journal homepage: https://jablm.acclmp.com/

Journal of Applied Biochemistry & Laboratory Medicine (2023) 04 (1):5-9

Original Article

Establishment of diagnostic cut off values of MCV, Fetal hemoglobin and Red Cell Width Distribution for HbE disease and HbE trait.

Biswas Teresa[1*], Thakuria Ghanashyam[2]
1.Laboratory Director & Consultant Biochemist, Bioscan Diagnostics, Guwahati, Assam.
2. Consultant Pathologist, Bioscan Diagnostics, Guwahati, Assam.

Keywords:
HbE disease, HbE trait, Fetal hemoglobin, mean corpuscular volume (MCV), Mean corpuscular hemoglobin concertration (MCHC), Red cell distribution width (RDW).

Article Cycle
Received: 2nd March, 2023
Editorial Review: 10th March, 2023
Peer review: 25th May, 2023
Accepted: 23rd June, 2023

How to cite this article
Biswas T, Thakuria G. Establishment of diagnostic cut off values of MCV, Fetal hemoglobin and Red Cell Width Distribution for HbE disease and HbE trait. Journal of Applied Biochemistry & Laboratory Medicine 2023; 04 (1):5-9.
Access link for this article
https://jablm.acclmp.com/2022/04/01_5-9.pdf

ABSTRACT

Background:
Hemoglobin E variant (HbE) is world's one of the commonest hemoglobin variant. HbE disorder and its trait variety become more lethal when it is co-existent with beta thalassemia or alpha thalassemia respectively and these combinations can lead to substantially increased death rates in patients due to cardiovascular effects. However, as far routine hematological investigations are concerned, HbE disease and HbE trait cannot be well differentiated by most of the routine hematological investigations.

Methodology:
The present study was designed and undertaken to assess the possibility to find out any hematological parameter that can be used to differentiate between the HbE disease and its trait status with maximum possible significant sensitivity and specificity. After fulfilling the inclusion and exclusion criteria routine hematological parameters i.e. MCV, Hb, MCHC, RDW and FHb were assessed in 66 HbE disease and 190 HbE trait patients. Routine hematological parameters were measured in them and compared using independent t test. An ROC analysis was done for each parameter between the two groups for obtaining an ideal cut off value for each with maximum sensitivity and minimum false positivity.

Results:
Values of MCV, Hb and FHb were significantly higher in the HbE disorder patients with FHb showing the highest significance of difference in the independent t test. No significant difference was observed in the values of MCHC. Results of ROC analysis showed that most optimum cut off values between the two states were observed for MCV, FHb and RDW at the levels of 65.7 fL, 2.75 % and 14.7 % respectively.

Conclusion:
The diagnostic cut off values of MCV, FHb and RDW can be used effectively for differentiating between the HbE homozygous (diseased state) and its trait condition (heterozygote state) for which the clinical features and routine hematological features overlap significantly.

INTRODUCTION

The inherent property that confers resistance to Plasmodium falciparum makes hemoglobin E (HbE) as world's one of the commonest hemoglobin variant[1]. Particularly, in the North Eastern part and the state of Bengal of India and some parts of South East Asia, its prevalence is substantially high[2-4]. In Orissa state of India, its prevalence is found to be about 1.4 percent in some tribal areas[5]. In Thailand, Laos and Cambodia the prevalence of HbE disease is upto 60 percent in some areas[6]. HbE disorder and its trait variety become more lethal when it is co-existent with beta thalassemia or alpha thalassemia respectively. These combinations can lead to substantially increased death rates in patients due to cardiovascular effects.

The genetic basis of HbE is the replacement of G by A at the 26th codon of the beta chain resulting into substitution of glutamate by lysine. Heterozygotes for this mutation are represented as HbE trait. The resulting mutation results in a suboptimal interaction at the alpha beta interface leading to increased instability during stressful conditions including oxidative stress. But genetic factors are not sufficient alone to explain the variable clinical outcome of HbE as people with similar mutations for HbE gene have been found to exhibit different clinical manifestations altogether.

*Correspondence:
Dr. Teresa Biswas, Laboratory Director & Consultant Biochemist,
Bioscan Diagnostics, Guwahati, Assam
Email: teresabiswasguha@gmail.com

Unlike many hemoglobinopathies, the natural history of HbE disease is widely diverse and it varies from totally asymptomatic to completely transfusion dependent[7]. However, HbE trait disease has only mild microcytic anemia that is often indistinguishable from iron deficiency anemia and needs definite hematological investigations for definite diagnosis.

However, as far routine hematological investigations are concerned, HbE disease and HbE trait cannot be well differentiated by most of the routine hematological investigations. Hemoglobin estimation has shown a significant overlap between HbE and its trait showing little or no change. The diagnostic parameters become more regressed towards normal pattern in HbE traits except showing mild iron overload. Both of these disorders are associated with microcytosis, target erythrocytes, insignificant changes in reticulocyte count with no or little anemia[8]. In some studies, no significant difference could be found in the mean values of Hb, MCV and MCHC between the HbE homozygotes and traits. Only the mean values of red cell distribution width (RDW) and Fetal hemoglobin showed significantly increased values in the HbE homozygotes[7]. In some other studies, significant differences between all parameters of Hb level, MCH, MCV, MCHC, RDW and fetal hemoglobin were found between the HbE homozygotes and traits[9]. But, the definite investigational parameter that can differentiate between these two disorders conclusively is the difference in the elution patterns of these two variants by HPLC technique. However, HPLC is costly and is not available in most of the clinical labs which run under middle to low financial set ups, whereas the routine blood tests are available in all of them. As the differences between the mean values of these routine hematological parameters between the HbE disease and its trait form are very subtle and overlapping, we aimed in our present study to find out any potential cut off value of these parameters that could differentiate these two closely related hemoglobin disorders with highest possible sensitivity and specificity in a routine laboratory set up.Our research question was to find out whether these cut off values can be established using the standard receiver operator characteristic curves (ROC) from sufficient number of data from the hematological investigations obtained under routine laboratory set up. We hypothesized that suitable cut off values can be detected for some of these parameters using the ROC curve analysis. Hence, the present study was designed and undertaken to assess the possibility to find out any hematological parameter that can be used to differentiate between the HbE disease and its trait status with maximum possible significant sensitivity and specificity.

Methodology:
The present non interfering observational study was carried out in a tertiary level diagnostic laboratory in Guwahati, Assam, India during a six month period of April 2022 to October 2022. Routine hematological parameters were determined in the patients suspected to be suffering from hemoglobin related disorders as evident from their clinical history and examination. Values of hemoglobin concentration, MCV, MCH, MCHC and RDW were measured by standard protocols using automated 5 part cell counters for the patients diagnosed as HbE homozygotes or HbE traits. Fetal hemoglobin was measured using the HPLC technique. Mean values for all parameters of both groups were ascertained. All cases attending the laboratory for blood investigations and/or diagnostic evaluation for hemoglobin related disorder were selected during a period of 6 months following certain inclusion and exclusion criteria.

Sample size calculation: All samples fulfilling the exclusion and inclusion criteria were selected using the method of convenience during the study period.

Inclusion criteria: Patients belonging to all age group were selected. Both male and female subjects were considered.
Exclusion criteria: Patients receiving blood transfusion for any purpose within last 4 months were not included. Patients suffering from any other hemoglobinopathies were excluded from the study. Patients suffering from other causes of anemia like iron deficiency, pregnant population, and individuals receiving any long term treatment for any purpose and patients suffering from any chronic inflammatory disorder, malignancy, metabolic and endocrinological disorders were not considered for the study.

Methodology: After fulfilling the selection criteria, the hematological parameters were measured by automated 5 part cell counter. Hemoglobin fractions were estimated by HPLC using Bio-Rad D10 HPLC analyzer.

Data analysis: Data obtained during the study period were analysed for their mean values by the independent t test after ascertaining their normal distribution pattern using the Smirnov Kolmogorov test. We further determined the cut off values of each hematological parameters between the HbE disease and HbE traits with maximum possible sensitivity and minimum false positivity using the receiver operator characteristic curve (ROC). All statistical analysis were performed using the SPSS software 20 for Windows. In all statistical analyses a p value of ≤ 0.05, was considered to be statistically significant.

Results: The present study was performed in 66 HbE homozygotes and 190 HbE trait patients after following the inclusion and exclusion criteria. As Smirnov Kolmogorov tests showed that the data followed normal distribution pattern so the independent sample t test was done to ascertain the significance of mean difference of study parameters between the two groups (Table 1).

Table 1: Independent sample t test for the hematological parameters between the HbE disease and trait.

	HbE disease (n = 66)	HbE trait (n = 190)	Mean difference	2 tailed significance (P value)
MCV, fL (Mean ± SD)	61.01 ± 4.39	72.92 ± 7.81	-11.91	<.001**
MCHC, g/dL (Mean ± SD)	33.37 ± 3.17	33.57 ± 2.12	-.19	.567
Hb, g/dL (Mean ± SD)	9.72 ± 1.48	10.24 ± 1.68	-.52	.027*
FHb, % (Mean ± SD)	4.76 ± 2.89	1.32 ± .97	3.44	<.001**
RDW, % (Mean ± SD)	16.51 ± 3.76	14.23 ± 2.14	2.28	<.001**

P value is considered to be significant at P <0.05
** Significant*

*** Highly significant.*

Table 1 showed significantly lower values of MCV (P<.001) and Hb (P=.027)) in the HbE disease patients in comparison to the traits. On the other hand, values of FHb (foetal haemoglobin), and RDW showed significantly higher values in the HbE disease (P<.001). No significant difference was observed between the values of MCHC between the two diseased states.

To continue the data analysis further, for obtaining a potential cut off value of these parameters that could help in differentiating the HbE disease from HbE trait, ROC curve was obtained for each the results of which have been illustrated in Figure 1 and 2 and their cut off values with corresponding sensitivity and false positivity have been shown in the Table 2. Our aim was to obtain maximum specificity (minimum false positivity) against an optimum sensitivity for each parameter to exclude the effect of overlapping of corresponding data between the HbE and its trait.

It is evident from these figures and table that the parameters having maximum area under curve (AUC), a good sensitivity and minimum false positivity were fetal hemoglobin and MCV with a corresponding value of 2.75 and 65.7 respectively. RDW also complied with these criteria albeit with a little more false positivity at its most optimum value of 14.7. On the other hand, the parameters like Hb and MCHC could not meet any such optimum criteria as they failed to have a good AUC, sensitivity and specificity for any corresponding value on the ROC curve.

Figure 1: Receiver operator characteristic curve to detect any potential cut off value of Hemoglobin concentration (Hb) and MCV between the HbE trait and Homozygote patients with maximum sensitivity and minimum false positivity.

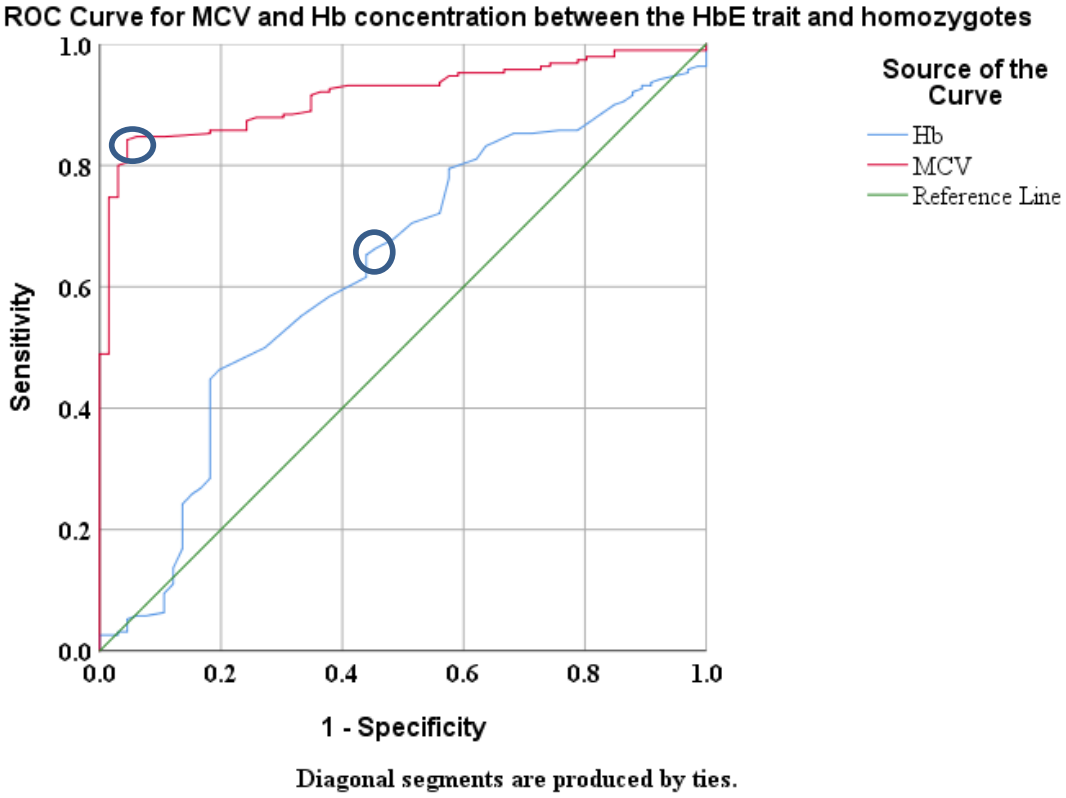

Figure 2: Receiver operator characteristic curve to detect any potential cut off value of fetal hemoglobin concentration (FHb), MCHC and red cell distribution width (RDW) between the HbE Homozygote and trait patients with maximum sensitivity and minimum false positivity.

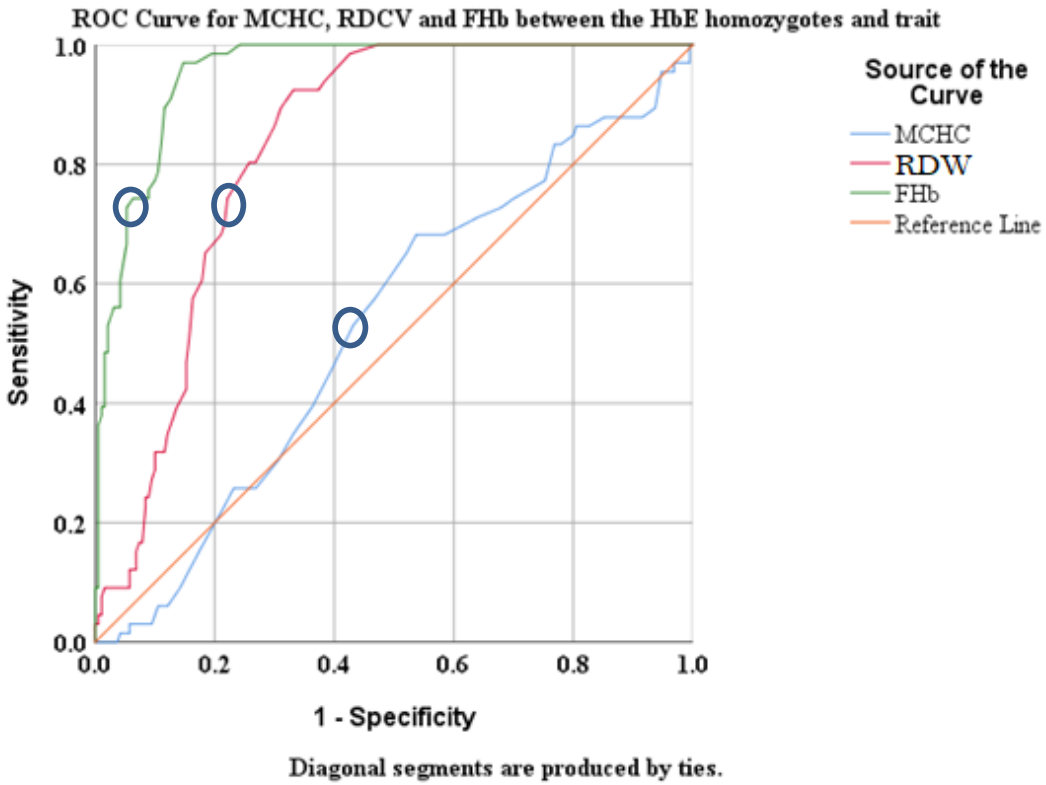

Table 2: ROC measurements for different hematological parameters.

	Area under curve (AUC)	Sensitivity	False positivity (1-specificity)	Corresponding value
MCV*	**0.916**	**0.842**	**0.045**	**65.7 fL**
MCHC	0.529	0.530	0.432	34.0 g/dL
RDW*	**0.829**	**0.742**	**0.221**	**14.7 %**
FHb*	**0.954**	**0.727**	**0.05**	**2.75 %**
Hb	0.626	0.653	0.439	9.8 g/dL

* Parameters showing optimum ROC analysis characteristics.

Discussion:

Results of the Table 1 and 2 indicate that the hematological values like blood hemoglobin concentration, fetal hemoglobin levels, MCV and RDW were significantly different in the HbE diseased patients in comparison to the HbE traits. MCHC values did not show any significant difference between these two states in the present study. It has been found in other studies that although there is RBC hypochromia and anemia in HbE disease, they are rarely found in the HbE trait. Decreased synthesis of the beta E mRNA due to a mutated codon 26 in HbE homozygous cases reduces the synthesis of globin chain resulting in mild to severe anemia [10, 11]. However, the effects of this mutation are minimal or absent in the HbE traits due to heterozygosity and that explains minimal or no anemia in these cases. Mild to moderate anemia in the HbE trait generally indicates an associated hemoglobin disorder like alpha thalassemia or iron deficiency anemia. As we excluded other hemoglobin related disorders for the present study, a significantly lower level of hemoglobin concentration in the HbE diseased patients in comparison to those with the HbE trait forms is justified. But as the range of hemoglobin in HbE varies widely from 3 to 13 g/dl[12], it shows significant overlap with that of HbE trait patients and so cannot elicit a suitable cut off value that can delineate both these disorders separately with optimum sensitivity and specificity. An AUC of 0.626, a sensitivity of 0.653 and a false positive value of 0.439 at its most optimum cut off value of 9.8 g/dl as obtained from our ROC curve analysis (Figure 1 and Table 2) justify this fact that hemoglobin concentration is not a suitable cut off indicator to differentiate between these two disorders.

On the other hand, FHb values that showed a significantly higher value in HbE disease (Table 1), also revealed an AUC of 0.954, sensitivity of 0.727 and a false positivity of 0.05 (Table 2) at the cut-off point of 2.75 as indicated by the ROC curve (Figure 2). These data suggested that FHb can be used as an excellent cut off marker for differentiating HbE disease and its heterozygous trait disorder. It has been reported that reactivation of the gamma genes in the beta globin gene cluster occurs in cases of HbE homozygotes that leads to an average fetal hemoglobin concentration > 4 g/dl in these

patients[13]. Due to this persistent reactivation of the gamma genes, levels of fetal hemoglobin show a definite increase in the HbE homozygotes with minimum overlapping with the HbE heterozygotes who show minimally elevated FHb values. Data in the Table 1 of our present study show the mean value of FHb as 4.76 and 1.32 g/dl in the HbE homozygotes and traits respectively which complies well with other studies. This definite and distinctive increase in FHb levels in HbE disease in comparison to HbE traits with minimum overlapping in between have enabled this parameter as an optimum cut off marker between these two conditions which is shown in our present study.

The role of MCV as a good cut off marker has also been reported in our present study. In contrast to the MCHC that showed a poor AUC (0.529), sensitivity (0.530) and specificity (Table 2) and thus failed to comply with the ideal criteria for a good cut off value, the optimum value of 65.7 fl of MCV showed a much better compliance as a good discriminating indicator with a high AUC of 0.916, sensitivity of 0.842 and a false positivity of 0.045. Our findings corroborate with reports of some other studies which found that at a value of 69 fl, MCV could discriminate well the mild thalassemia conditions like HbE disorders[14].

The red cell distribution width or RDW signifies the coefficient of variation of red blood cell volume distribution that also indicated their size. RDW has been found to be increased significantly in thalassemia and a cut off value of 15.15 has been reported to be used as a screening marker for thalassemia[15]. In our present study, we have successfully determined the diagnostic cut off value for RDW at a level of 14.7 that can successfully differentiate between the HbE disease and its trait condition with an acceptable AUC of 0.83, sensitivity of 0.74 and a false positivity as low a 0.22 (Figure 2, Table 2).

Conclusion:

In conclusion, the present study helps substantially in determining the diagnostic cut off values of some routine hematological parameters for differentiating between the HbE homozygous (diseased state) and its trait condition (heterozygote state) for which the clinical features and routine hematological features overlap significantly. The outcome of the present study conclusively indicate that the diagnostic cut off values MCV, FHb and RDW can be successfully used to differentiate between the HbE diseased and HbE trait condition in supplementation to HPLC or in its absence.

Acknowledgement: Nil

Conflict of interest: Nil

References:

1. Chotivanich K, Udomsangpetch R, Pattanapanyasat K, Chierakul W, Simpson J, Looareesuwan S, et al. Hemoglobin E: a balanced polymorphism protective against high parasitemias and thus severe P falciparum malaria. Blood. 2002;100(4):1172-6Available from: https://www.ncbi.nlm.nih.gov/pubmed/12149194.

2. Rees DC, Styles L, Vichinsky EP, Clegg JB, Weatherall DJ. The hemoglobin E syndromes. Ann N Y Acad Sci. 1998;850:334-43Available from: https://www.ncbi.nlm.nih.gov/pubmed/9668555.

3. Lukens JN. Abnormal hemoglobins: General principles. In:Greer JP, Foerster J, Lukens JN, editors. Wintrobe's clinical hematology. December, 11th ed. Lippincott Williams & Wilkins Publishers; December 2003. p 1247–1262.

4. Beutler E. The sickle cell diseases and related disorders. In:Beutler E MD, Lichtman MA MD, Coller BS MD, Kipps TJ MD PhD, Seligsohn U MD, editors. Williams hematology. 6th ed. McGraw-Hill Professional; November 28, 2000. p 581–606.

5. Balgir RS. The spectrum of haemoglobin variants in two scheduled tribes of Sundargarh district in north-western Orissa, India. Ann Hum Biol. 2005;32(5):560-73Available from: https://www.ncbi.nlm.nih.gov/pubmed/16316913.

6. Weatherall DJ, Clegg JB. Inherited haemoglobin disorders: an increasing global health problem. Bull World Health Organ. 2001;79(8):704-12Available from: https://www.ncbi.nlm.nih.gov/pubmed/11545326.

7. Vichinsky E. Hemoglobin e syndromes. Hematology Am Soc Hematol Educ Program. 2007:79-83Available from: https://www.ncbi.nlm.nih.gov/pubmed/18024613.

8. Lukens JN. Abnormal hemoglobins: General principles. In: Greer JP, Foerster J, Lukens JN, editors. Wintrobe's clinical hematology. December, 11th ed. Lippincott Williams & Wilkins Publishers; December 2003. p 1247–1262.

9. Kishore B, Khare P, Gupta RJ, Bisht S, Majumdar K. Hemoglobin E disease in North Indian population: a report of 11 cases. Hematology. 2007;12(4):343-7Available from: https://www.ncbi.nlm.nih.gov/pubmed/17654063.

10. Orkin SH, Kazazian HH, Jr., Antonarakis SE, Ostrer H, Goff SC, Sexton JP. Abnormal RNA processing due to the exon mutation of beta E-globin gene. Nature. 1982;300(5894):768-9Available from: https://www.ncbi.nlm.nih.gov/pubmed/7177196.

11. Traeger J, Wood WG, Clegg JB, Weatherall DJ. Defective synthesis of HbE is due to reduced levels of beta E mRNA. Nature. 1980;288(5790):497-9Available from: https://www.ncbi.nlm.nih.gov/pubmed/7442796.

12. Fucharoen S, Ketvichit P, Pootrakul P, Siritanaratkul N, Piankijagum A, Wasi P. Clinical manifestation of beta-thalassemia/hemoglobin E disease. J Pediatr Hematol Oncol. 2000;22(6):552-7Available from: https://www.ncbi.nlm.nih.gov/pubmed/11132229.

13. Ruangrai W, Jindadamrongwech S. GENETIC FACTORS INFLUENCING HEMOGLOBIN F LEVEL IN beta-THALASSEMIA/HB E DISEASE. Southeast Asian J Trop Med Public Health. 2016;47(1):84-91Available from: https://www.ncbi.nlm.nih.gov/pubmed/27086429.

14. Nuchprayoon I, Sukthawee B, Nuchprayoon T. Red cell indices and therapeutic trial of iron in diagnostic work-up for anemic Thai females. J Med Assoc Thai. 2003;86 Suppl 2:S160-9Available from: https://www.ncbi.nlm.nih.gov/pubmed/12929984.

15. Song QL, Guo YL, He YJ, He C, Zhang T, Cai Y, et al. [Diagnostic Cut-Off Value of RDW for Screening Thalassemia and the Combined Determination of MCV, MCH, HBA (2) and RDW]. Zhongguo Shi Yan Xue Ye Xue Za Zhi. 2021;29(3):847-52Available from: https://www.ncbi.nlm.nih.gov/pubmed/34105482.