

Evaluation of VACUETTE® CAT Serum Fast Separator Blood Collection Tube for Routine Chemistry Analytes

Background:

Greiner-Bio-One, Austria has been selling plastic evacuated tubes (VACUETTE®) for venous blood collection since 1986.

VACUETTE® CAT Serum Fast Separator blood collection tubes contain thrombin in addition to the blood clotting activator in order to accelerate the clotting process.

Due to the rapid clotting process within 5 minutes after blood collection and the following centrifugation, the VACUETTE® CAT Serum Fast Separator blood collection tubes enable faster turnaround times similar to plasma tubes. The tubes are suitable for the usage for routine chemistry analyses on patients who are not on heparin or other thrombin inhibitor therapy.

The VACUETTE® CAT Serum Fast tube is offered as a gel separator tube. The gel has a specific gravity, forms a stable barrier between the blood cells and the serum during centrifugation and provides stability for most analytes up to 48h when measured out of the primary tube stored at 4-8°C.

Study Objective:

The study has been carried out to demonstrate that VACUETTE® CAT Serum Fast Separator blood collection tubes are suitable for routine chemistry analysis for up to 48h after centrifugation in comparison to VACUETTE® CAT Serum Separator Blood Collection tubes.

Study design and procedure:

Venous whole blood was collected from 50 healthy donors using a VACUETTE® SAFETY Blood Collection Set (Item #450085) into the following tubes:

Sample A: VACUETTE® CAT Serum Separator tube (Prod. No. 456073)

Sample B: VACUETTE® CAT Serum Fast Separator tube (Prod. No. 456310)

All samples were inverted 8 times. Samples A were centrifuged after 30 min clotting time at 1800g for 10 min at 20°C (centrifuge: Eppendorf 5810R). Samples B were centrifuged after 5 min using the same centrifugation setting. Initial analysis was done within 4h after centrifugation at room temperature storage on an AU680 and DxI800 from Beckman Coulter. All samples were stored in an upright position at 4-8°C for being analyzed again after 24h and 48h.

Analytes on Beckman Coulter AU680 and DxI800:

No.	Testing	Abbreviation
1	Alanine Aminotransferase	ALT, GPT
2	Albumin	ALB
3	Alkaline Phosphatase	ALP
4	Aspartate Aminotransferase	AST, GOT
5	Calcium	Ca
6	Chloride	Cl
7	Carcinoembryonic antigen	CEA
8	Cholesterol	CHOL

9	C-reactive Protein	CRP
10	Creatine Kinase MB	CK
11	Creatinine	Krea
12	Ferritin	FERR
13	Free Thyroid stimulating hormone	fTSH
14	Free Triiodthyronine	fT ₃
15	Folic acid	Fol
16	Gamma Glutamyltransferase	GGT
17	Glucose	Gluk
18	Immunoglobulin G	IgG
19	High Density Lipoprotein	HDL
20	Inorganic Phosphate	IP
21	Iron	Fe
22	Lactate Dehydrogenase	LDH
23	Lipase	LIP
24	Low Density Lipoprotein	LDL (cal)
25	Magnesium	Mg
26	Potassium	K
27	Progesterone	Prog
28	Prostate-specific Antigen	PSA
29	Sodium	Na
30	Total Bilirubin	TBIL
31	Total Protein	TP
32	Triglyceride	TG
33	Troponin I	Trop
32	Urea	UREA
33	Uric Acid	UA
34	Vitamin B ₁₂	Vit B ₁₂
35	Vitamin D ₃	Vit D ₃
36	Transferrin	Trans
37	Beta-HCG	HCG
38	Total T ₄	T ₄

Comparison analysis was performed at all time points of determination. Statistics was performed with the t-test ($\alpha = 0.05$) using StatSoft Software, Version 12.

Clinical evaluation was based on the allowed recommendation by the German Medical Association (RILIBÄK). ^[6].

Results:

The initial mean values of all analytes are summarized in Table 1, the 24h-values in Table 2, and the 48h-values in Table 3.

Comparing the initial values of both tubes, no significant deviations have been found for Lip, Mg, LDH, IP, Urea, Fe, ALP, ALT, AST, GGT, ALB, Ca, CHOL, Krea, HDL, TG, UA, CRP, FERR, IgG, LDL, Fol, fT₃, T₄, TSH, Vit B₁₂, Vit D₃, Trop. High deviations resulting from very low absolute values have been found for HCG, CEA, CK-MB, Prog, PSA. The slight deviation found for TBil is caused by the light sensitivity of that analyte. The systematical deviation of

glucose up to 17% (sample B indicates higher values) is due to the inhibition of glycolysis in sample B caused by the rapid clotting process.

Furthermore, systematic deviations due to the faster clotting process have been found for sodium (up to 2%), potassium (up to 9%), chloride (up to 3%), total protein (up to 7%) or Trans (up to 8%). Those deviations are in accordance to literature ^{[1], [2], [3], [4]}.

One value after 48h for donor 7 (beta HCG) has been regarded as outlier due to a possible microclot effect on the analyzer.

Comparing the stability of the analytes over time, the troponin stability is limited to 6h in the tube with thrombin in comparison to the reference serum tube in order to avoid false positive troponin results ^[5]. No significant deviations have been found for all other analytes.

The bar plots illustrating the mean values for all analytes after initial, 24h and 48h measurement have been shown in the Annex.

Conclusion:

The equivalent clinical performance of the VACUETTE® CAT Serum Fast Separator Blood Collection Tube in comparison to the VACUETTE® CAT Serum Separator Blood Collection Tube has been demonstrated for routine biochemical analytes on a Beckman Coulter analyzer at initial time and after 48h (except Troponin I for 6h) for healthy donors.

Provided a clear serum after centrifugation, the utilization of the VACUETTE® CAT Serum Fast Separator tube enables a faster turnaround time in the laboratory due to the rapid clotting process minimizing the cell lysis in the tube within 5 minutes on the basis of the thrombin additive. From the rapid clotting process resulting systematic bias found for some analytes such as the electrolytes sodium, potassium, chloride or total protein in comparison to laboratory reference values as described by literature have to be considered.

References:

[1] W.-Y. Ng, C-P. Yeo, Thrombin-Accelerated Quick Clotting Serum Tubes: An Evaluation with 22 Common Biochemical Analytes. *Advances in Hematology* (2013),

[2] S.J.Steindel, Evaluation of a Thrombin Containing Blood Collection Tube. *Clin. Chem.*, Vol. 26, 1, (1980)

[3] M. Kojancic, J. Cargonja, A.Delic-Knezevic. Evaluation of the BD Vacutainer RST blood collection tube for routine chemistry analytes: clinical significances of differences and stability study *Biochemica Medica* 2014; 24: 368-75

[4] Huyghe T et al. Studies on the use of BD Vacutainer SSTII and RST in general practice: investigation of artefactual hyperkalemia. *Ann Clin Biochem* 2014; 51: 30-37

[5] Koch et al, BD rapid serum tubes reduce false positive plasma troponin T results on the Roche Cobas e411 analyzer. *Clin Biochem* 2012; 45: 842-44

[6] Guideline from the medical association in Germany for quality assurance of laboratory tests. *German Medical Journal*. Vol 105, Issue 7, 2008

Table 1 Initial values within 4 h after blood collection

	Valid N	Mean	Std.Dev.
LIP [U/l]	50	18,06	10,39
Mg [mmol/l]	50	0,80	0,05
LDH [U/l]	50	145,60	30,31
IP [mmol/l]	50	1,00	0,16
UREA [mg/dl]	50	23,41	6,34
Fe [umol/l]	50	18,90	6,77
ALP [U/l]	50	60,66	15,26
ALT [U/l]	50	32,14	61,06
AST [U/l]	50	33,06	55,97
CK-MB [U/l]	50	11,94	10,37
GGT [U/l]	50	22,52	26,63
ALB [g/l]	50	42,91	2,98
TBIL [mg/dl]	50	0,68	0,28
CA [mmol/l]	50	2,27	0,09
CHOL [mg/dl]	50	210,80	48,19
KREA [umol/l]	50	0,76	0,12
GLUK [mg/dl]	50	92,44	16,60
HDL [mg/dl]	50	59,59	14,53
TP [g/l]	50	66,03	4,23
TG [mg/dl]	50	114,24	44,32
UA [mg/dl]	50	4,68	0,92
CRP [mg/l]	50	2,34	1,93
FERR [ng/ml]	50	76,42	107,58
IgG [mg/dl]	50	993,76	209,38
Trans [ng/ml]	50	268,62	39,15
LDL cal [mg/dl]	50	128,40	43,04
Na [mmol/l]	50	137,98	1,79
K [mmol/l]	50	4,03	0,28
Cl [mmol/l]	50	105,04	2,34
CEA [ng/ml]	50	1,29	0,99
Fol [ng/ml]	50	7,51	3,96
FT3 [pg/ml]	50	3,31	0,30
fTSH [uIU/ml]	50	1,71	0,73
Prog [ng/ml]	50	1,93	3,91
PSA [ng/ml]	50	0,36	0,54
HCG [mIU/ml]	50	0,30	0,39
T ₄ [ng/ml]	50	76,82	11,77
VitB ₁₂ [pg/ml]	50	313,66	255,84
Vit D ₃ [ng/ml]	50	19,71	6,54
Trop I [µg/l]	50	0,00	0,00

Table 2: Values after 24 h after blood collection and storage at 4-8°C

Analyte	Valid N	Mean	Std.Dev.
LIP [U/l]	50	18,56	10,41
Mg [mmol/l]	50	0,84	0,06
LDH [U/l]	50	143,42	30,02
IP [mmol/l]	50	1,01	0,16
UREA [mg/dl]	50	23,35	6,28
Fe [umol/l]	50	19,02	6,78
ALP [U/l]	50	60,84	15,62
ALT [U/l]	50	31,68	60,23
AST [U/l]	50	33,26	55,81
CK-MB [U/l]	50	12,16	10,79
GGT [U/l]	50	22,50	26,46
ALB [g/l]	50	43,08	2,93
TBIL [mg/dl]	50	0,66	0,28
CA [mmol/l]	50	2,28	0,09
CHOL [mg/dl]	50	212,68	48,03
KREA [umol/l]	50	0,77	0,12
GLUK [mg/dl]	50	92,78	16,62
HDL [mg/dl]	50	58,96	14,45
TP [g/l]	50	65,92	4,27
TG [mg/dl]	50	114,84	44,55
UA [mg/dl]	50	4,68	0,92
CRP [mg/l]	50	2,33	1,92
FERR [ng/ml]	50	76,12	107,20
IgG [mg/dl]	50	999,08	210,68
Trans [ng/ml]	50	269,22	40,09
LDL cal [mg/dl]	50	130,84	42,54
Na [mmol/l]	50	138,34	1,88
K [mmol/l]	50	4,07	0,27
Cl [mmol/l]	50	105,46	2,26
CEA [ng/ml]	50	1,35	0,95
Fol [ng/ml]	50	7,49	3,92
FT3 [pg/ml]	50	3,20	0,32
fTSH [uIU/ml]	50	1,72	0,73
Prog [ng/ml]	50	1,81	3,62
PSA [ng/ml]	50	0,35	0,52
HCG [mIU/ml]	50	0,32	0,43
T ₄ [ng/ml]	50	79,21	12,70
VitB ₁₂ [pg/ml]	50	298,72	257,70
Vit D ₃ [ng/ml]	50	18,70	5,98
Trop I [µg/l]	50	0,06	0,39

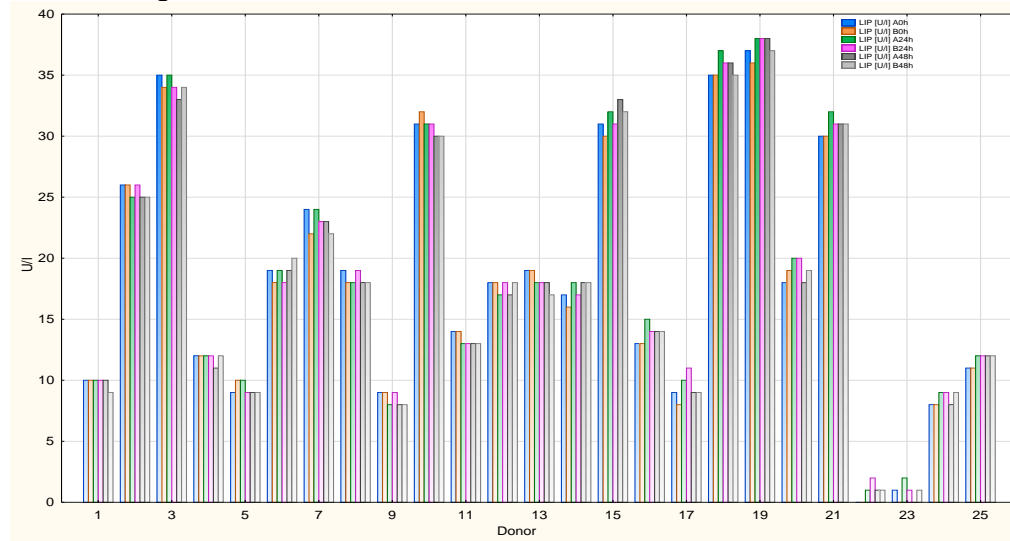
Table 3: Values after 48 h after blood collection and storage at 4-8°C

Analyte	Valid N	Mean	Std.Dev.
LIP [U/l]	50	18,10	10,43
Mg [mmol/l]	50	0,86	0,06
LDH [U/l]	50	138,50	28,95
IP [mmol/l]	50	1,01	0,16
UREA [mg/dl]	50	23,25	6,22
Fe [umol/l]	50	19,07	6,74
ALP [U/l]	50	60,46	15,59
ALT [U/l]	50	31,24	59,35
AST [U/l]	50	33,38	55,79
CK-MB [U/l]	50	11,74	10,68
GGT [U/l]	50	22,44	26,88
ALB [g/l]	50	42,89	2,72
TBIL [mg/dl]	50	0,62	0,26
CA [mmol/l]	50	2,28	0,09
CHOL [mg/dl]	50	212,08	47,90
KREA [umol/l]	50	0,77	0,12
GLUK [mg/dl]	50	91,86	16,36
HDL [mg/dl]	50	58,25	13,92
TP [g/l]	50	65,89	4,20
TG [mg/dl]	50	114,58	44,62
UA [mg/dl]	50	4,66	0,92
CRP [mg/l]	50	2,34	1,93
FERR [ng/ml]	50	75,98	107,58
IgG [mg/dl]	50	995,68	211,33
Trans [ng/ml]	50	269,62	40,21
LDL cal [mg/dl]	50	130,86	42,35
Na [mmol/l]	50	138,14	1,82
K [mmol/l]	50	4,11	0,28
Cl [mmol/l]	50	104,84	2,20
CEA [ng/ml]	50	1,33	1,03
Fol [ng/ml]	50	7,58	3,82
FT3 [pg/ml]	50	3,22	0,30
fTSH [uIU/ml]	50	1,73	0,73
Prog [ng/ml]	50	1,87	3,74
PSA [ng/ml]	50	0,34	0,52
HCG [mIU/ml]	50	0,49	1,07
T ₄ [ng/ml]	50	81,10	12,40
VitB ₁₂ [pg/ml]	50	291,86	257,96
Vit D ₃ [ng/ml]	50	20,06	6,51
Trop I [µg/l]	50	0,39	1,03

Annex:

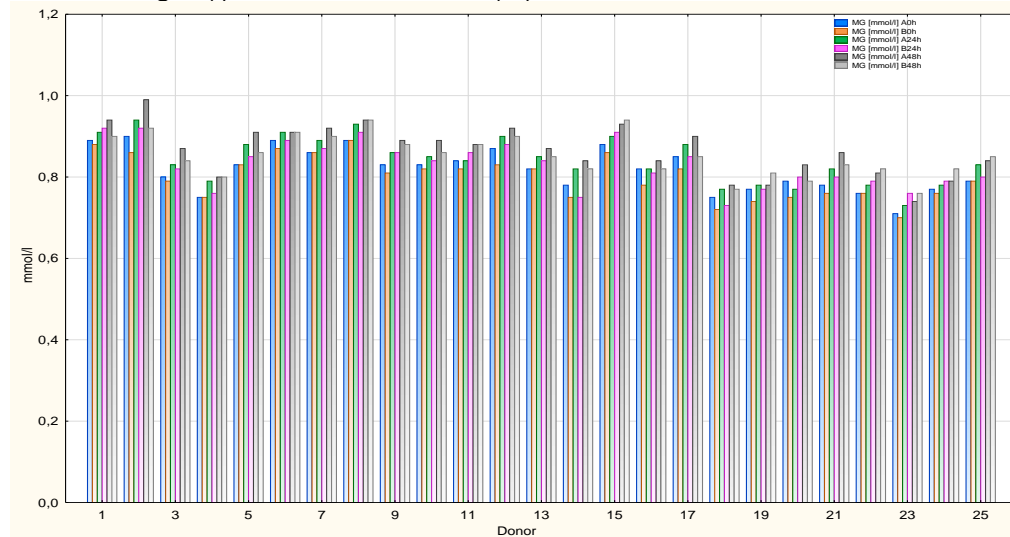
Lipase

Normal range: < 67 U/l

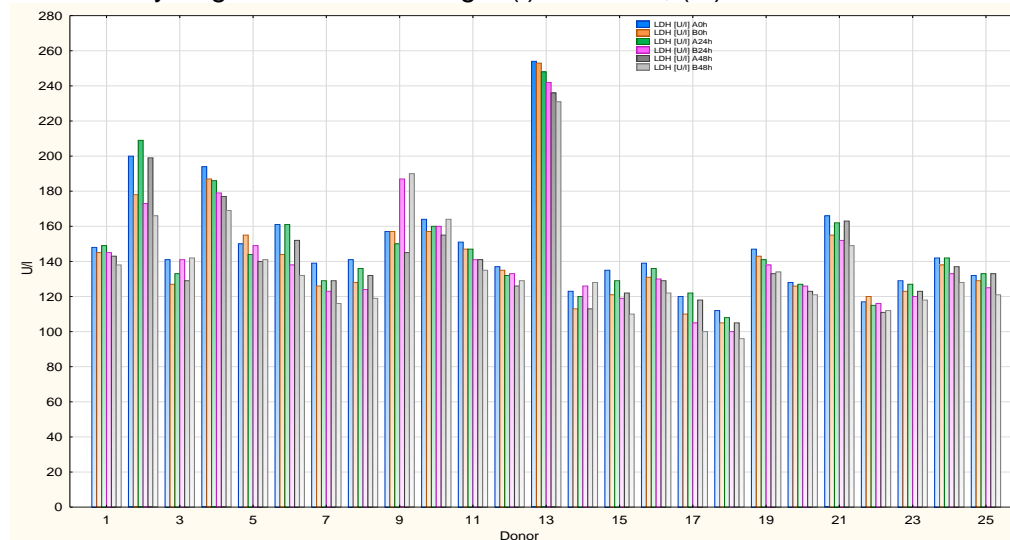


Magnesium

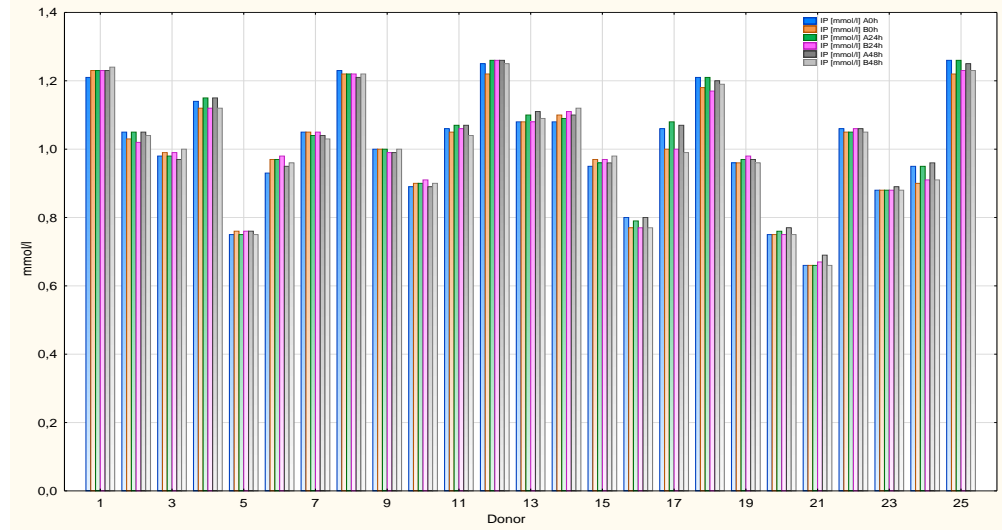
Normal range: (f) 0.73 - 1.06 mmol/l; (m) 0.77 - 1.03 mmol/l



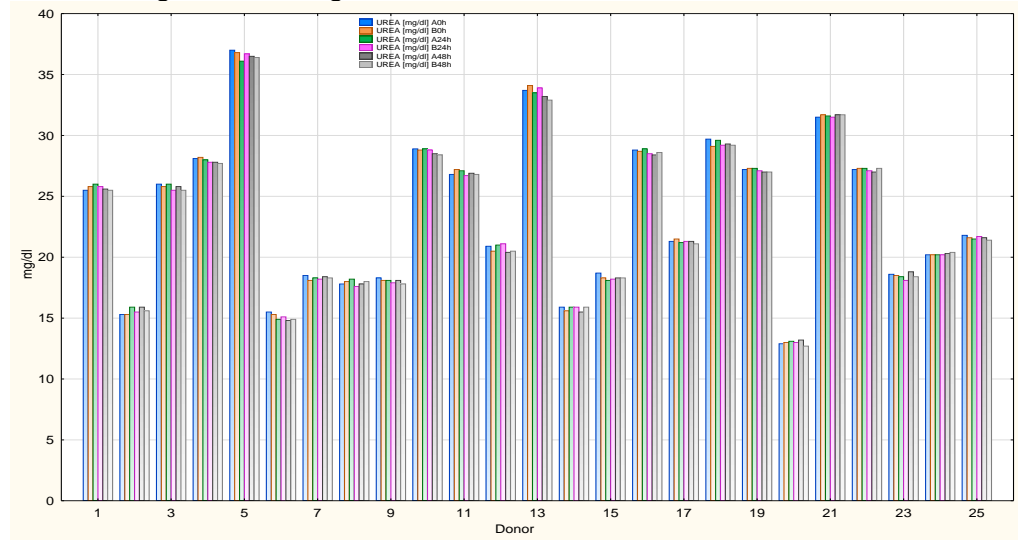
Lactat-Dehydrogenase Normal range: (f) < 247 U/l; (m) < 248 U/l



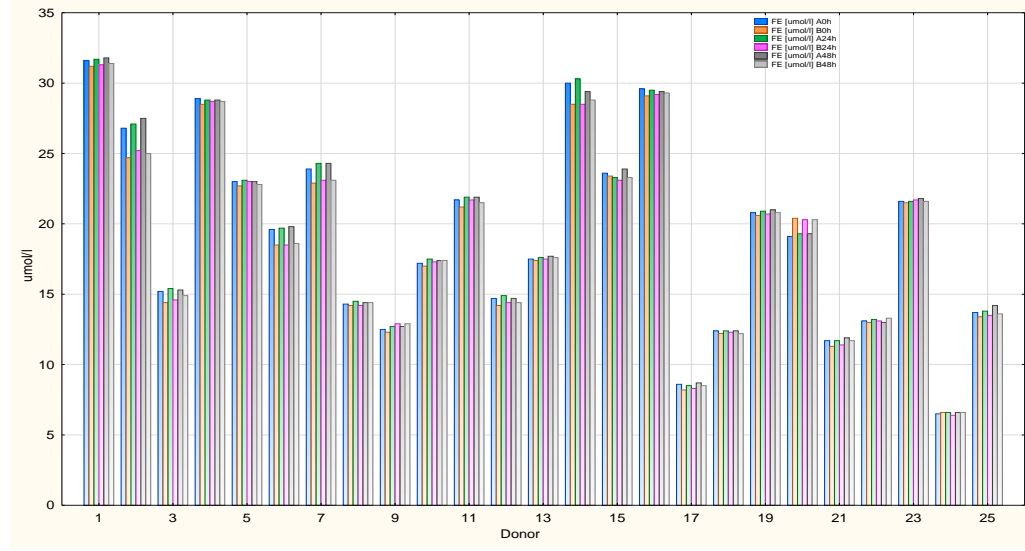
Phosphate inorganic
 Normal range: 0.81 - 1.45 mmol/l



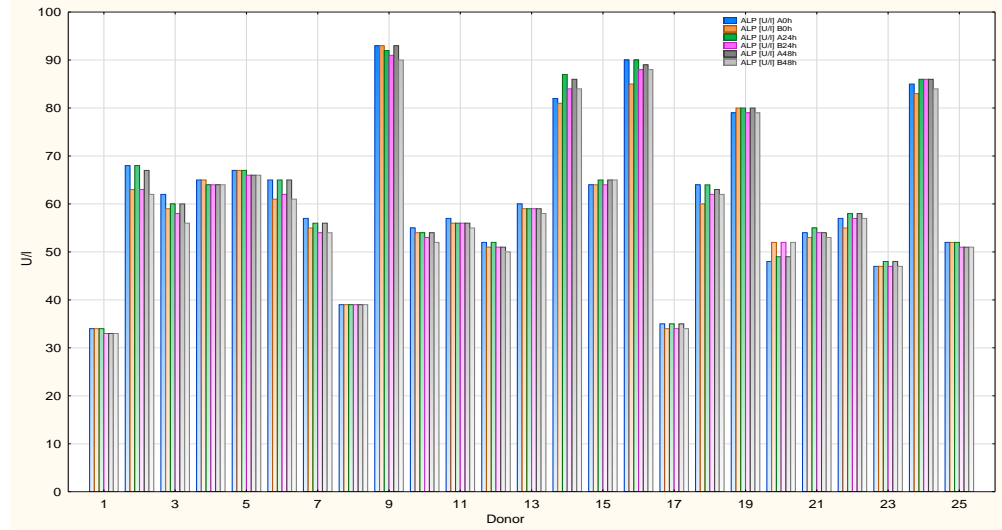
Urea
 Normal range: 17 - 43 mg/dl



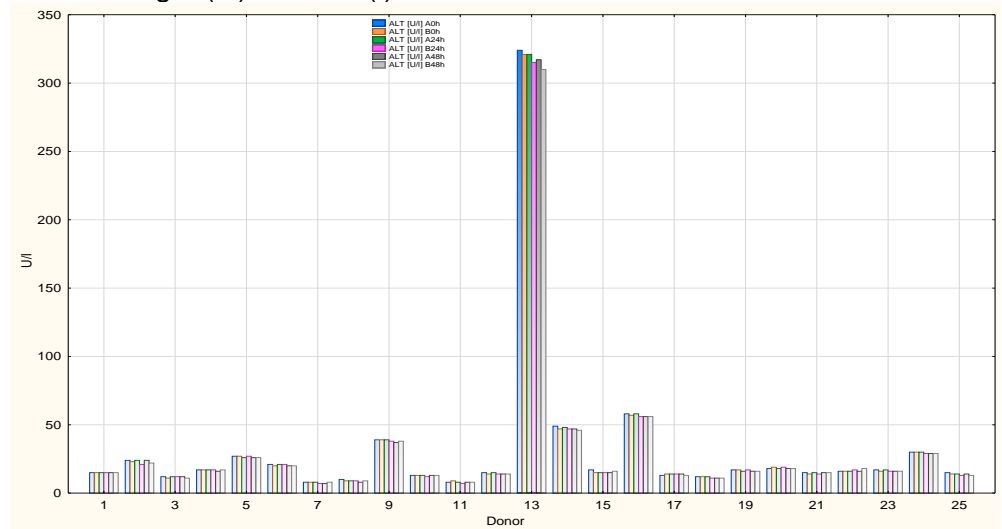
Iron
 Normal range: (f) 12.5 - 32.2 μ mol/l (m) 10.7 - 32.2 μ mol/l



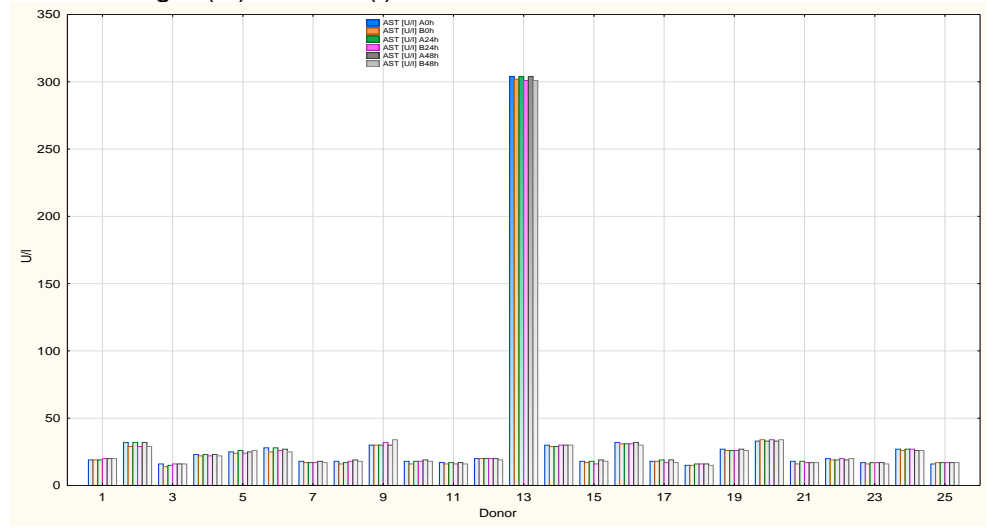
Alkaline phosphatase
 Normal range: 60 - 200 U/l



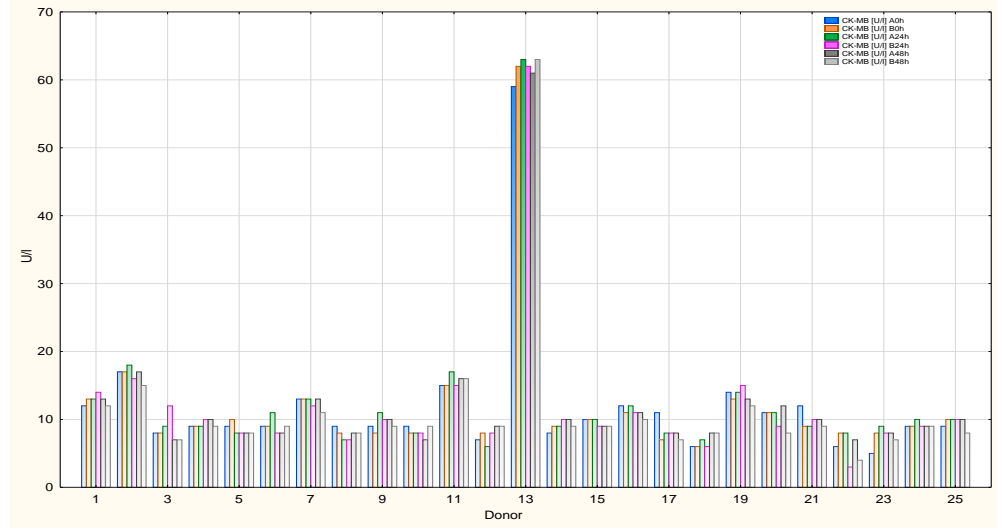
Alanine transaminase
 Normal range: (m) < 50 U/l (f) < 35 U/l



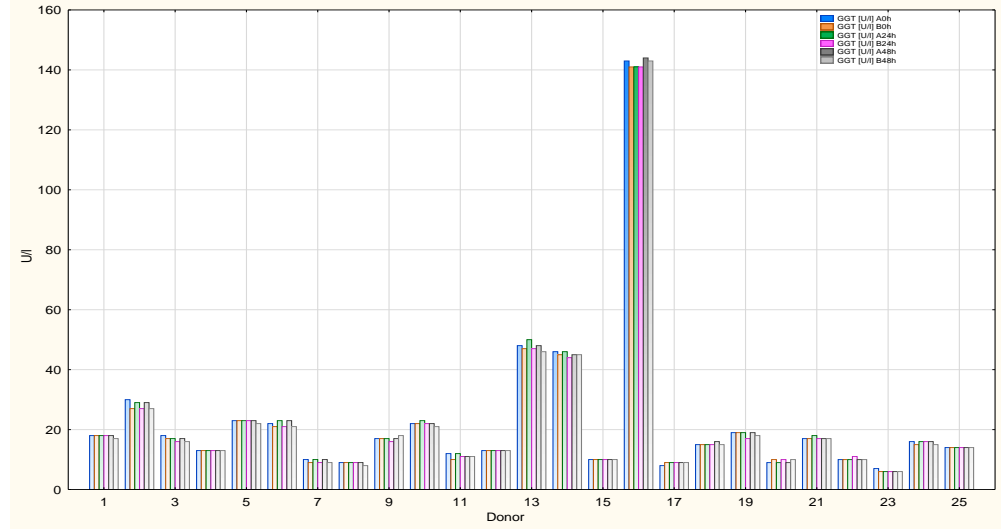
Aspartate transaminase
 Normal range: (m) < 50 U/l (f) < 35 U/l



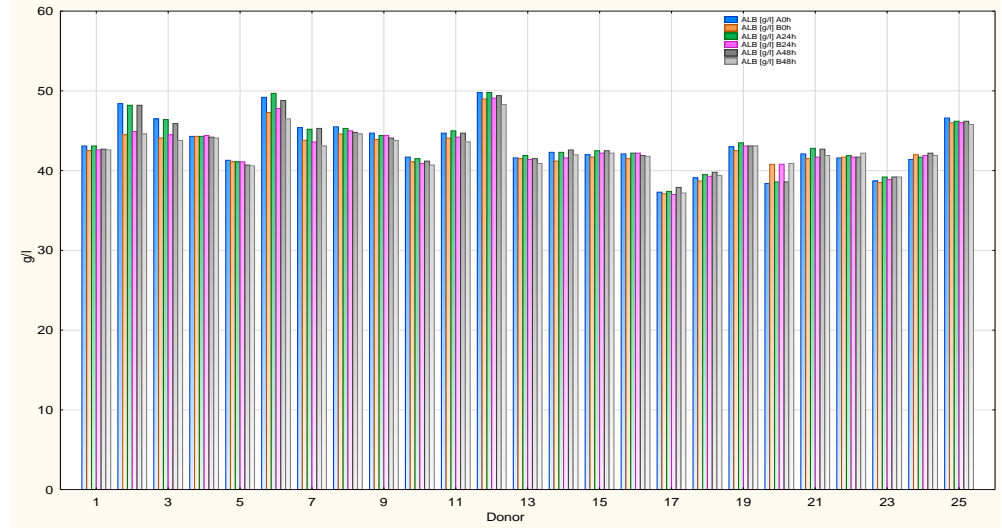
Creatine kinase enzyme activity
 Normal range: < 24 U/l



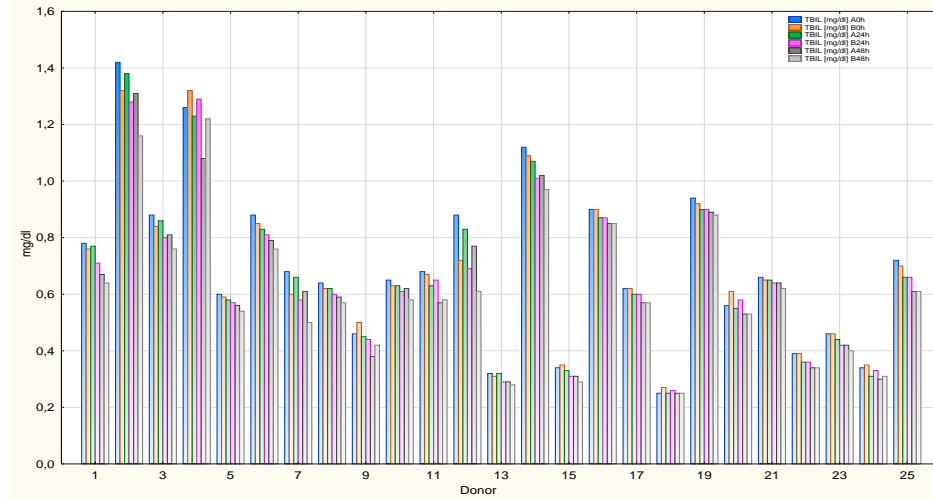
Gamma glutamyl transpeptidase
 Normal range: (f) < 55 U/l (m) < 38 U/l



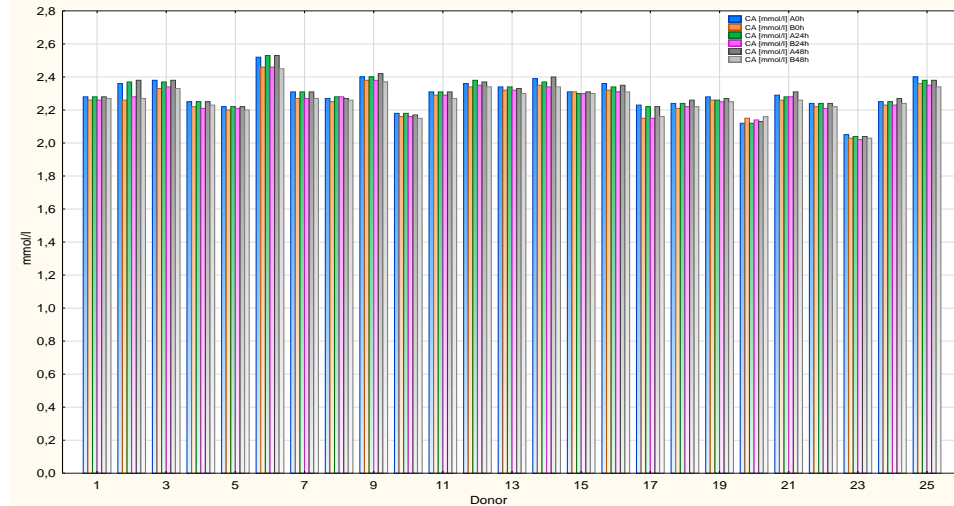
Albumin
 Normal range: 35 - 52 g/l



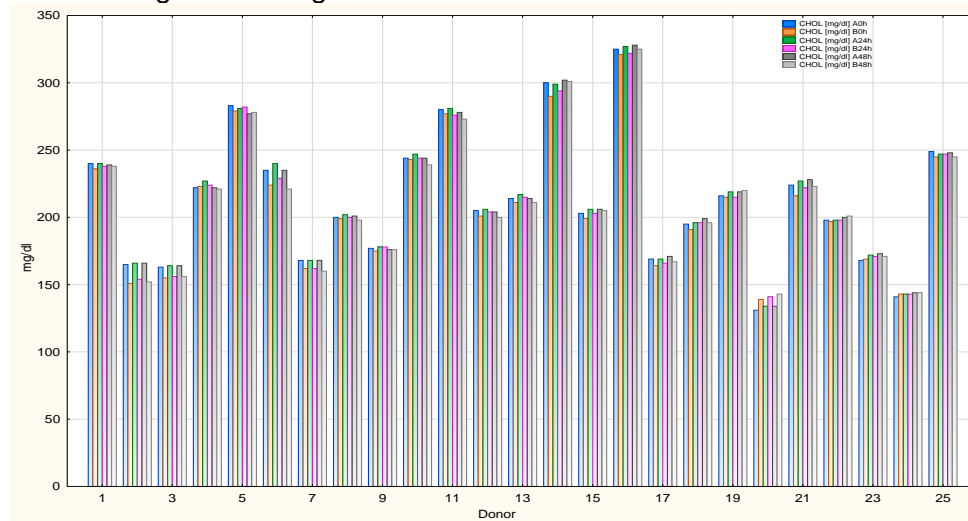
Total Bilirubin
 Normal range: 0.3 - 1.2 mg/dl



Calcium (total)
 Normal range: 2.20 - 2.65 mmol/l

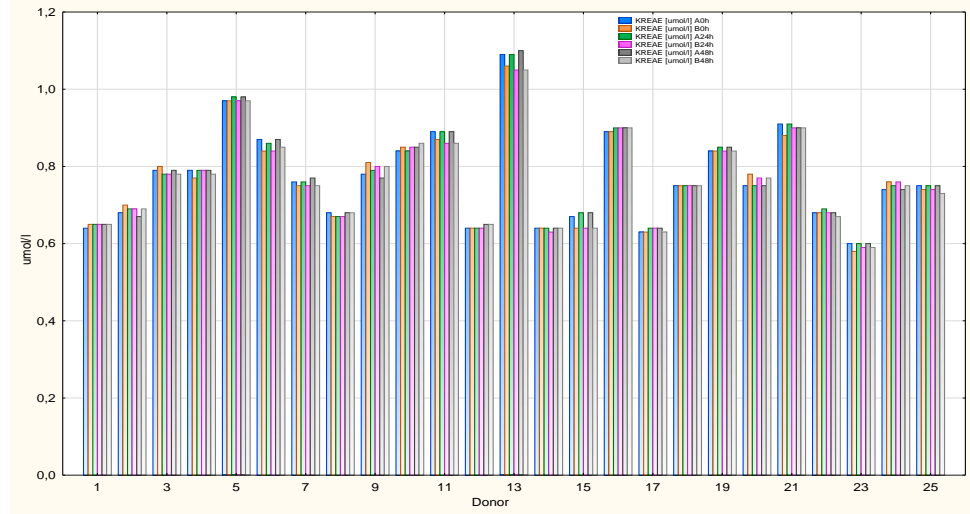


Cholesterol
 Normal range: < 200 mg/dl



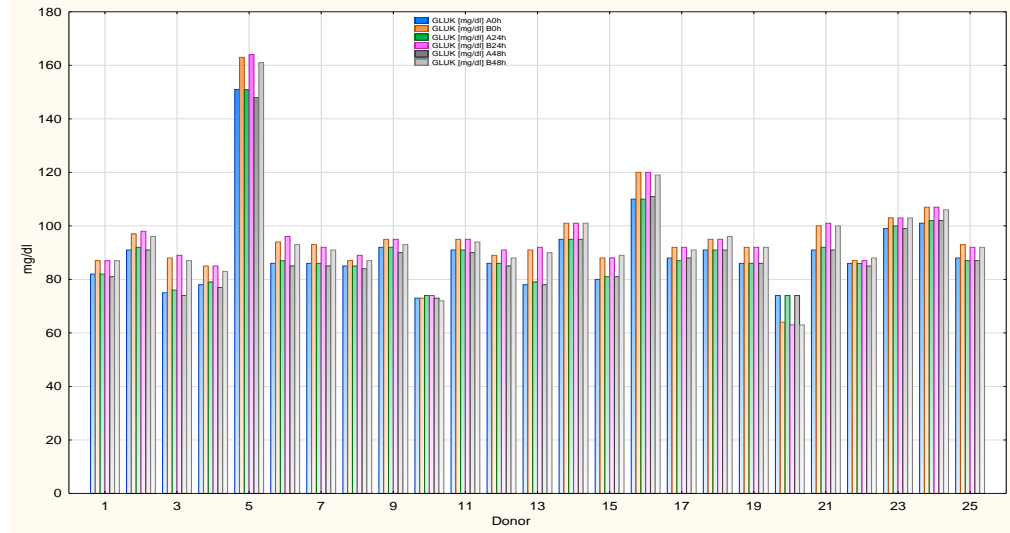
Creatinine

Normal range: (f) 0.72 - 1.18 mg/dl; (m) 0.55 - 1.02 mg/dl



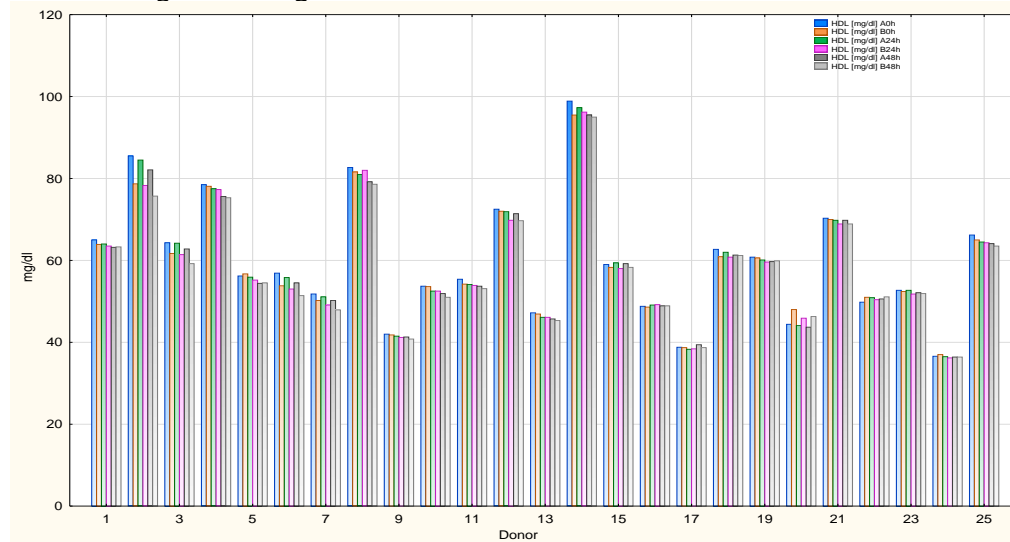
Glucose

Normal range: 74 - 106 mg/dl

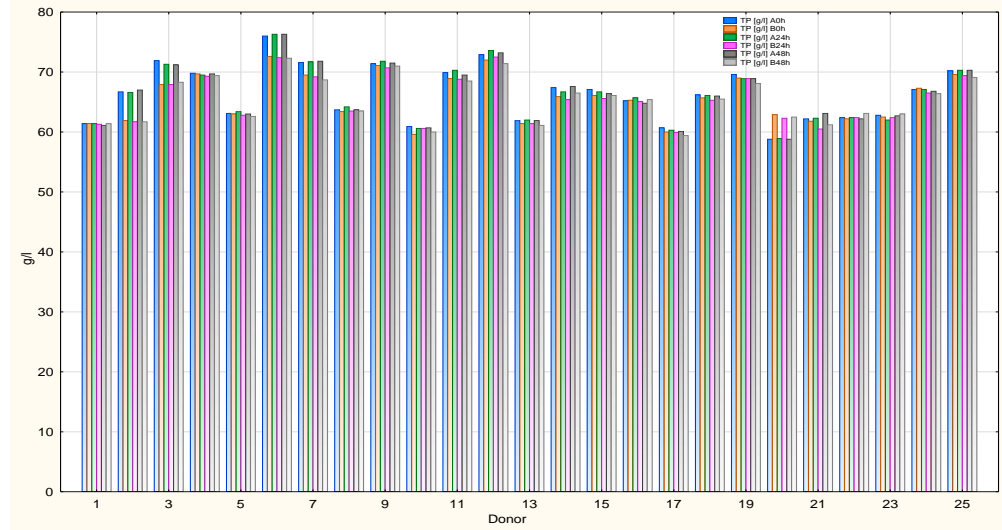


High Density Lipoprotein

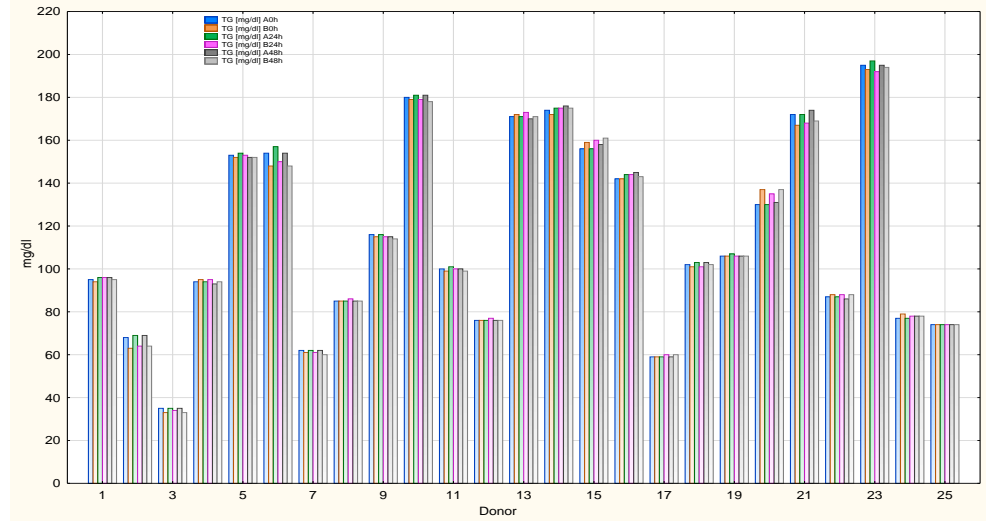
Normal range: ≥ 40 mg/dl



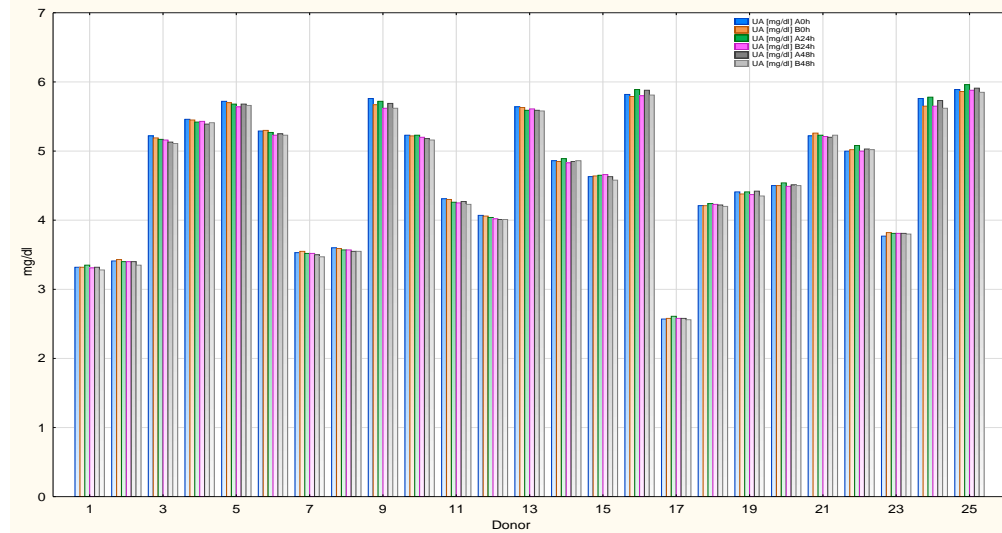
Total Protein
Normal range: 66 - 83 g/l



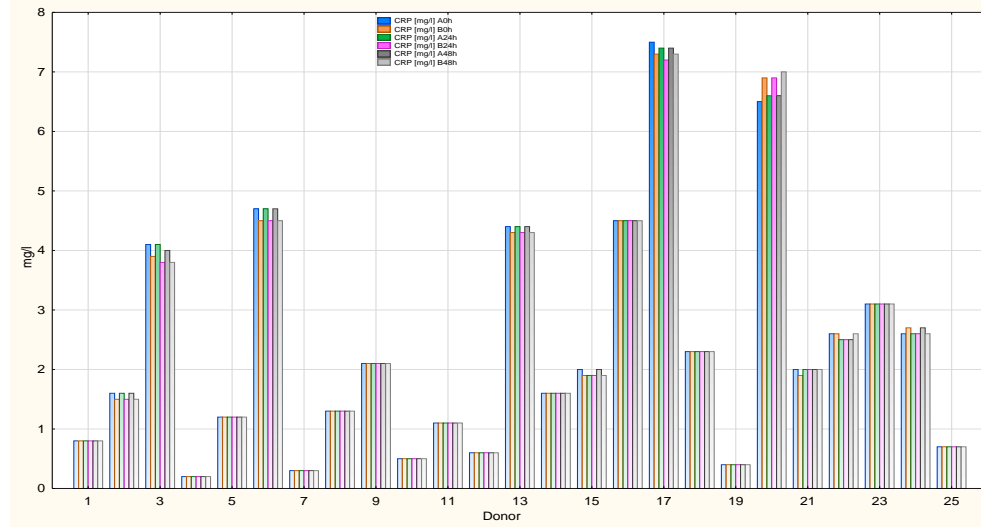
Triglyceride
Normal range: normal ≤ 150 < borderline high < 200 high < 500 very high



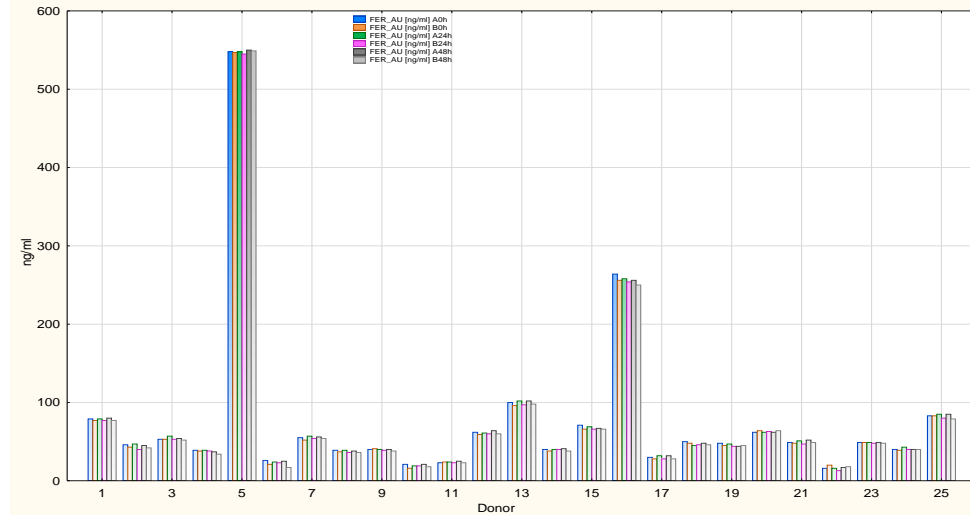
Uric acid
Normal range: (f) 3.5 - 7.2 mg/dl, (m) 2.6 - 6.0 mg/dl



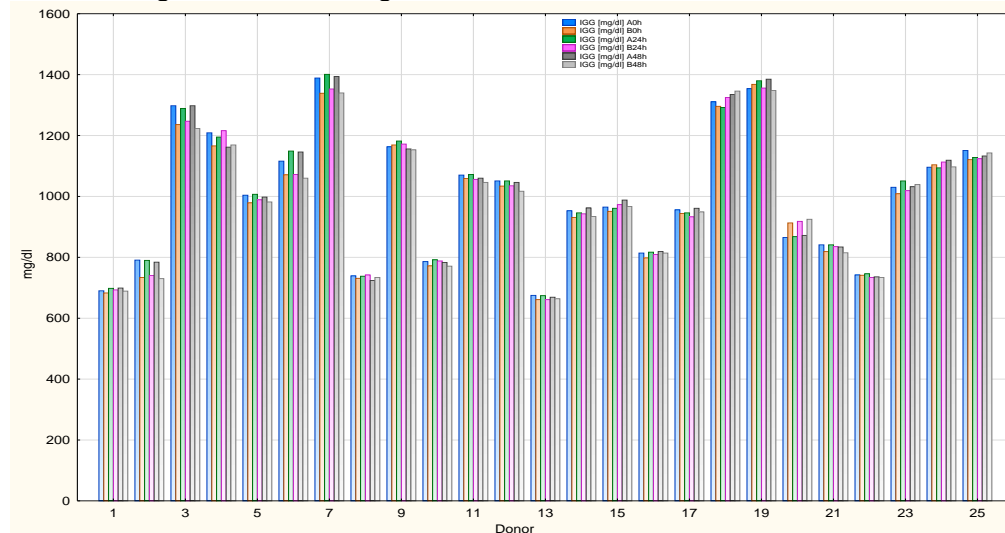
C-reactive protein
Normal range: <5 mg/l



Ferritin
Normal range: (f) 20 - 250 µg/l; (m) 10 - 120 µg/l

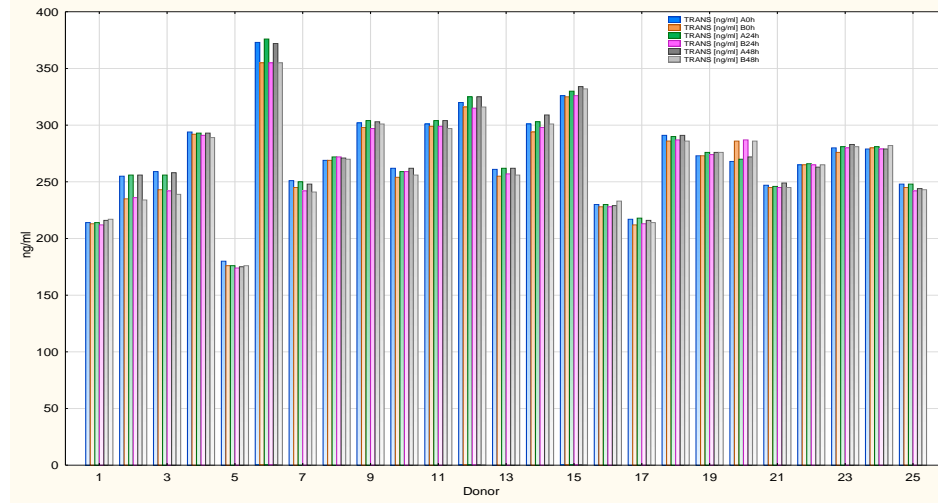


Immunoglobulin G
Normal range: 700 - 1600 mg/dl



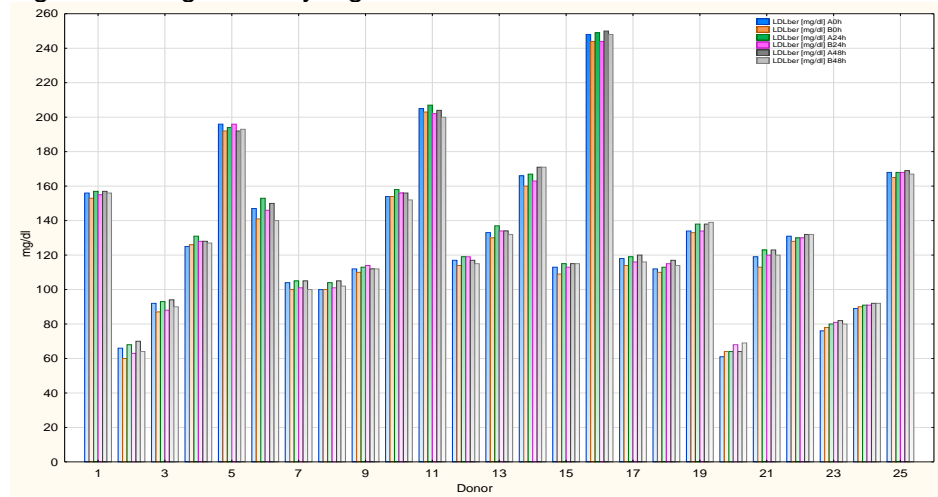
Transferrin

Normal range: 200 - 300 mg/dl



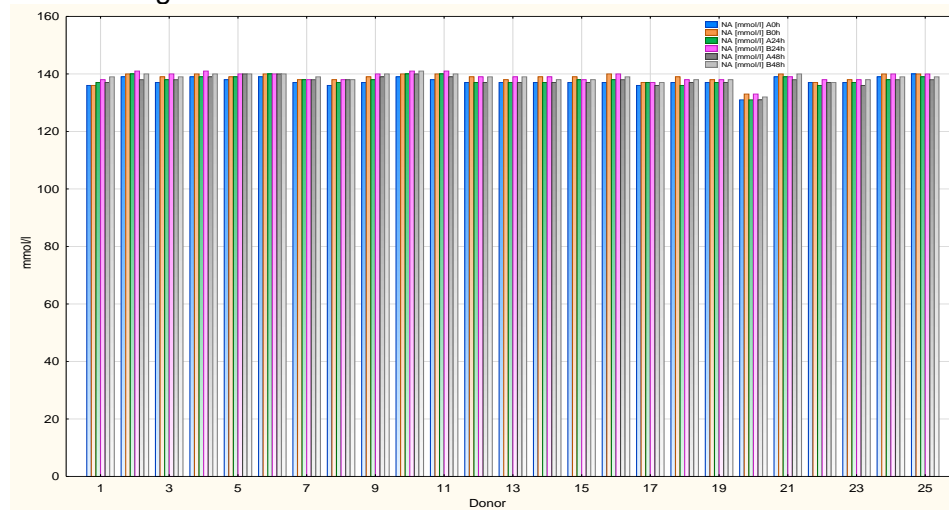
LDL, calculated

Normal range: optimal < 100mg/dl < near optimal < 129 mg/dl < borderline high < 159 mg/dl < high < 190 mg/dl < very high

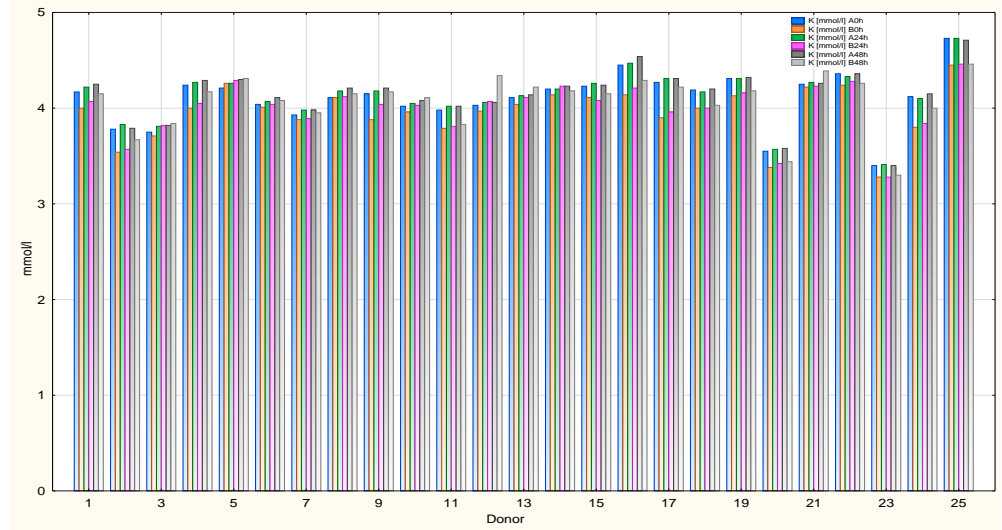


Sodium

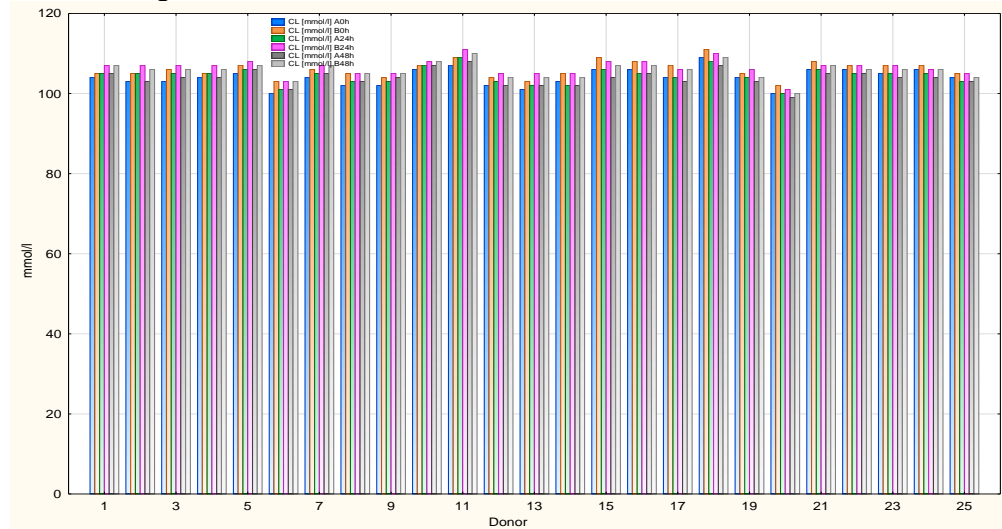
Normal range: 136 - 146 mmol/l



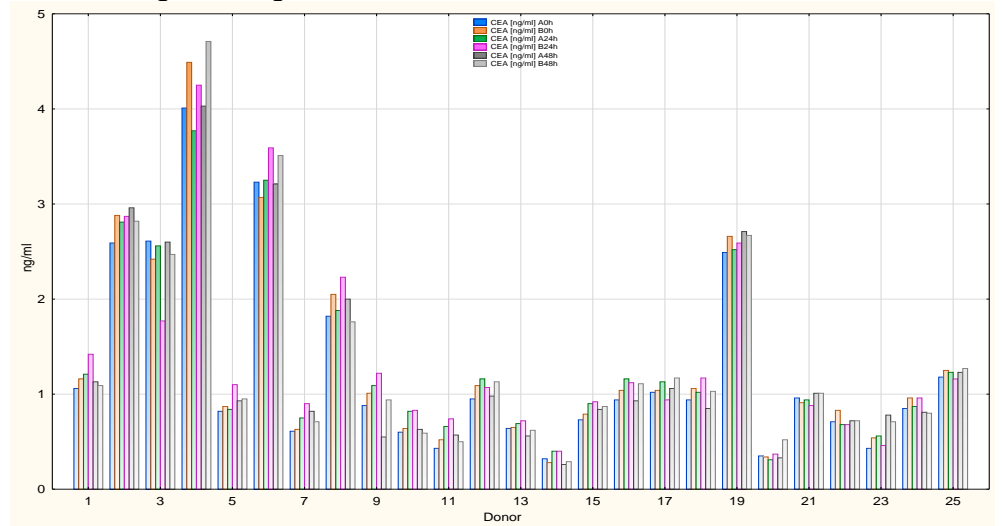
Potassium
 Normal range: Serum 3.5 - 5.1 Plasma 3.4 - 4.5 mmol/l



Chloride
 Normal range: 101 - 109 mmol/l

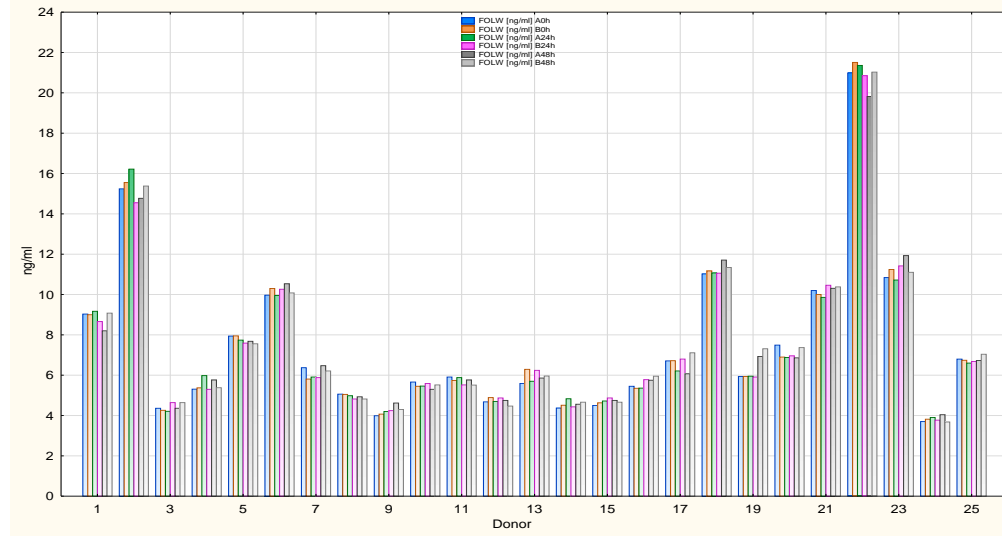


Carcinoembryonic antigen
 Normal range: < 3 ng/ml



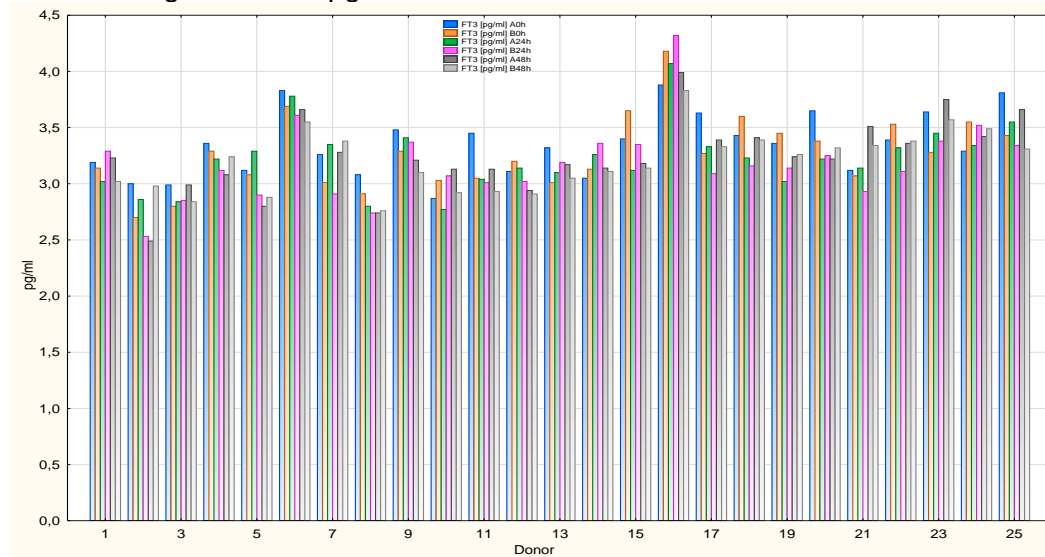
Folic Acid

Normal range: folate deficiency = < 4ng/ml Serum



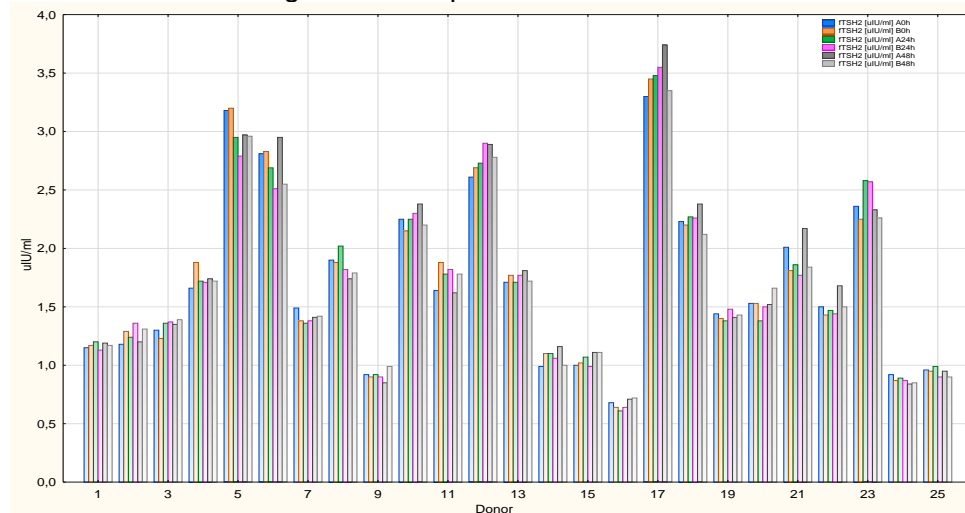
Free Triiodothyronin

Normal range: 2.5 - 3.9 pg/ml



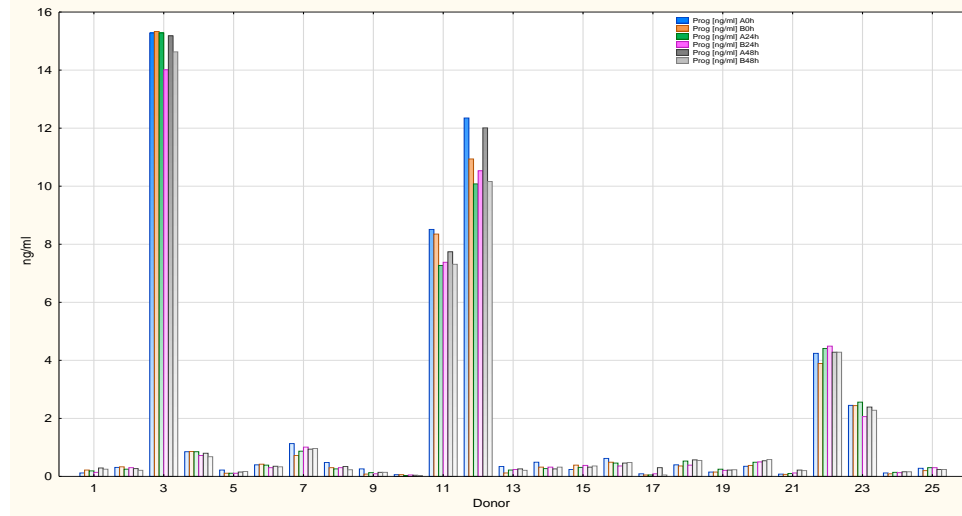
Free Thyrotrope

Hormone Normal range: 0.1 - 3.5 μ U/ml



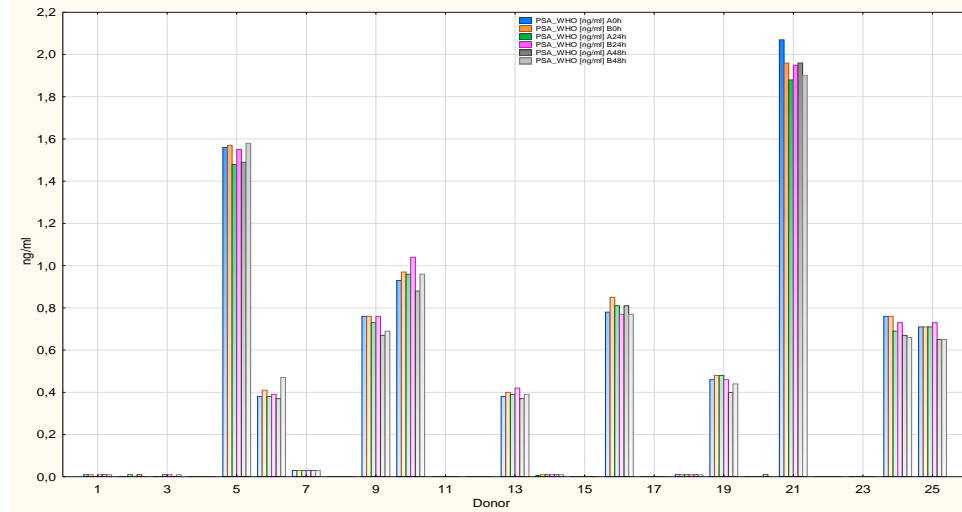
Progesterone

Normal range: (f) 0.07 - 1.38 ng/ml(m)



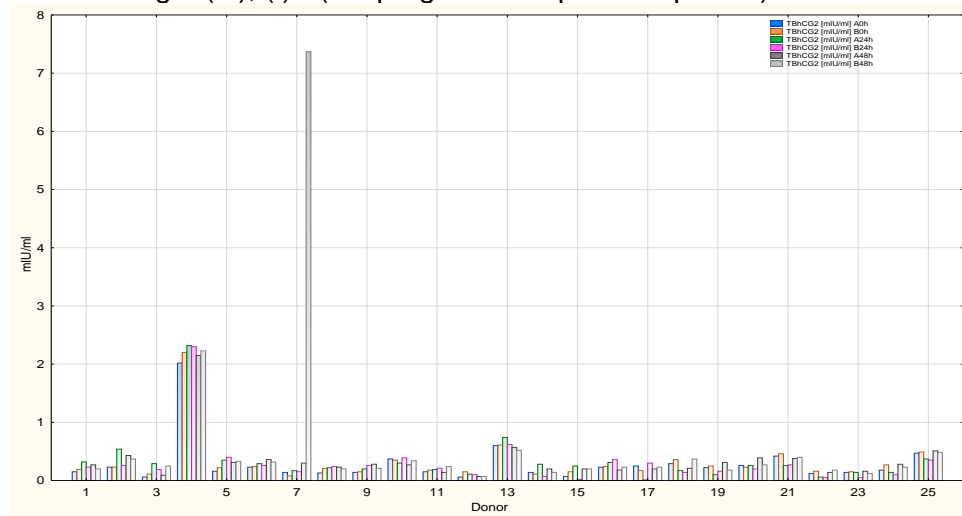
Prostate-specific antigen

Normal range: < 1.8 ng/ml

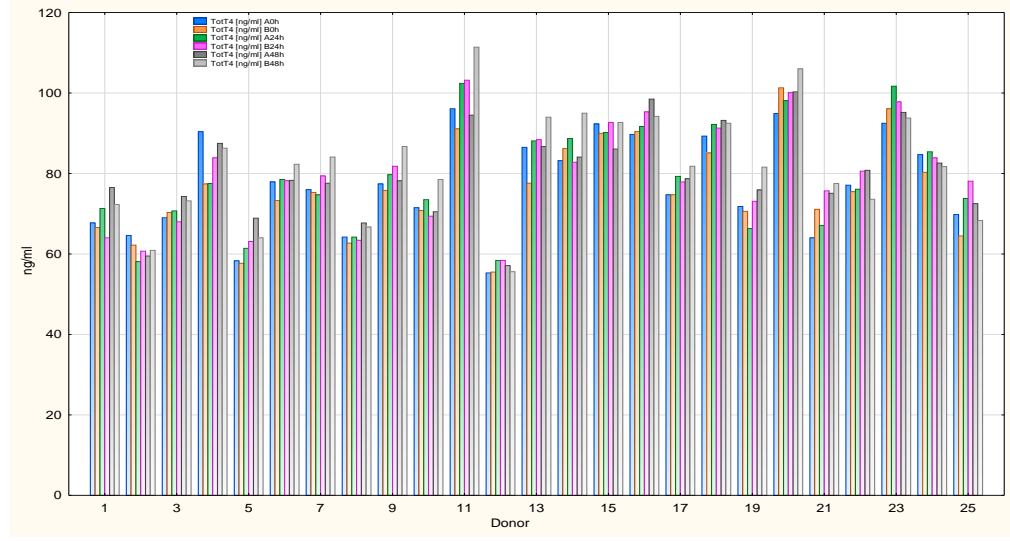


Human beta chorionic gonadotropin

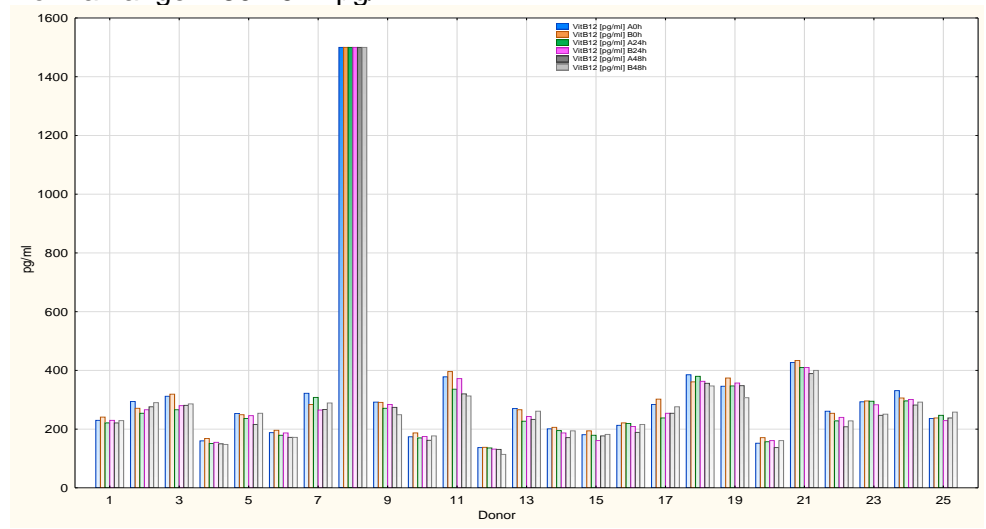
Normal range: (m), (f) - (nonpregnant and premenopausal) < 5 IU/l



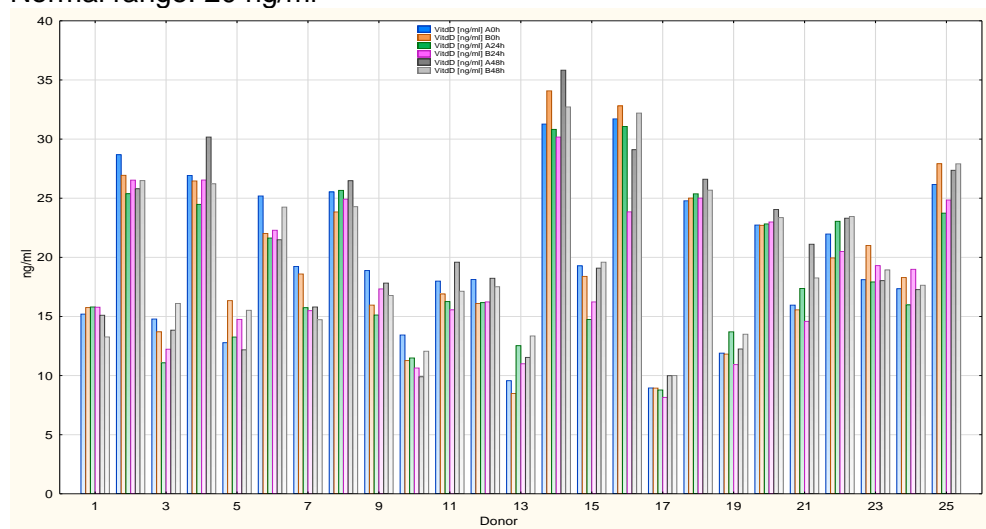
Total Thyroxin
 Normal range: 6.09 - 12.2 µg/dl



Vit B₁₂ (Cobalamine)
 Normal range: 180 - 914 pg/ml



Vitamin D₃
 Normal range: 20 ng/ml



Troponin I

Normal range: 0.1-0.2 µg/l

