of leukaemia and blast cell population. Our study was done in a purpose to identify the significance of correlation between RBC histograms and peripheral smear studies in various anemias such as microcytic hypochromic type, macrocytic type, dimorphic type and normocytic normochromic anemia type. The analysis and results showed that there is a significant correlation. However, there is a complex histogram pattern distribution in dimorphic anemia that makes the histogram pattern analysis a non-independent parameter; peripheral smear must be done in such cases as they are in good correlation with histograms. This concludes that peripheral smear examination is an important diagnostic tool even in the period of molecular and automated analysis. As a supplementary to peripheral smear examination RBC histogram and other criteria such as MCV, MCH, MCHC and RDW can be used in diagnosis of various anemias.

CONSENT

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

ETHICAL APPROVAL

The study was approved by the Institutional Ethics Committee of Sree Balaji Medical College and Hospital.

ACKNOWLEDGEMENTS

The encouragement and support from Bharath University, Chennai is gratefully acknowledged. For provided the laboratory facilities to carry out the research work.
COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Sullivan E. Hematology Analyzer: From workhorse to thoroughbred. Lab Med [ Internet]. 2006;37( 5):273 – 8 .
2. Novis DA, Walsh M, Wilkinson D, St. Louis M, Ben- Ezra J. Laboratory productivity and the rate of manual peripheral blood smear review: A College of American Pathologists Q-Probes study of 95 141 complete blood count determinations performed in 283 institutions. Archives of Pathology and Laboratory Medicine. 2006; 130. 596 – 601 .
3. Kakkar N, Makkar M. Red Cell Cytograms Generated by an ADVIA 120 Automated Hematology Analyzer: Characteristic Patterns in Common Hematological Conditions. Lab Med [ Internet]. 2009;40(9) :549 – 55 .
4. Radtke H, Meyer T, Kalus U, Röcker L, Salama A, Kiesewetter H, et al. Rapid identification of iron deficiency in blood donors with red cell indexes provided by Advia 120 . Transfusion. 2005;45(1):5–10 .
5. Marković M, Majkić-Singh N, Subota V, Mijušković Z. Reticulocyte hemoglobin content in the diagnosis of iron deficiency anemia. Clin Lab.2004;50(7–8):431 – 6 .
6. Bain BJ. Diagnosis from the Blood Smear. N Engl J Med [ Internet]. 2005;353(5):498 – 507 .
7. Wayne A. National Committee for Clinical Laboratory Standards. 2 nd ed. NCCLS Approved s tandard M 27 - PA. Villanova, PA; 1997 .
8. Benoist B de,Mc LeanE.Egill,Cogswell M. Worldwide prevalence of anaemia 1993 - 2005 . WHO Global Database on Anaemia. Worldd Preval anaemia 1993 - 2005 WHO Glob database anaemia [ Internet]. 2008 ; vi + 41 10 . Goel A, Deepak D, Gaur N. Study of relationship of tobacco smoking with haemoglobin concentration in healthy adults. J Pharm Biomed Sci. 2010;1(19 ).
9. Henry Foy, M. LayrisseMM. WHO Technical Report Series on Iron Deficiency Anaemia. Geneva; 1959 .
10. Who, Chan M. Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. Geneva, Switz World Heal Organ [ Internet]. 2011;1 – 6 .
11. ICMR. Indian Council of Medical Research. Evaluation of the National Nutritional Anaemia Prophylaxis Programme. Task Force Study. 1989 ;
12. Panel E. Recommendation for reference method for determination by centrifugation of packed cell volume of blood. International Committee for Standardization in Haematology Expert Panel on Blood Cell Sizing. J Clin Pathol[ Internet]. 1980;33( 1):1 – 2 .
13. Coulter WH. Meansfor counting particles suspended in a fluid 2656508. pdf. Chicago; 1953 .
14. CoulterWH.Highspeedautomaticblood cell counter and s ize analyzer. Proc Natl Electron Conf. 1956;1034 – 1040 .
15. Brecher G, Schneiderman M,Williams Gz. Evaluation of electronic Red Blood Cell Counter. Am J Clin Pathol. 1956; 26(12): 1439 – 49 .
16. Richar WJ, Breakell ES. evaluation of an electronic particle counter for the counting of white blood cells. 2017;31( 5):384–93 .
17. Grant Ji, Britton Mc Jr Kt. Measurement of red blood cell volume with the electronic cell counter. Am J Clin Pathol. 2020;33 : 138 - 43 .
18. Feichtmeir Tv, Nigon K, Hannon Ma, Bird Db Cl. Electronic counting of erythrocytes and leukocytes. ots rd tr. Hydrochloric acid for stromatolysis of erythrocytes in Coulter leukocyte counting. Tech Bull Regist Med Technol. 2021;31 :49 - 52 .
19. Hatch A, Balazs T. the use of cetavlon in a diluents for counting leukocytes in the Coulter electronic counter.Acomparison with some currently use diluents. AmJ Clin Pathol. 1961;36:220 – 3 .
20. D’ ANGELO G LM. A practical diluent for electronic white cell counts. Tech Bull Regist Med Technol. Prudene which. Accuracycontrol of blood cell counts with the coulter counter. Am J MedTechnol.1964;( 30 ) :1 – 35 .
21. WISECUP WG CB. Evaluation and calibration of an electronic particle counter for enumeration of multispecies blood cells. Am J Clin Pathol. 1963;39:349– 54 .
22. Maeda H et al. Experiences With The Use Of The Coulter Counter, Model A. PMID 14201625. 1964;(12):294 – 300.

23. Thom R. Method and results by improved electronic blood cell sizing - Modern concept in hematology. 1972 191 - 199.

24. Thom R. Measurement blood cell size distribution using hydrodynamic focusing system. Sysmex J. 1985;8:43 – 56.

25. Lokwani DP. The ABC of CBC: Interpretation of Complete Blood Count and Histograms [ Internet]. The ABC of CBC: Interpretation of Complete Blood Count and Histograms. 2013:8.

26. Tatsumi N, Tsuda I, Akira F, Takubo T, Hayashi M, Matsumoto H. Principle of blood cell counter - Development of Electric Impedance Method. Sysmex J Int. 1999;9 (1): 8 – 20.

27. Cook JD, Finch CA, Smith NJ. Evaluation of the iron status of a population. Blood. 1976;48 (3): 449 – 55.

28. SP. Iron Deficiency Anemia in moderate to severely anaemic patients. pdf [ Internet]. 2009.

29. Yogender P, Sujatha R, Rangaswamy R, Sreekantha and AS. The Study of Iron Related Parameters in Iron Deficiency Anaemia in Pregnancy. RJPBCS. 2014;4 (5):980.

30. De Maeyer E, Adiels - Tegman M. The prevalence of anaemia in the world. World Health Stat Q [ Internet]. 1985;38(3):302 – 16.

31. Micronutrient deficiency: Battling iron deficiency anaemia: the challenge. WHO. 2004.

32. Sultan AH. Anemia among female college students attending the University of Sharjah, UAE: prevalence and classification. J Egypt Public Heal Assoc [ Internet]. 2007;82(3–4):261 – 71.

33. Krause JR, Costello RT, Krause J PL. Use of the TechniconH- 1 in the characterization of leukemias. Arch Pathol Lab Med. 1988;112( 9 ):889 – 94.

34. Bessman JD, Gilmer PR, Gardner FH. Improved classification of anemias by MCV and RDW ( Bessman et at. - Am Soc Clin Pathol- 198 ) 3 . Am Soc Clin Pathol. 1983;80( 3 :322 – 6.

35. Richard A. Savage M. What i s the significance of twodistinct RBC populations as ide from the obvious t ransfusion? We recently had a CBC RBC histogram with two distinct population peaksand the patient had not been t ransfused in the last year. Captodayonline; 2005.

36. Rees MI, Worwood M, Thompson PW, Gilbertson C, May A. Red cell dimorphism in a young man with a constitutional chromosomal translocation t Br J Haematol [ Internet]. 1994;87( 2 ):386 – 95.

37. Bessman D. Erythropoiesis during recovery f rom iron deficiency: normocytes and macrocytes. Blood: [ Internet]. 1977; 50 (6):987 – 93.

38. Bessman D. Erythropoiesis during recovery f rom macrocytic anemia: macrocytes, normocytes, and microcytes. Blood [ Internet]. 1977;50 (6):995 – 1000.

39. Bessman JD, Banks D. Spurious macrocytosis, a common clue to erythrocyte cold agglutinins. Am J Clin Pathol. 1980; 74 (6):797 – 800.

40. Aslinia F, Mazza JJ, Yale SH. Megaloblastic anemia and other causes of macrocytosis. Clinical Medicine and Research. 2006; 4236 – 41.

41. Rowan RM, Fraser C, Gray JH, Mc Donald GA. The Coulter Counter Model S Plus -- the shape of things tocome.Clin Lab Haematol [ Internet]. 1979;11(1):29–40.

42. Hoff brand A; PAHM. Essential Haematology. Verwilghen RL. Recommendations for reference method for haemoglobinometry in human blood ( ICSH s tandard 1986) and specifications for international haemoglobin bincyanide reference preparation (3 rd edition): International committee for standardization in haematology; Expert Clin Lab Haematol. 1987; 9 (1):73 – 9.

© 2021 Kannan et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/66768