Original Article

Biological Reference Intervals for Hematological Parameters Including Novel Research Parameters in Population of Eastern India

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Background : Biological Reference Intervals (BRI) are used for a comparative decision-making process by describing the distribution of results derived from healthy reference population. Biological reference intervals should be derived from the local population to take into account regional variations.

Aims and Objectives : Our aim was to establish a biological reference interval for routine hematological parameters along with novel hematology parameters from health check-up of young working in-house staff of our hospital.

Materials and Methods: This is a cross sectional study carried out in our hospital using samples collected for routine health check-up of hospital staff using the Mindray BC-6800 Plus automated analyser from December, 2022 to June, 2023.

Results : A total of 474 male participants and 464 female participants were included and reference intervals for reportable and non-reportable novel hematologic parameters established.

Discussion : The study has established comparability of parameters with other Indian and International studies and shows difference in values from other population in India specifically for platelet parameters supporting the findings of Inherited Macrothrombocytopenia. We have also established the reference ranges of additional Novel Research hematological parameters with increasing utility in modern hematology practice and intend to study them further for their utility in diagnostic and therapeutic management of patients.

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Key words **: Biological reference ranges, Inherited Macrothrombocytopenia, Novel hematological parameters.**

Biological reference intervals (BRI) are used for a comparative decision-making process by describing the distribution of results derived from healthy reference population. The values aid in the interpretation of laboratory results for patient care[1-3]. Most common parameter used by clinicians for management, diagnosis and monitoring of their patients is a CBC[3].

Hematologic interpretations rely on the BRI derived from population to be served. Although age and sex are the two most common categorizing criteria for BRI, variations are also seen during physiological conditions like pregnancy or exercise[4-6].

Only few clinical laboratories have the resources to establish BRIs as they require data from healthy local population. As reference values in-use are used from authoritative textbooks or western references[5], adopting from dissimilar population without considering local ethnicity may lead to mismanagement with increase in cost and risk patient safety. Therefore the need to establish hematological BRIs in the eastern population of the country.

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Editor's Comment :

- The eastern population is ethnically different from other population in our country especially in platelet parameters which is also called as Bengal Macrothrombocytopenia. So, the need to establish reference ranges in our population for routine parameters as well as for many novel hematology parameters available in our analyser, so as to use them as reference points for further studies in this field.

Aims and Objectives

Aims : To establish a biological reference interval for routine hematological parameters from blood samples collected from yearly health checkup of young working (<40 years) in-house population of our hospital.

Objectives : Establish biological reference interval for reportable hematologic parameters (WBC, Neu%, Lym%, Mon%, Eos%, Bas%, RBC, HGB, HCT, MCV, MCH, MCHC, RDW-CV, RDW-SD, PLT, MPV, PDW, PCT) along with novel research hematological parameters available for evaluation (P-LCC, P-LCR, HFC#, HFC%, WBC-D, WBC-N, TNC-D, TNC-N, NLR, Micro#, Micro%, Macro#, Macro%, PLT-I, PLR, PDW-SD, Neu-X, Neu-Y, Neu-Z, Lym-X, Lym-Y, Lym-Z, Mon-X, Mon-Y, Mon-Z) for reference for future studies.

Materials and Methods

(1) A cross sectional study was carried out in our hospital of apparently healthy adults of both sexes of hospital staff from December, 2022 to June, 2023.

(2) **Inclusion into the study was based on the following criteria :** aged <40 years adults; subject to health check up with normal records of vital signs and physical examination.

(3) **Exclusion criteria :** age >40 years, having co-morbidities.

(4) As per CLSI recommendation[6] a sample size of minimum of 120 participants is required. Total of 938 persons, 474 males and 464 females were recruited for the study.

(5) **Sample collection :** 3-ml venous blood was collected from antecubital vein under aseptic conditions into Dipotassium Ethylene Diamine tetra acetic acid (K_2EDTA) Vacutainer tube (Becton Dickinson, PL6 7BP, UK) for hematological analysis.

(6) **Laboratory assays :** The whole blood EDTA samples were analysed within two hours using Mindray BC-6800 Plus automated hematology analyzer (Mindray Bio-medical electronics Co, Ltd, Shenzhen, China), for both reportable (for in-vitro diagnostic use in clinical laboratories) and non-reportable/ research use hematologic parameters.

Reportable parameters : The parameters used in this study are white Blood Cell Count (WBC), Neutrophil percentage (Neu%), Lymphocyte percentage (Lym%), Monocyte percentage (Mon%), Eosinophil percentage (Eos%), Basophil percentage (Bas%), Red Blood Cell Count (RBC), Hemoglobin (HGB), Hematocrit(HCT), Mean Cell Volume (MCV), Mean Cell Hemoglobin (MCH), Mean Cell Hemoglobin Concentration (MCHC), Red Cell Distribution Width-Coefficient of Variation (RDW-CV), Red Cell Distribution Width-Standard Deviation (RDW-SD), Platelet Count (PLT), Mean Platelet Volume (MPV), Platelet Distribution Width (PDW), Plateletcrit (PCT).

Non-reportable/Research Use only parameters: Platelet-Large Cell Count (P-LCC), Platelet-Large Cell Ratio (P-LCR), High Fluorescent Cell percentage (HFC%), White Blood Cell Count -DIFF (WBC-D), White Blood Cell Count-WNB (WBC-N), Total Nucleated Cell Counts-DIFF (TNC-D), Total Nucleated Cell Counts-WNB (TNC-N), Neutrophil-to-lymphocyte Ratio (NLR), Microcyte percentage (Micro%), Macrocyte percentage (Macro%), Platelet-to-lymphocyte ratio (PLR), Platelet Distribution Width Standard Deviation (PDW-SD), mean neutrophil distribution-side scatter intensity (Neu-X), Neutrophil distribution-side fluorescent light intensity (Neu-Y), mean neutrophil distribution- forward scatter intensity (Neu-Z), mean Lymphocyte distribution- side scatter intensity (Lym-X), mean Lymphocyte distribution-side fluorescent intensity (Lym-Y), mean Lymphocyte distribution- forward scatter intensity (Lym-Z), mean monocyte distribution-side scatter intensity (Mon-X), mean Monocyte distribution-side fluorescent light intensity (Mon-Y), mean Monocyte distribution - forward scatter intensity (Mon-Z).

The principles used by the analyzer for measurement are :

• Sheath flow impedance method, laser scatter and SF Cube cell analysis technology (3D analysis using information from scatter of laser light at two angles and fluorescence signals) for cell differentiation and counting;

• Colorimetric method for HGB measurement.

Well-trained experienced laboratory personnel performed all tests according to Standard Operating Procedures (SOPs).

(7) **Quality control :** The analyzer is calibrated annually and daily Internal Quality Control (IQC) run as per QC protocol with 3 level (low, normal and high) commercially available controls with daily monitoring using LJ charts and Westgard rules. Laboratory is also enrolled in a proficiency testing program for hematology.

(8) **Statistical analysis :** Quantitative data has been represented as mean & SD and qualitative data has been represented as percentages. 97.5 percentile and 2.5 percentile formed the upper and lower limit of reference range respectively of the population. The biological reference interval is Mean±2*SD for those parameters whose p-value for normality test (Shapiro Wilk's test) is greater than 0.05 (p-value>0.05). The group comparisons were based either on parametric or non-parametric statistics depending on whether the distribution is normal or not. For comparison of each of the parameters between two groups (across males and females), the non-parametric Mann-Whitney test is being used.

Results

Demographic characteristics: A total of 474male participants and 464 female participants were included in the study.

Table1 shows the reference intervals for reportable and non-reportable novel hematologic parameters of our study population.

Table 2 shows comparison between our study and other Indian and International studies.

Figs 1-3 show comparative representation of 3 key parameters between various studies.

Discussion

Biological reference ranges for WBC counts across all Indian and International studies are comparable with appropriate differential counts (Fig 1).

The Hemoglobin reference ranges are comparable with the Indian multi-centric study[7] but slightly less than the international study values especially the lower

Table 1 — *Biological Reference ranges of Males and Females in Eastern Indian Population – Reference range (Mean± 2*SD) and Percentile (2.5 - 97.5). Mann-Whitney test for 2 groups used for comparison*

BRI Tests	Male (N=474) Reference range	Female (N=464) Reference range
WBC	4.28-10.16	4.38-10.50
NEU#	2.25-6.4	2.01-7.25
LYM#	1.10-3.8	1.19-3.37
MON#	0.23-0.72	0.23-0.68
EOS#	0.03-0.85	0.03-0.86
BAS#	0.01-0.08	0-0.06
IMG#	0-0.05	0-0.03
NEU%	42.1-74.3	43.3-76.6
LYM%	17.3-45.3	16.67-44.6
MON%	4.0-9.7	3.40-9.44
EOS%	0.6-12.7	0.40-9.67
BAS%	0.1-1.01	0.1-0.8
IMG%	0-0.7	0-0.4
RBC	4.14-5.382	3.65-5.12
HGB	12.4-16.9	10.3-14.3
HCT	37.9-51.1	32.5-43.4
MCV	78.7.3-99.7	73.4-96.8
MCH	25.4-33.1	23.21-31.9
MCHC	31.4-35.0	30.4-34.3
RDW-CV	12.5-15.3	12.3-16.44
RDW-SD	39.14-50.0	38.7-50.3
PLT	85.3-355	72.74-384.66
MPV	9.1-16.4	8.65-16.20
PDW	15.7-17.0	15.4-16.9
PCT	0.14-0.34	0.14-0.42
P-LCC	44.50-121.5	46.15-135.8
P-LCR	20.2-68.2	16.55-68.21
HFC#	0-0.053	0-0.06
HFC%	0-0.7	0-0.84
WBC-D	4.38-10.47	4.43-10.9
WBC-N	4.28-10.16	4.38-10.50
TNC-D	4.38-10.47	4.43-10.9
TNC-N	4.28-10.16	4.38-10.50
NLR	0.96-4.06	0.98-4.5
MICRO#	0.02-0.26	0.02-0.54
MICRO%	0.48-5.0	0.4-11.6
MACRO#	0.09-0.29	0.05-0.19
MACRO%	1.68-6.2	1.05-4.7
PLT-I	81.3-355.0	92.6-380.4
PLR	38.4-189.7	42.66-215.5
PDW-SD	10.2-29.0	9.55-28.2
NEU-X	301.6-397.3	306.53-397.7
NEU-Y	404.6-500.3	416.3-514.8
NEU-Z	1553.8-1832.5	1549.3-1824.0
LYM-X	92.0-105.9	91.1-105.4
LYM-Y	665.4-785.7	685.1-797.4
LYM-Z	921.6-981.1	927.9-987.8
MON-X	199.1-223.8	203.2-227.9
MON-Y	962.3-1122.6	982.6-1149.5
MON-Z	1201.9-1309.5	1223.4-1321.1

limits and also lower than studies from Mumbai[8], Chennai[10]and Delhi[9], the possible explanation could be prevalence of Thalassemia traits in the eastern population. The RBC count reference ranges also show wider range possibly both due to prevalence of traits and smoking reflected also in other RBC parameters like HCT and MCV. RDW the newly reportable parameter shows slightly higher upper limit values compared to international studies but is comparable with other Indian studies (Fig 2).

Platelet counts are conspicuously lower than the values from all the studies, Indian and International due to the unique finding of Macrothrombocytopenia[13-15] in the eastern population characterized by decreased platelet count (mild to moderate thrombocytopenia), macroplatelets (giant platelets) and no bleeding manifestations. This also corroborates with difference in other novel platelet parameters values like PDW and PCT from the other studies (Fig 3).

Earlier called as Harris Syndrome seen in mediterranean population and also called as Asymtomatic Constitutional Macrothrombocytopenia (ACMT)[13] is now called as Inherited Macrothrombocytopenia as it is linked to genetic molecular sequences. It is also referred to as Bengal Macrothrombocytopenia[15] by many hematologists in the southern and western parts of the country as they have specially found it is patients from Bengal. All novel platelet parameters like MPV, PDW, PCT also show increased values compared to all the other studies which corroborates with Inherited Macrothrombocytopenia, thus emphasizing the

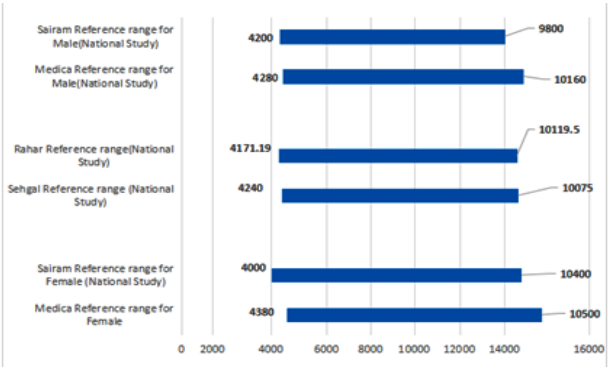

Fig 1 — Distribution of Reference range of WBC across Indian and International Studies

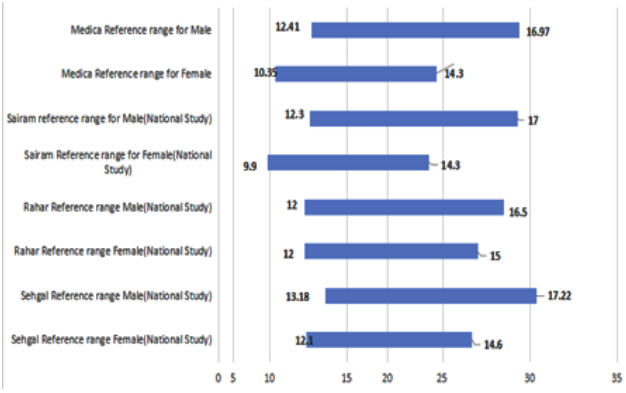

Fig 2 — Distribution of Reference range of HGB across Indian and International Studies

Table 2 — *Comparison between our study and other Indian and International studies*

Parameters	Present study BRI (Male)	Present study BRI (Female)	*Sairam et al*, apollo[7] males (n = 7,478)	*Sairam et al*, apollo[7], females (n = 3,187)	Textbook reference (Europe/ N.America)[5]	*Sehgal et al*, PD Hinduja 2013[8] (n=100)	*Rahar et al*, Delhi 2020[9] (n=150)	*Subhashree et al* 2012 (n= 500)[10]	*Sachdev et al*2014 (n=945)[11]	*Hur et al, Korea 2020*[12] *(n=1238)*
WBC (cells/ uL)	4.28-10.16	4.38-10.50	4200 to 9800	4000 to 10400	4100 to 10000	4240 to 10075	4171.19 to 10119.54	*n/a*	*n/a*	*n/a*
Neu%	42.13-74.33	43.33-76.64	42 to 74	44 to 75	40 to 80	*n/a*	*n/a*	*n/a*	*n/a*	*n/a*
Lym%	17.26-45.32	16.67-44.59	18 to 45	18 to 45	20-to 40	*n/a*	*n/a*	*n/a*	*n/a*	*n/a*
Mon%	4.0-9.7	3.40-9.44	2 to 10	2 to 9	2 to 10	*n/a*	*n/a*	*n/a*	*n/a*	*n/a*
Eos%	0.6-12.7	0.40-9.67	1 to 8	1 to 8	1 to 6	*n/a*	*n/a*	*n/a*	*n/a*	*n/a*
Bas%	0.1-1.01	0.1-0.8	0-0	0 to 0	<1 to 2	*n/a*	*n/a*	*n/a*	*n/a*	*n/a*
IMG%	0.0-0.7	0.0-0.4	*n/a*	*n/a*	*n/a*	*n/a*	*n/a*	*n/a*	*n/a*	*n/a*
RBC (x10^6/ uL)	4.14-5.82	3.65-5.12	4.5 to 5.5	3.5–5.2	4.5 to 5.5 (male); 3.8 to 4.8 (female)	male: 4.56 to 6.16 female: 4.20 to 5.39	male: 4.14 to 5.49 female: 4.04 to 5.43	*n/a*	*n/a*	*n/a*
HGB (gm/dL)	12.41-16.97	10.35-14.30	12.3 to 17	9.9–14.3	13 to 17 (male); 12 to 15 (female)	male: 13.18 to 17.22 female: 12.1to 14.6	male: 12-16.5 female: 12-15	*n/a*	*n/a*	*n/a*
HCT (%)	37.9-51.1	32.5-43.40	37 to 51	30 to 43	40 to 50 (male); 36 to 46 (female)	male: 40.24 to 53.48 female: 37.33 to 46.05	male: 36-49.6 female: 36-44.6	*n/a*	*n/a*	*n/a*
MCV (fL)	78.73-99.71	73.4-96.84	78 to 97	72 to 96	83 to 101	81.13 to 93.45	80.66 to 95.31	*n/a*	*n/a*	*n/a*
MCH (pg)	25.4-33.11	23.21-31.94	26 to 33	23 to 32	27 to 32	male: 26.12 to 30.67 female: 25.5 to 30.2	male: 26 to 34.2 female: 25.6 to 33.4	*n/a*	*n/a*	*n/a*
MCHC (gm/dL)	31.38-35.0	30.42-34.34	31 to 36	30 to 35	31.5 to 34.5	male: 30.88 to 34.96 female: 30.47 to 33.95	male: 31.5 to 35.8 female: 30 4 to 35.1	*n/a*	*n/a*	*n/a*
RDW-CV (%)	12.5-15.3	12.30-16.44	*n/a*	*n/a*	11.6 to 14	12.3 to 15.14	12.36 to 15.71	males: 12.23% to 15.36% females: 12.3%-15.85%	*n/a*	*n/a*
RDW-SD (fL)	39.14-50.01	38.7-50.34	*n/a*	*n/a*	*n/a*	*n/a*	*n/a*	*n/a*	*n/a*	*n/a*
PLT (x10^3/ uL)	85.3-355	72.74-384.66	130 to 380	130 to 420	150 to 410	male: 153 to 366 female: 182 to 409	male: 150 to 388 female: 164 to 420	*n/a*	150 to 520	*n/a*
MPV (fL)	9.1-16.4	8.65-16.20	*n/a*	*n/a*	*n/a*	9.32-11.99	9.24 to 14.49	males: 7.9 fL to 13.7 fL females: 8 fL to 13.28 fL	8.6 to 15.5	8.4 to 12.2
PDW	15.7-17.0	15.4-16.9	*n/a*	*n/a*	*n/a*	9.44-15.02	9.38 to 21.28	males: 9 fL - 16.56 fL females: 8 fL -13.28 fL	8.3 to 25.0	15.5 to 16.7
PCT (%)	0.14-0.34	0.14-0.42	*n/a*	*n/a*	*n/a*	0.18-.40	male: 0.17-0.44 female: 0.21 to 0.47	*n/a*	0.15 to 0.62	0.2 to 0.4
P-LCC (10^3/uL)	44.50-121.5	46.15-135.8	*n/a*	*n/a*	*n/a*	*n/a*	*n/a*	*n/a*	*n/a*	39 to 101
P-LCR%	20.2-68.2	16.55-68.21	*n/a*	*n/a*	*n/a*	*n/a*	*n/a*	*n/a*	*n/a*	14.5 to 40.9

importance of establishing the region-specific reference intervals in the laboratory reporting system for our ethnic population.

Here, we have also established the reference ranges of additional Novel research hematological parameters with increasing utility in modern hematology practice like Platelet-large Cell Count (P-LCC), Platelet-large Cell Ratio (P-LCR), High Fluorescent Cell Percentage (HFC%), White Blood Cell Count -DIFF (WBC-D), White Blood Cell Count-WNB (WBC-N), Total Nucleated Cell Counts-DIFF (TNC-D), Total nucleated cell Counts-WNB (TNC-N), Neutrophil-to-Lymphocyte Ratio (NLR), Microcyte Percentage (Micro%), Macrocyte percentage (Macro%), Platelet-to-lymphocyte ratio (PLR), Platelet Distribution Width Standard Deviation (PDW-SD), mean neutrophil distribution-side scatter intensity (Neu-X), neutrophil distribution-side fluorescent light intensity (Neu-Y), mean neutrophil distribution- forward scatter intensity (Neu-Z), mean lymphocyte distribution- side scatter intensity (Lym-X), mean lymphocyte distribution-side fluorescent intensity (Lym-Y), mean lymphocyte distribution- forward scatter intensity (Lym-Z), mean monocyte distribution-side scatter intensity (Mon-X), mean monocyte distribution-side fluorescent light intensity (Mon-Y). mean Monocyte distribution - forward scatter intensity (Mon-Z). Few of these like NLR, PLR have already found utility in COVID patients. These can be studied further for evaluation of various diseases.

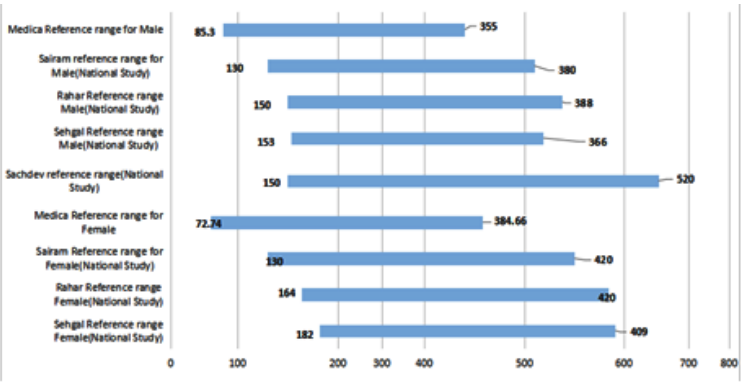

Fig 3 — Distribution of Reference range of PLT across Indian and International Studies

Conclusion

We have established the region-specific reference intervals in the laboratory reporting system for our ethnic population which is different from other population in India and also corroborates with findings of other studies from Eastern region especially in platelet parameters. We have also established the reference ranges of additional Novel Research hematological parameters with increasing utility in modern hematology practice and intend to study them further for their utility in diagnostic and therapeutic management of patients.

References

1 Aytekin M, Emerk K — Accurate reference intervals are required for accurate diagnosis and monitoring of patients. *eJIFCC* 2008; **19(2):** 1-5.

2 Katayev A, Balciza C, Seccombe DW — Establishing reference intervals for clinical laboratory test results: Is there a better way? *Am J Clin Pathol* 2010; **133(2):** 180-6.

3 Jacob EA — Complete Blood Cell Count and Peripheral Blood Film, Its Significant in Laboratory Medicine: A Review Study. *Am J Lab Med* 2016; **1(3):** 34-57. Higgins, Chris. (2012). An Introduction to Reference Intervals (1)-Some Theoretical Considerations. Point of Care. 11. 2–5. 10.1097/POC.0b013e318246a59c.

4 Zeh CE, Odhiambo CO, Mills LA — Laboratory Reference Intervals in Africa. 2012.

5 Imelda bates- Reference ranges and normal values. In Barbara J. Bain, Imleda Bates, Michael A. Laffan- Dacie Lewis Practical Haematology, 12th Edition, page 8-17

6 EP28 Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory, 3rd Edition, CLSI

7 Sairam S, Domalapalli S, Muthu S, Swaminathan J, Ramesh VA, Sekhar L, *et al* — Hematological and biochemical parameters in apparently healthy Indian population: defining reference intervals. *Indian J Clin Biochem* 2014; **29(3):** 290-7.

8 Sehgal KK, Tina D, Choksey U, Dalal RJ, Shanaz KJ — Reference range evaluation of complete blood count parameters with emphasis on newer research parameters on the complete blood count analyzer Sysmex XE-2100. *Indian J Pathol Microbiol* 2013; **56:** 120-4.

9 Rahar S, Vijay K, Swati R, Deepika G — Haematology reference range evaluation for novel research parameters on the complete blood count analyzer sysmex XN-1000. *Hamdan Medical Journal* 2022; **15(2):** 83-8.

10 A R S, Parameaswari PJ, Shanthi B, Revathy C, Parijatham BO — The reference intervals for the haematological parameters in healthy adult population of chennai, southern India. *J ClinDiagn Res* 2012; **6(10):** 1675-80.

11 Ritesh S, Tiwari AK, Shalini G, Vimarsh R, Sethi M — Establishing biological reference intervals for novel platelet parameters (immature platelet fraction, high immature platelet fraction, platelet distribution width, platelet large cell ratio, platelet-X, plateletcrit, and platelet distribution width) and their correlations among each other. *Indian J Pathol Microbiol* 2014; **57(2):** 231-5. doi: 10.4103/0377-4929.134676.

12 Kim H, Hur M, Kim SW, Moon HW, Yun YM — Reference intervals for clinically reportable platelet parameters on the Mindray BC-6800Plus hematology analyzer. *Clin Chem Lab Med* 2020; **58(10):** e213-e215.

13 Kunjali Naina HV, Nair SC, Daniel D, George B, Chandy M — Asymptomatic constitutional macrothrombocytopenia among West Bengal blood donors. *The American Journal of Medicine* 2002; **112(9):** 742-3. doi: 10.1016/s0002-9343(02)01114-2.

14 Edupuganti HS, Krishnamurthy V — Prevalence of constitutional macrothrombocytopenia in the immigrants of Northern and Eastern states of India. Department of Pathology, JSS Medical College, JSS Academy of Higher Education and Research, Mysuru, Karnataka, India, Year 2020; **63(4):** 593-6.

15 Ali S, Ghosh K, Shetty S — Differential expression of genes involved in Bengal macrothrombocytopenia (BMTCP). *Blood Cells Mol Dis* 2015; **55(4):** 410-4.