

Stability of **VACUETTE**[®] Lithium Heparin Separator tubes with modified centrifugation conditions

Background:

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

The anticoagulant heparin activates antithrombins, thus blocking the coagulation cascade and producing a whole blood/plasma sample making it ideal for rapid analysis and analysis of blood from patients on anticoagulant therapy.

VACUETTE[®] Lithium Heparin Separator tubes contain a barrier gel in the tube. The specific gravity of this material lies between the blood cells and plasma.

During centrifugation the gel moves upwards providing a stable barrier separating the plasma from cells. Plasma may be aspirated directly from the collection tube, eliminating the need for manual transfer to another container for analysis.^[1]

Study Objective:

In order to shorten turn-around-time (TAT), laboratory professionals very often centrifuge a variety of blood collection tubes under the same centrifuge settings. This study was carried out to compare two different centrifugation conditions including stability of analytes for 48 hours in order to improve laboratory efficiency by standardizing centrifugation conditions.^[2,3]

Study design and procedure:

Venous blood was collected from 40 presumably healthy donors into four **VACUETTE**[®] Lithium Heparin Separator tubes (456087, 13/100, 5 ml) using **VACUETTE**[®] Safety Blood Collection Sets with Holder (item # 450085). Two different **VACUETTE**[®] gel tubes (Sample A US P-Gel and Sample B European Gel) were included in this test. A discard tube was used to guarantee appropriate filling of the tubes. The order of collection was randomized. All tubes were inverted 8 times immediately after blood collection. Tubes were then centrifuged with a Rotanta 460R centrifuge from Hettich at two different centrifugation conditions (see Table 1).

Table 1: Centrifugation

Sample	RCF (x g)	Time	Temp [°C]
Sample A P-Gel	2200g	15 min	20 °C
Sample A P-Gel	1800g	10 min	20 °C
Sample B Gel	2200g	15 min	20 °C
Sample B Gel	1800g	10 min	20 °C

All sample tubes were analyzed for the components listed below at the initial time point within 2 hours of blood collection and after 48 hours on the Siemens Advia analyzer and Abbott Architect analyzer using accompanying reagents from the instrument manufacturers. Between measurements, the samples were stored in the refrigerator at 4-8 °C.

The following parameters were measured:

- Alanine Aminotransferase (ALT)
- Alkaline Phosphatase (ALP)
- Aspartate Aminotransferase (AST)
- Beta-HCG
- Blood Urea Nitrogen (BUN)
- Calcium
- Chloride
- Cholesterol
- Cholinesterase (CHE)
- Creatinine

- Gamma Glutamyltransferase (GGT)
- Glucose
- Iron
- Lactate Dehydrogenase (LDH)
- Magnesium
- Phosphate
- Potassium
- Sodium
- Total Bilirubin
- Total Protein
- Triglyceride
- Troponin I
- Thyroid stimulating hormone (TSH)
- Urea
- Uric acid
- Vitamin B₁₂
- Free Triiodothyronin (fT₃)
- Free Thyroxin (fT₄)

Results:

Comparison analysis was performed at the initial time point and at 48 hours. Statistical evaluation was performed with the T-test ($\alpha = 0.05$) using StatSoft, Version 9. Clinical evaluation was based on the allowed recommendation by the German Medical Association (RILBÄK).^[4] No clinically significant differences were observed for either gel type with the two centrifugation settings of 2200g for 15 minutes and 1800g for 10 minutes at the initial time point or after 48 hours. However, Glucose values were affected by decomposition in cells after 48 hours. The use of a glycolysis inhibitor to compensate for this biochemical process would have been beneficial.

Outlier testing was performed and showed that donor sample 14 (Sample A) for Alkaline Phosphatase at 2200g for 15 min was an outlier and was, therefore, excluded from statistical analysis. For Lactate Dehydrogenase, an analysis error occurred with donor sample 23 at the centrifugation setting of 2200g for 15 minutes at 48 hours (Sample B); donor sample 34 at 1800g for 10 min (Sample B) and donor sample 36 at 1800g for 10 min (Sample B) at the initial time point and 48 hours, all of which were excluded from statistical analysis.

Conclusion:

In this trial, the stability of the biochemical analytes was demonstrated when using the centrifugation conditions as tested (2200g for 15 min at 20°C as well as 1800g for 10 min at 20°C) for **VACUETTE**[®] Lithium Heparin Separator tubes to optimize TAT in routine laboratories. These centrifugation conditions for **VACUETTE**[®] Lithium Heparin Separator tubes showed comparable results for the biochemical parameters tested at the initial time point and at 48 hours. Differences in results were not clinically significant.

References:

[1] Instructions for Use. Evacuated Blood Collection System. For in vitro Diagnostic Use. Rev. 13.

[2] O'Keane M. P., Evaluation of three different specimen types (serum, plasma, lithium heparin and serum gel separator) for analysis of certain analytes: clinical significance of differences in results and efficiency in use. Clin. Chem. Labor. Med. (44), 5 662-668

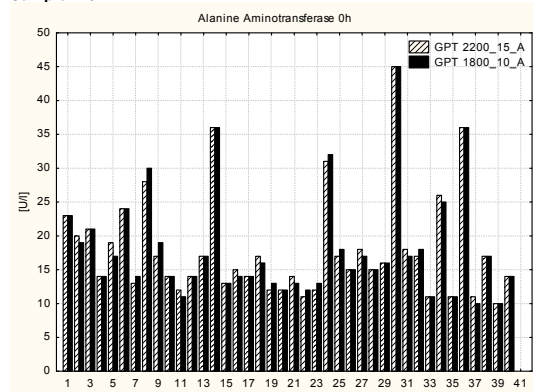
[3] Thomas L., Laboratory and Diagnosis. Indication and Evaluation of Laboratory Results for Medical Diagnosis. 7th edition. TH-Books.

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

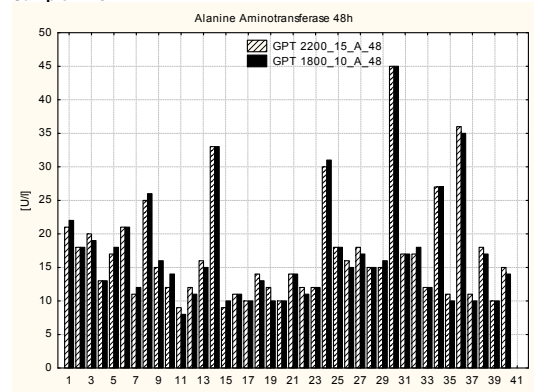
Alanine Aminotransferase (ALT, GPT)

Reference range: 0-45 U/l

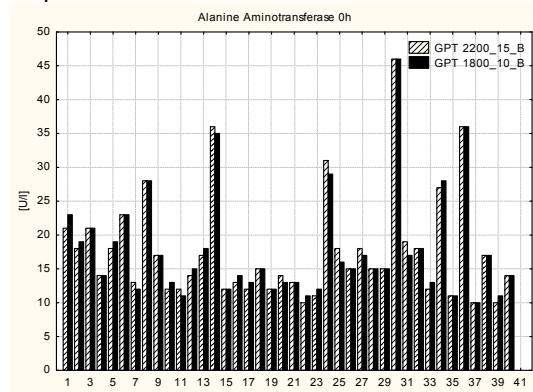
Sample A 0h



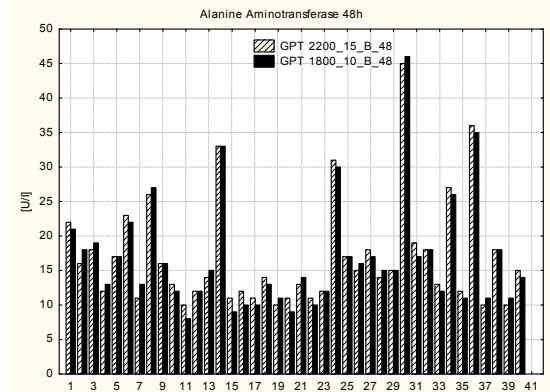
Sample A 48h



Sample B 0h



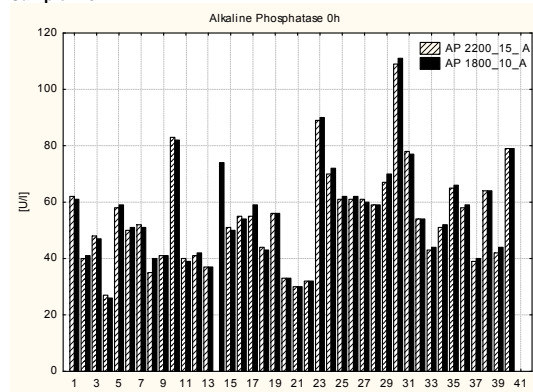
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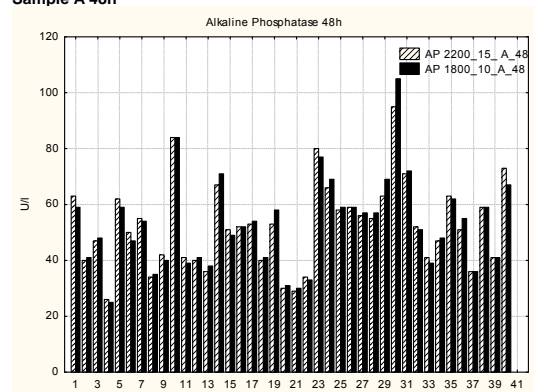
Alkaline Phosphatase:

Reference range: 30-120 U/l

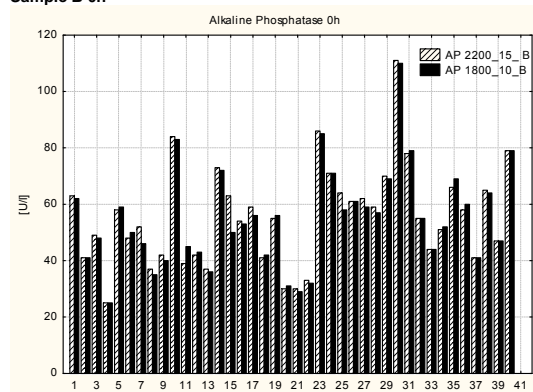
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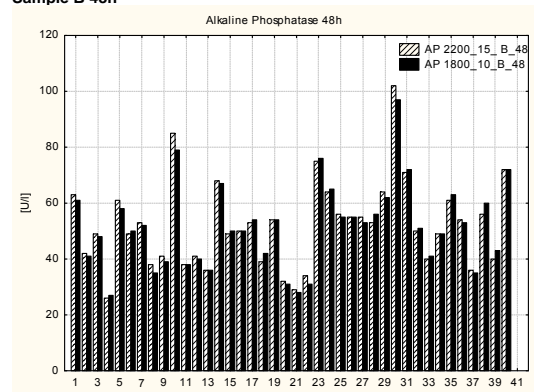
Sample A 48h



Sample B 0h

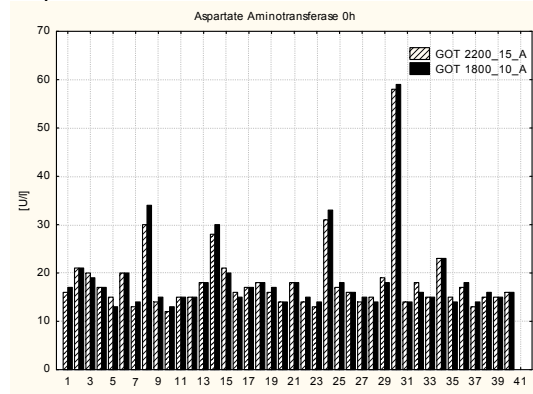


Sample B 48h

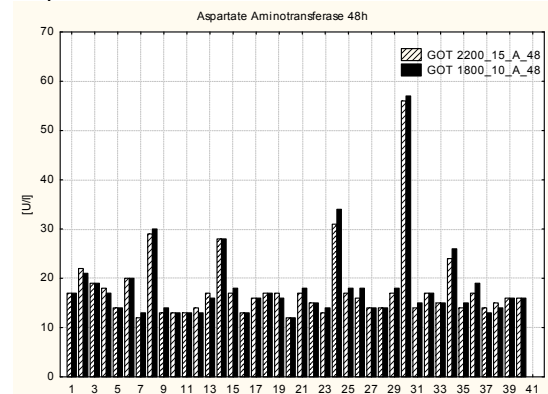


Aspartate Aminotransferase (AST, GOT)
Reference range: 0-35 U/l

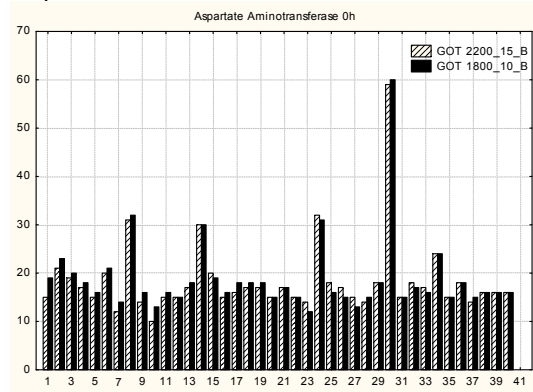
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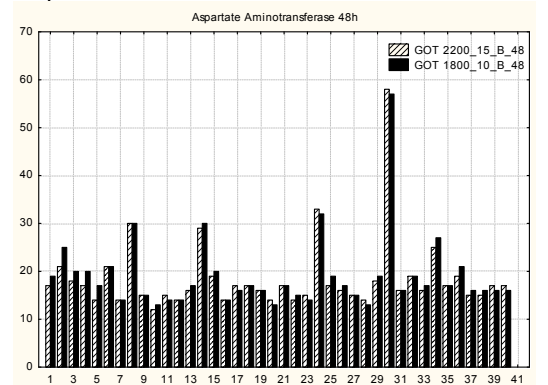
Sample A 48h



Sample B 0h



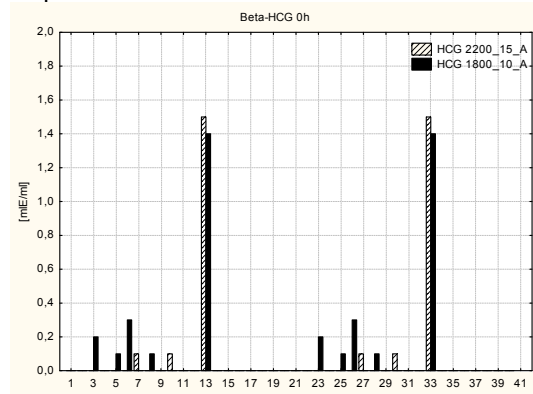
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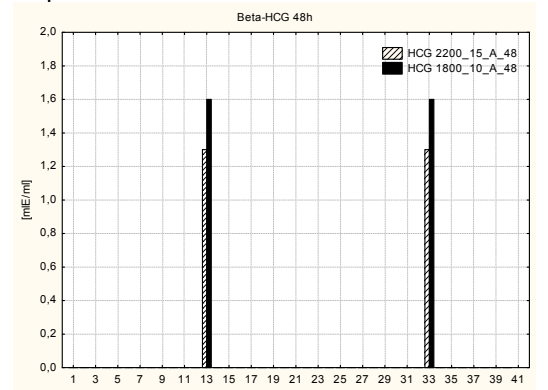
Beta-HCG

Reference range: 0-0.1 mIE/ml

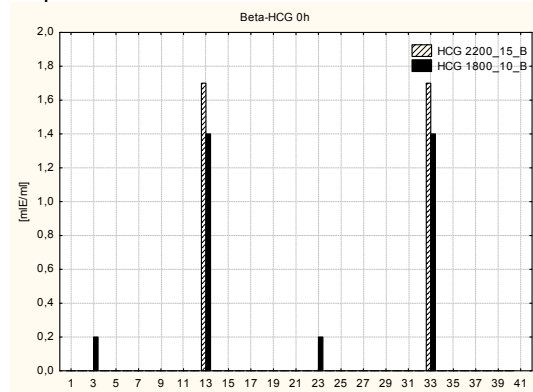
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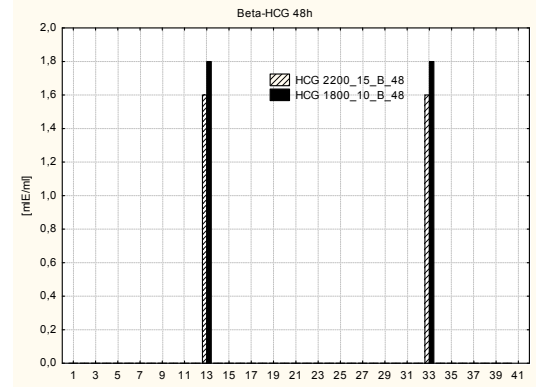
Sample A 48h



Sample B 0h



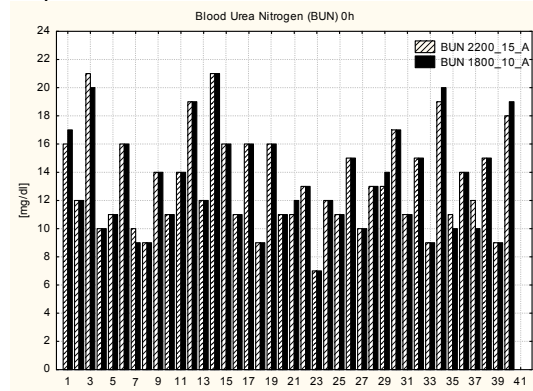
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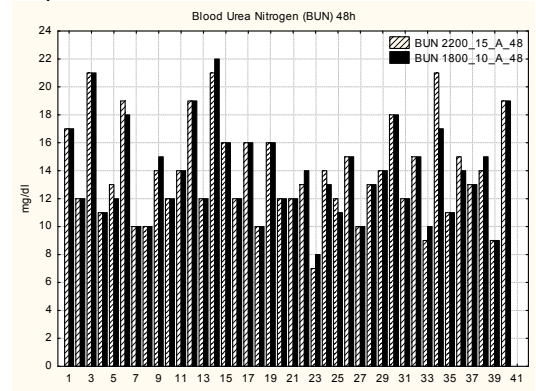
Blood Urea Nitrogen (BUN)

Reference range: 7-23 mg/dl

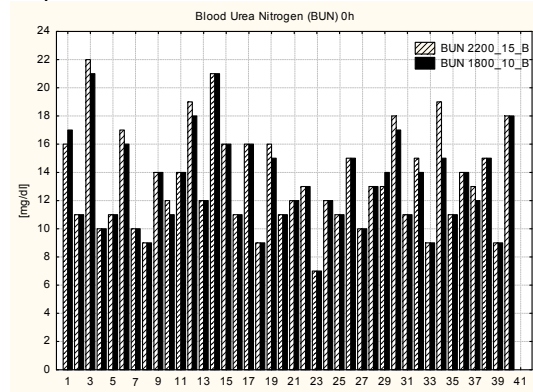
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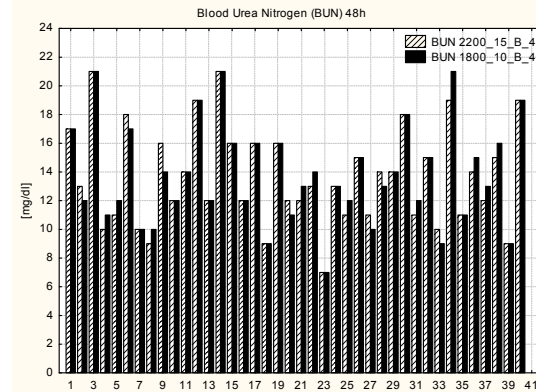
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Sample B 0h



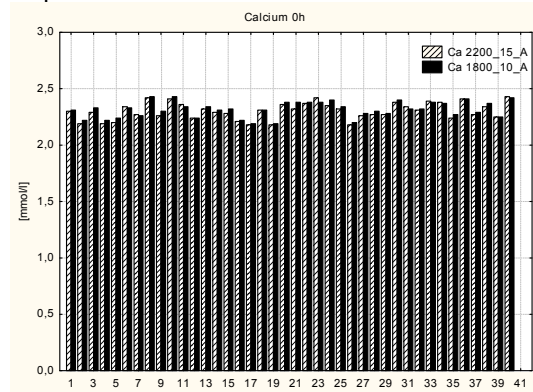
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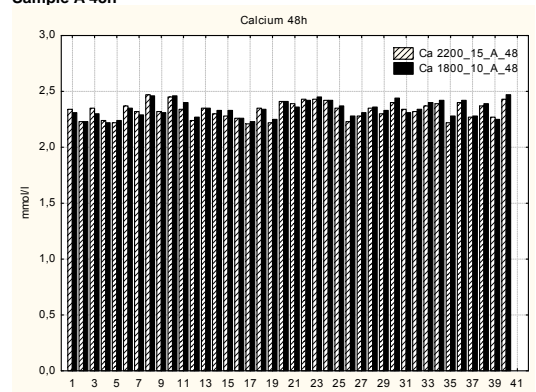
Calcium

Reference range: 2.1-2.7 mmol/l

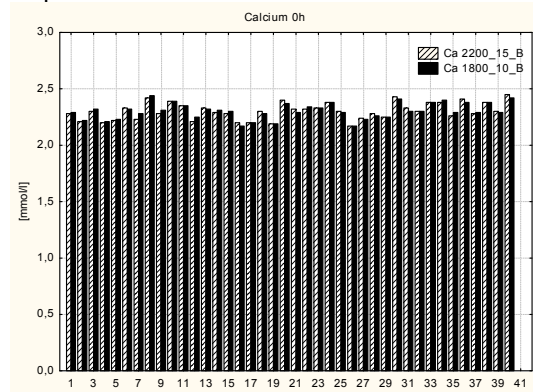
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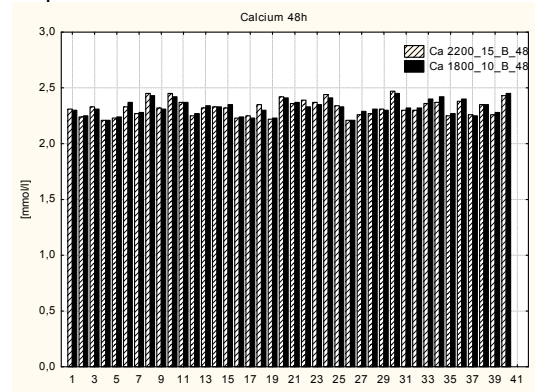
Sample A 48h



Sample B 0h



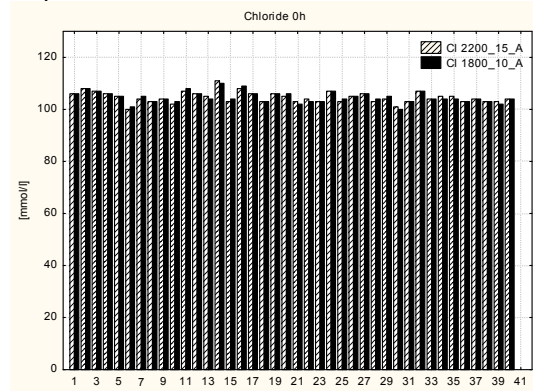
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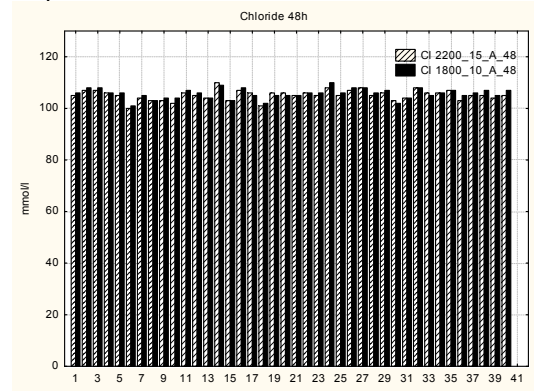
Chloride

Reference range: 95-105 mmol/l

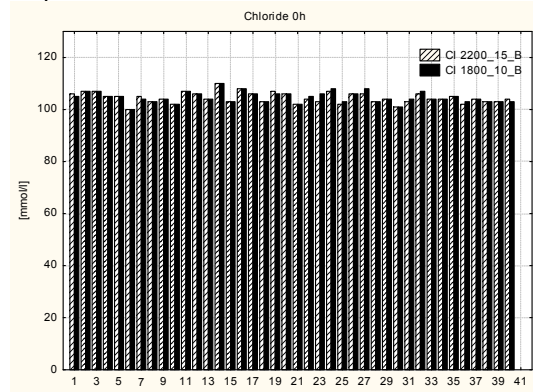
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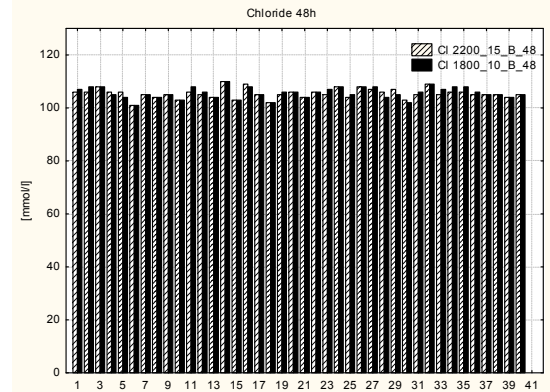
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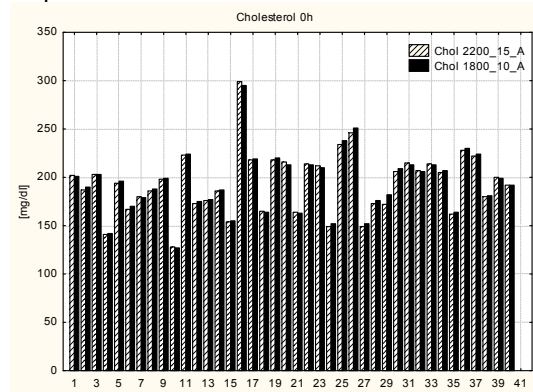
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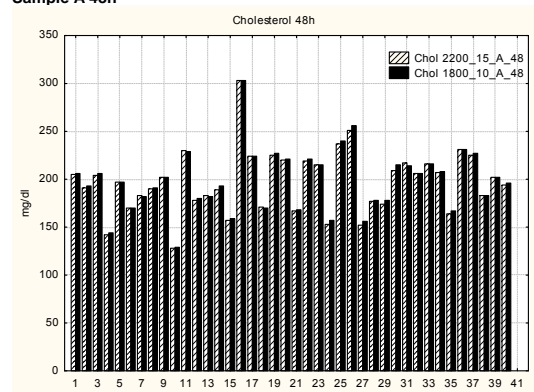
Cholesterol

Reference range: 100-200 mg/dl

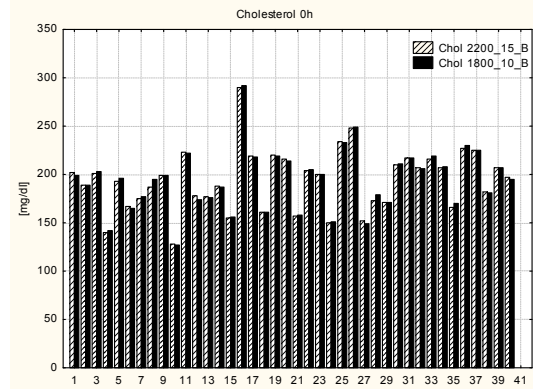
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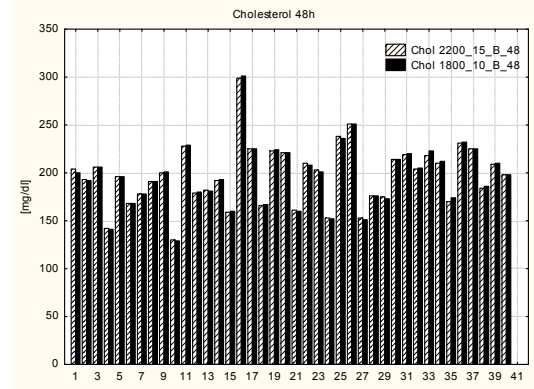
Sample A 48h



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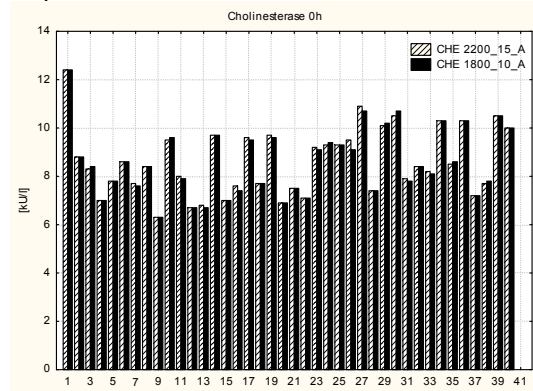
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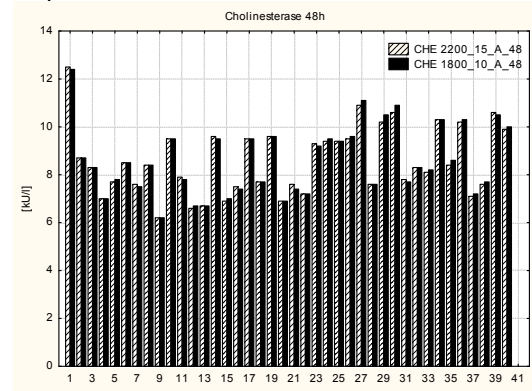
Cholesterinesterase

Reference range: 4.5-11.5 kU/l

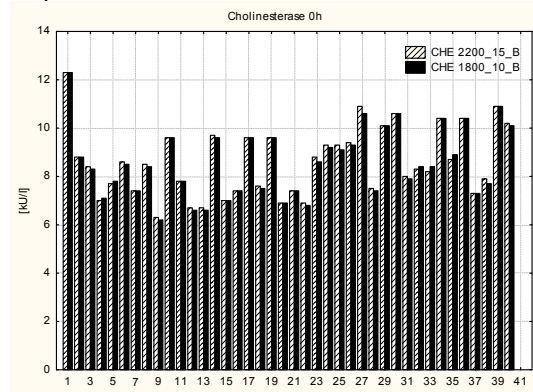
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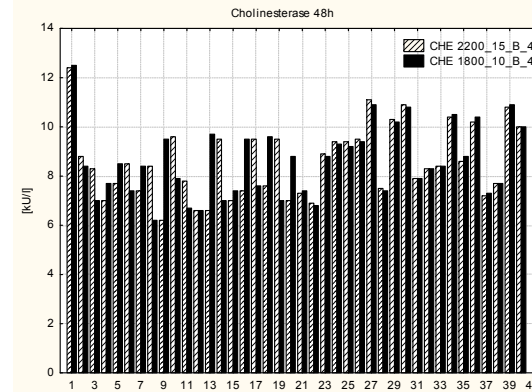
Sample A 48h



Sample B 0h



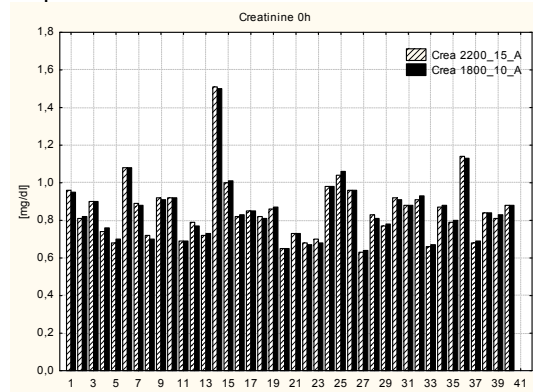
Sample B 48h



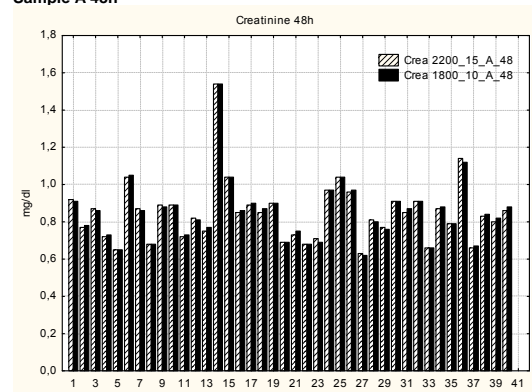
Creatinine

Reference range male: 0.55-1.17 mg/dl

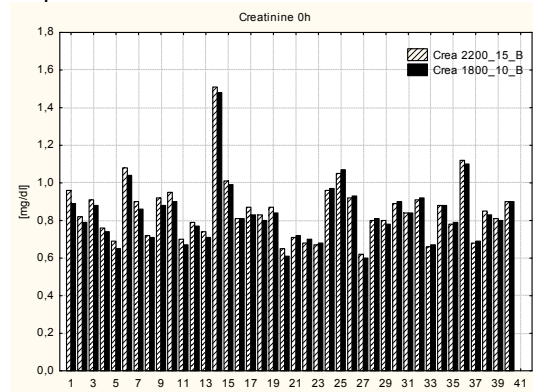
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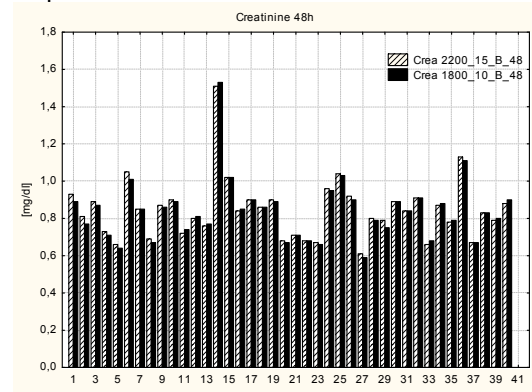
Sample A 48h



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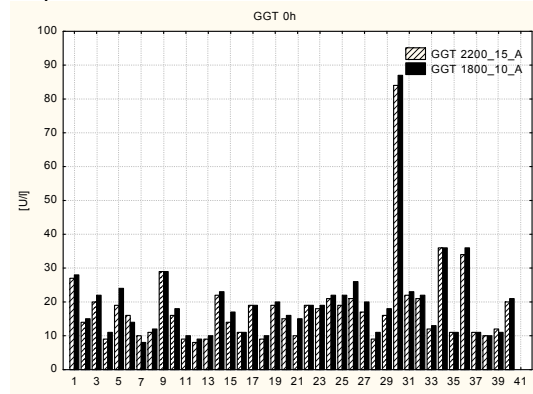


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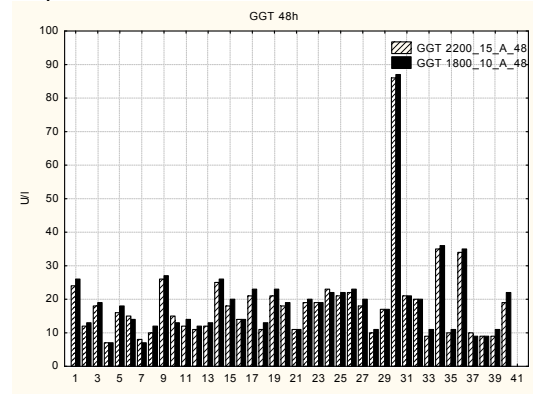


Gamma Glutamyltransferase (GGT)
Reference range: 0- 55 U/l

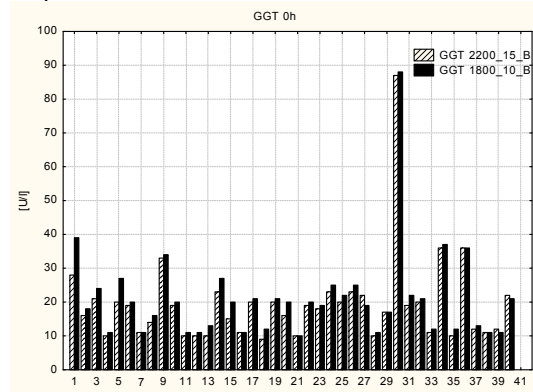
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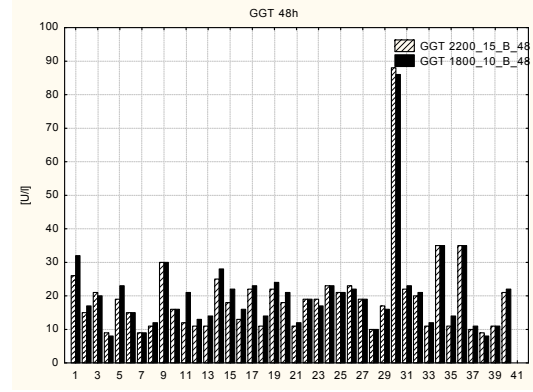
Sample A 48h



Sample B 0h



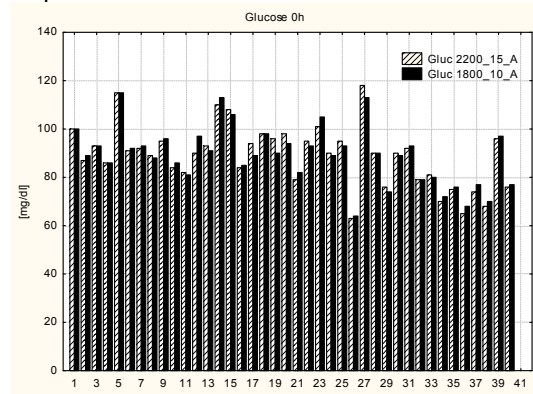
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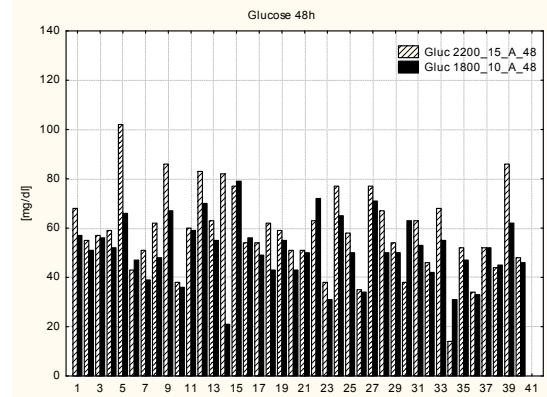
Glucose

Reference range: 60-110 mg/dl

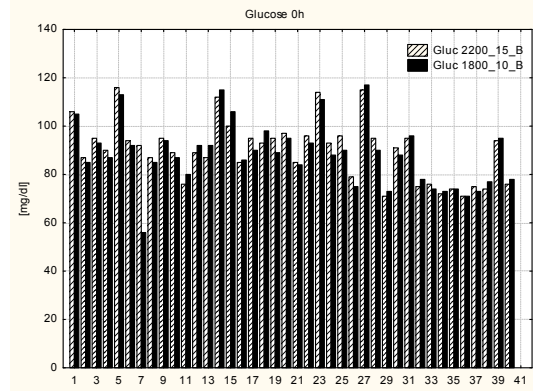
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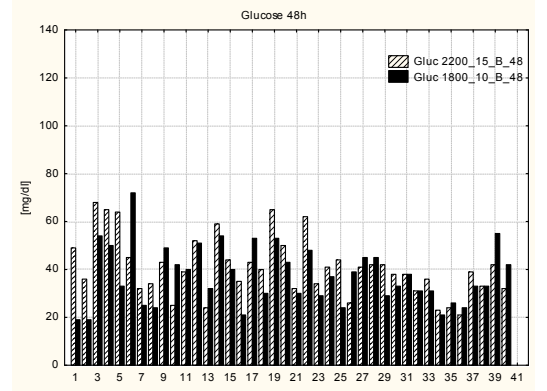
Sample A 48h



Sample B 0h



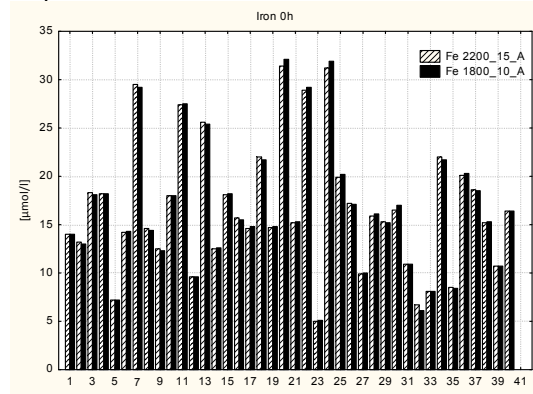
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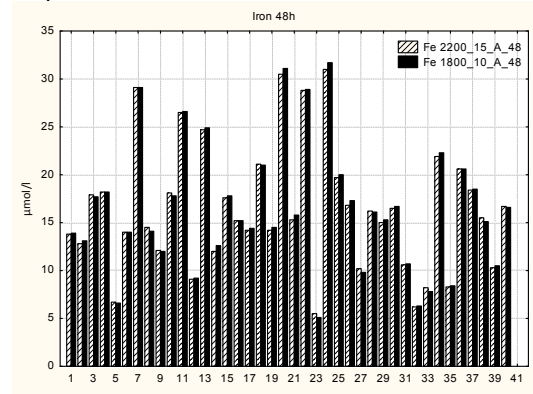
Iron

Reference range: 14.3-32.2 µmol/l

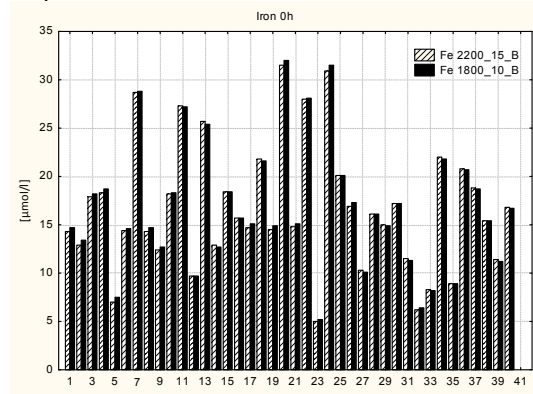
Sample A 0h



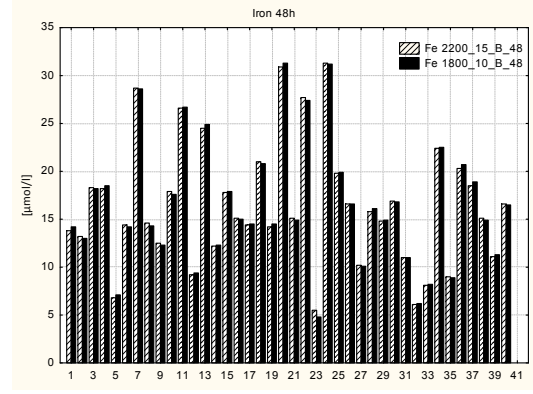
Sample A 48h



Sample B 0h



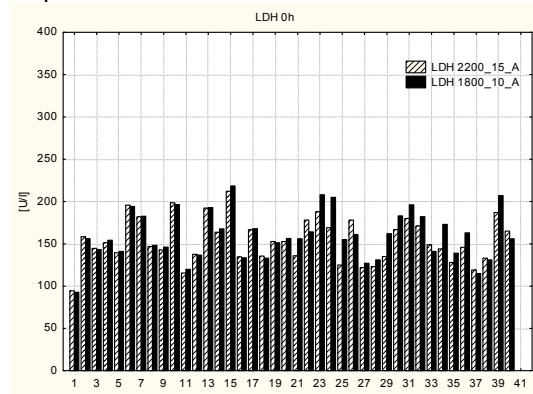
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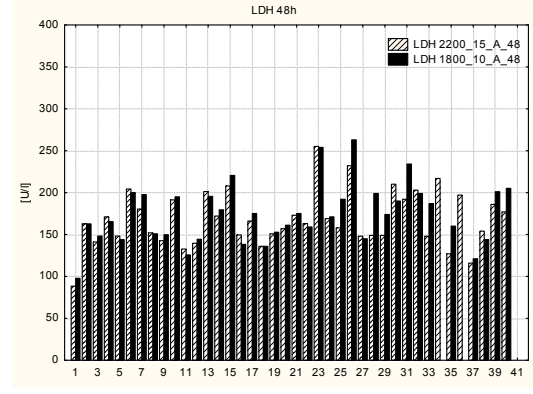
Lactate Dehydrogenase

Reference range: 0-248 U/l

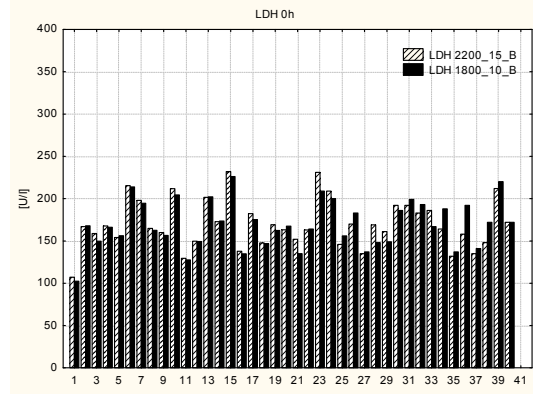
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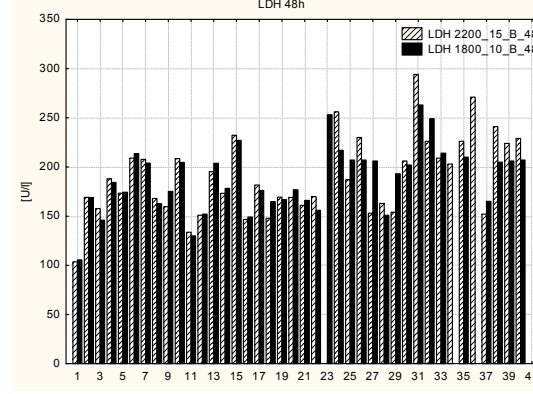
Sample A 48h



Sample B 0h



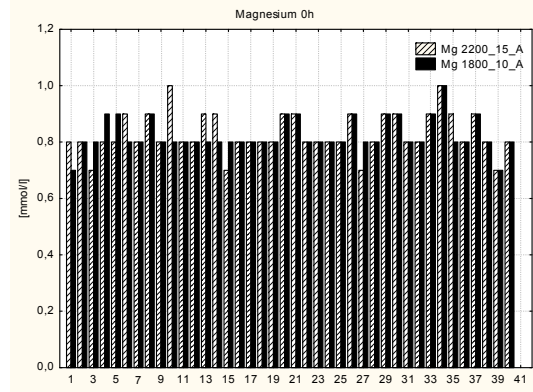
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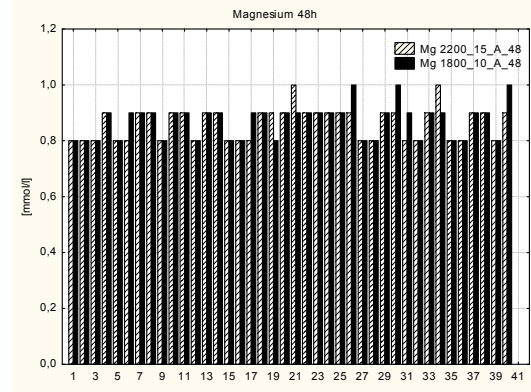
Magnesium

Reference range: 0.7-1.0 mmol/l

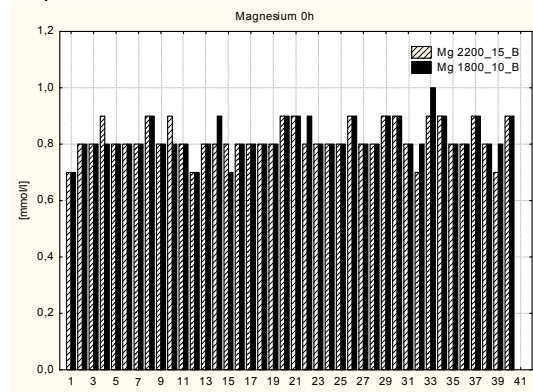
Sample A 0h



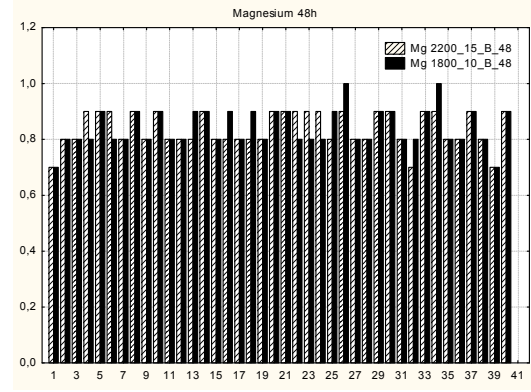
Sample A 48h



Sample B 0h



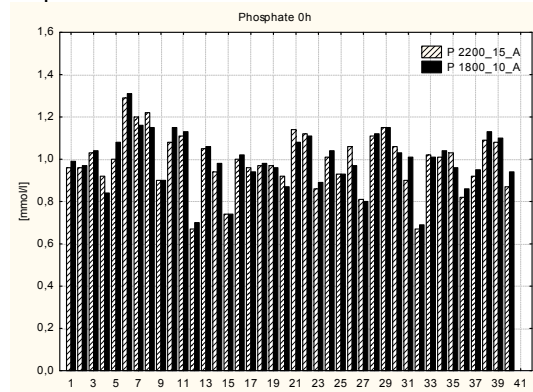
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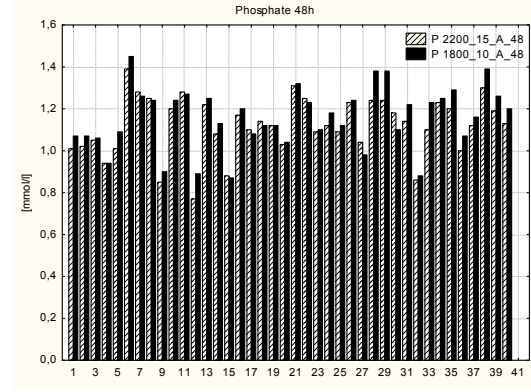
Phosphate

Reference range: 0.77-1.45 mmol/l

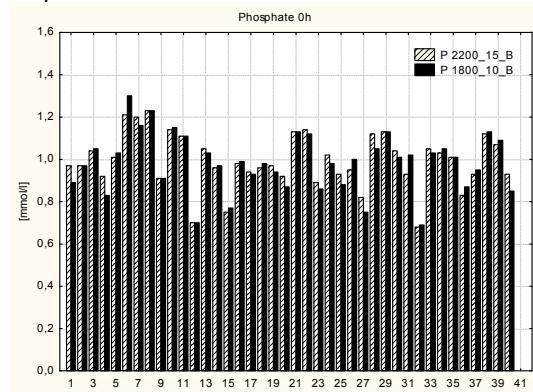
Sample A 0h



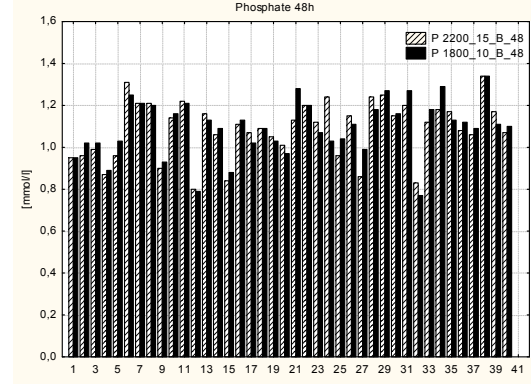
Sample A 48h



Sample B 0h



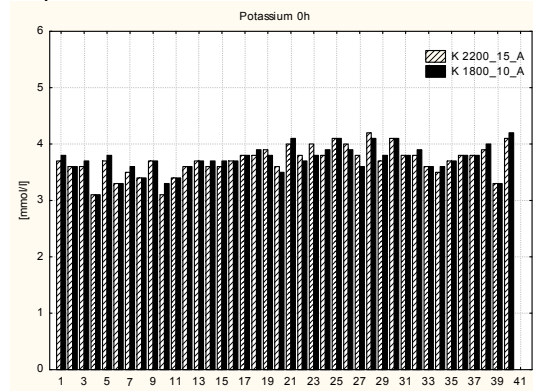
Sample B 48h



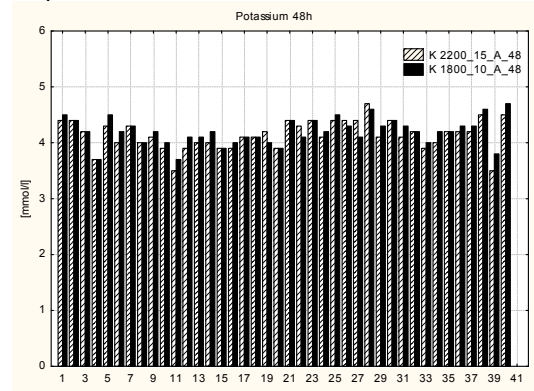
Potassium

Reference range: 3.5-5.3 mmol/l

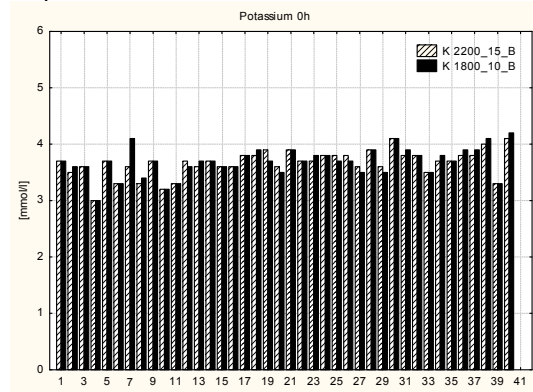
Sample A 0h



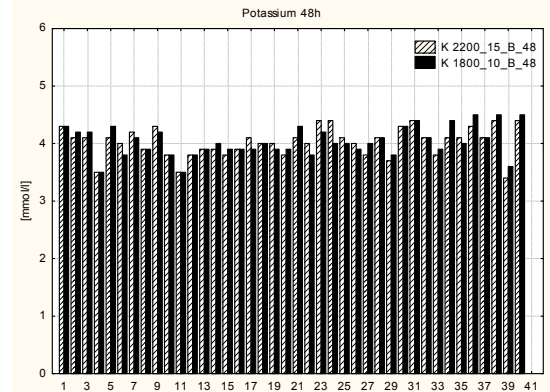
Sample A 48h



Sample B 0h



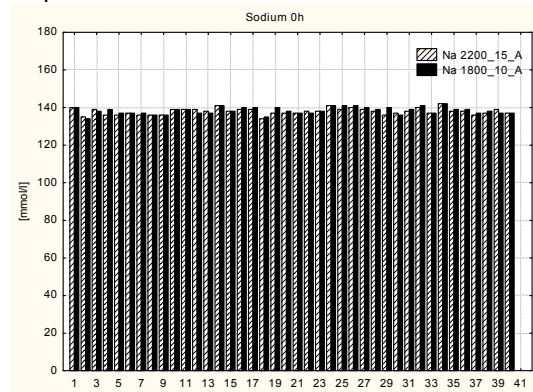
Sample B 48h



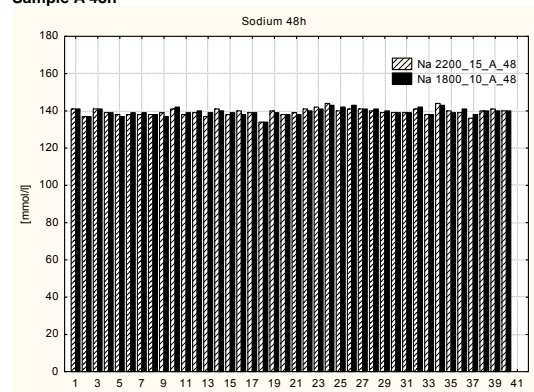
Sodium

Reference range: 135-150 mmol/l

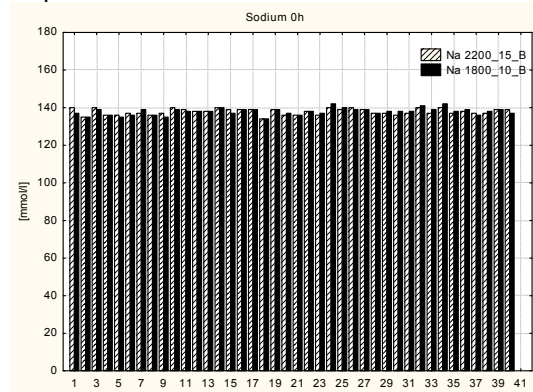
Sample A 0h



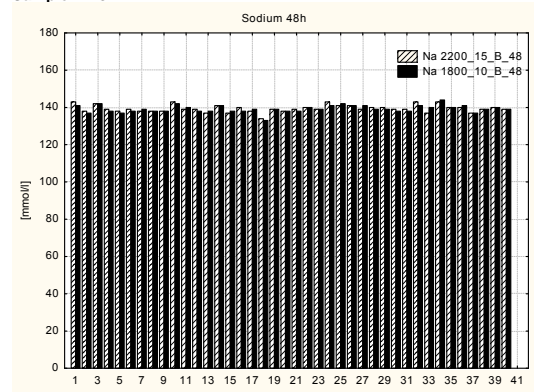
Sample A 48h



Sample B 0h



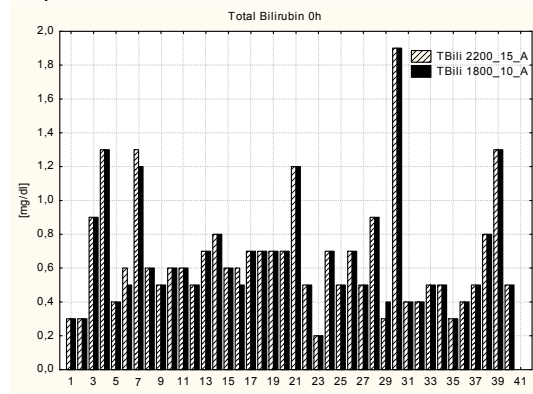
Sample B 48h



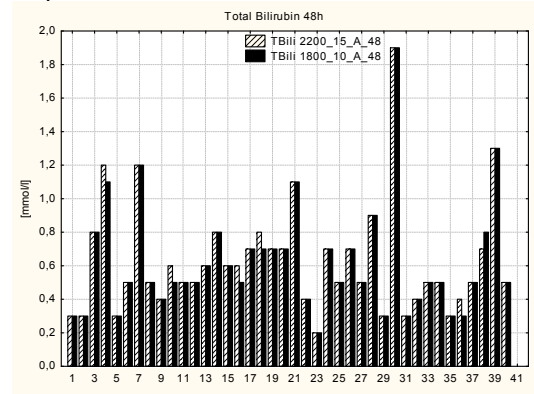
Total Bilirubin

Reference range: 0.1-1.2 mg/dl

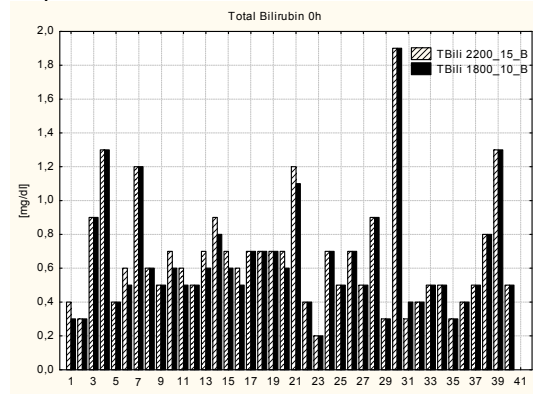
Sample A 0h



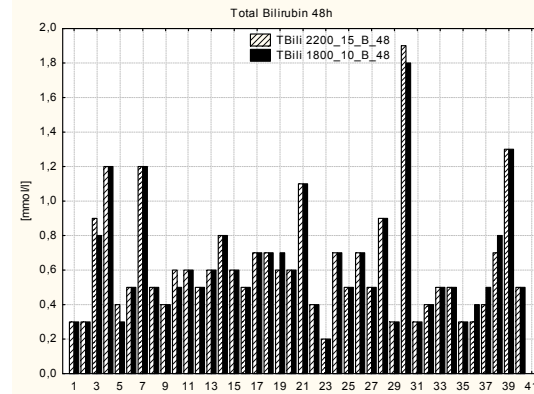
Sample A 48h



Sample B 0h



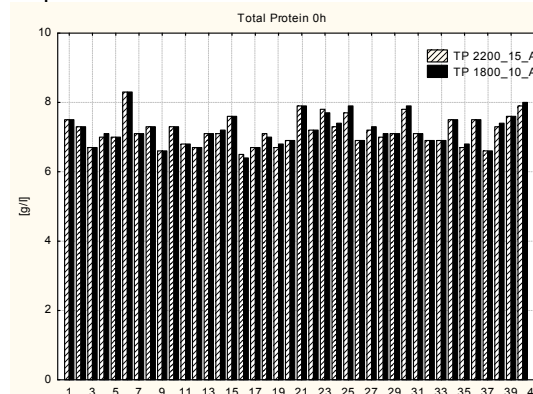
Sample B 48h



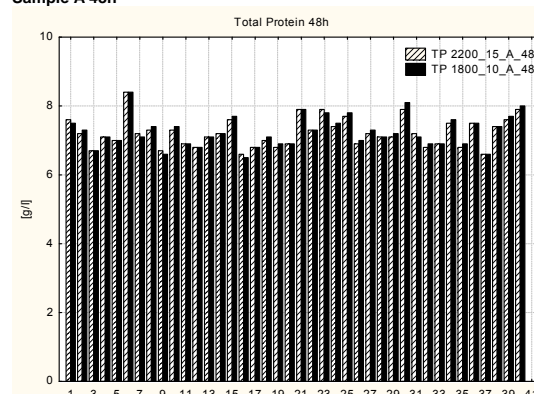
Total Protein

Reference range: 6-8 g/l

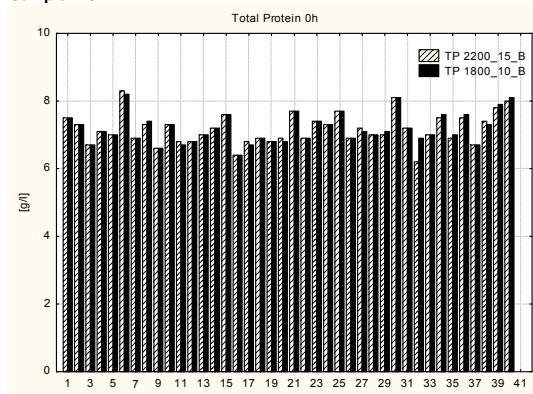
Sample A 0h



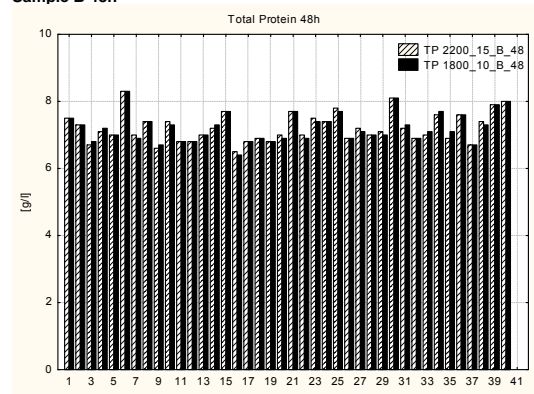
Sample A 48h



Sample B 0h



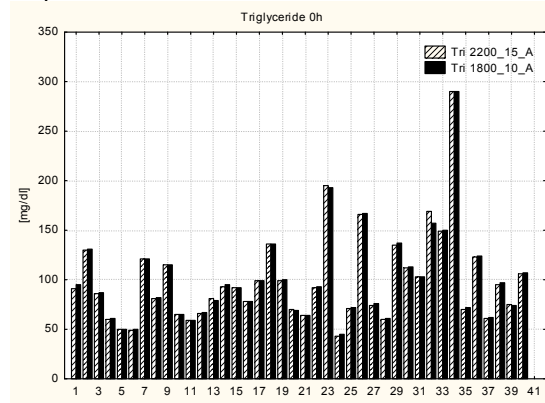
Sample B 48h



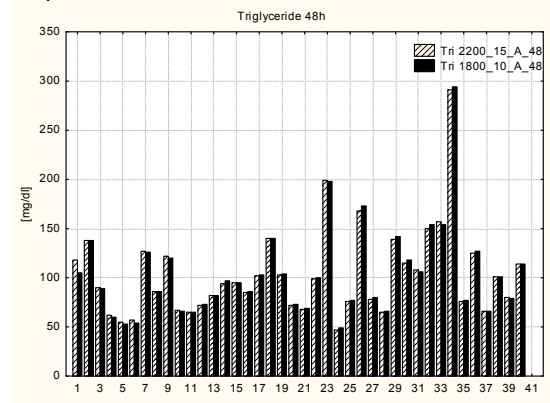
Triglyceride

Reference range: 25-180 mg/dl

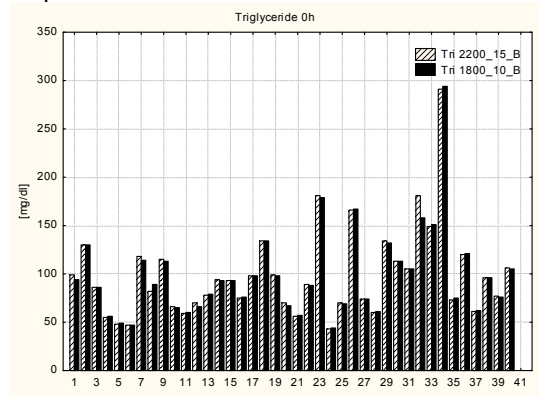
Sample A 0h



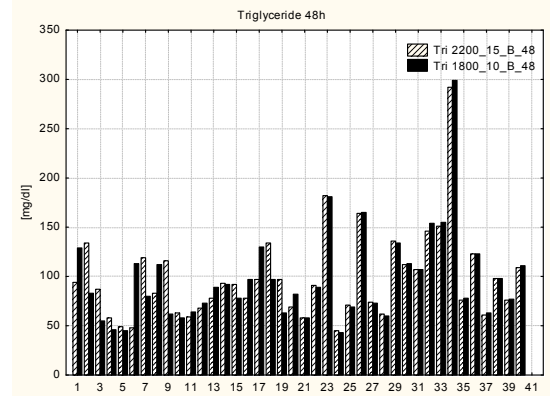
Sample A 48h



Sample B 0h



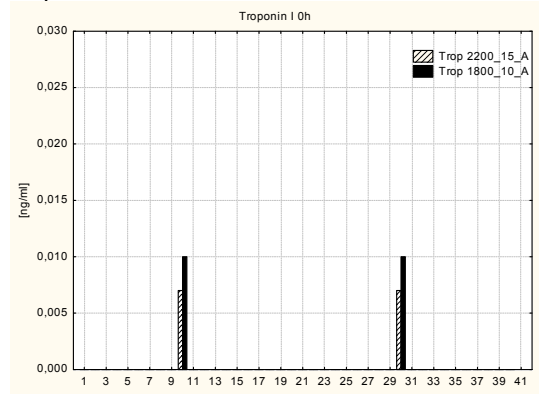
Sample B 48h



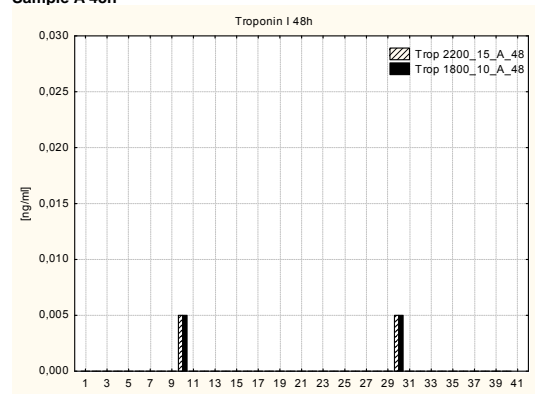
Troponin I

Reference range: 0-0.028 ng/ml

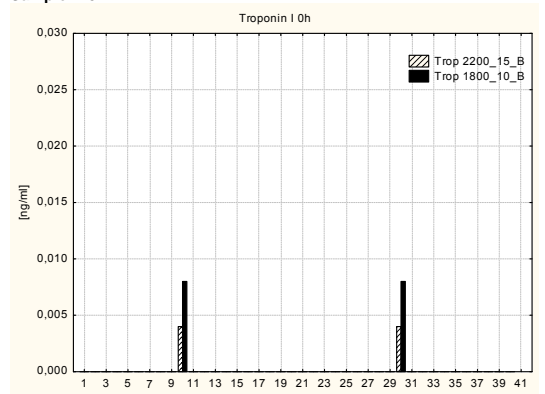
Sample A 0h



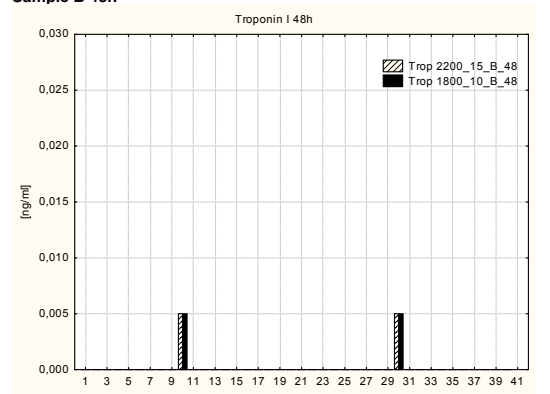
Sample A 48h



Sample B 0h

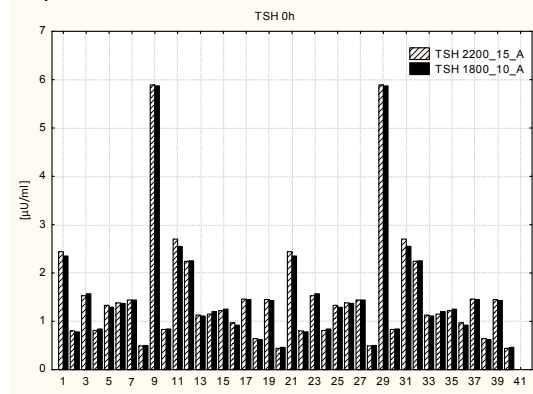


Sample B 48h

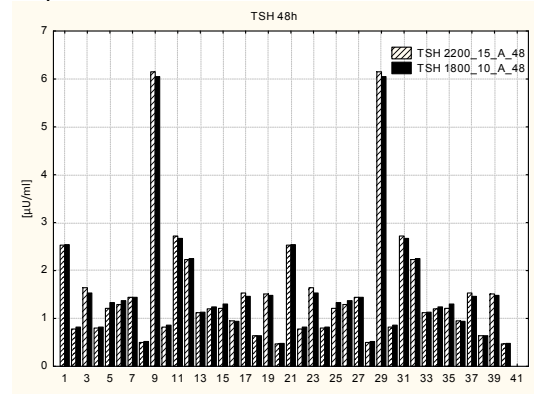


Thyroid stimulating hormone (TSH)
Reference range: 0.47-3.5 µU/ml

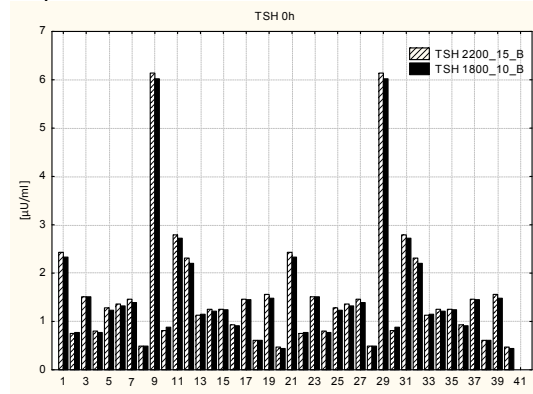
Sample A 0h



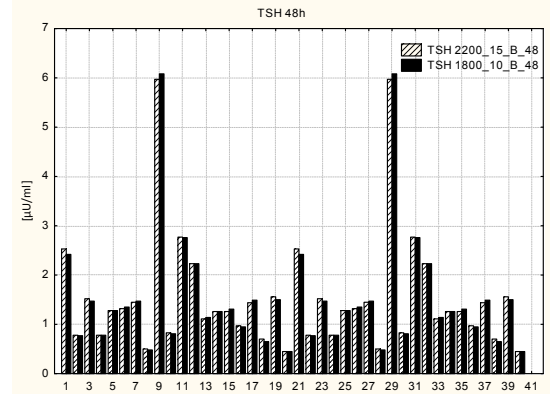
Sample A 48h



Sample B 0h



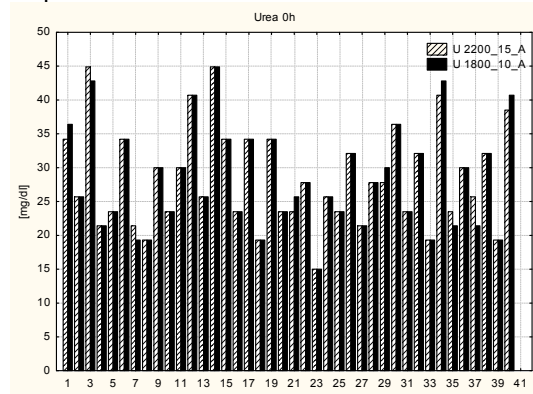
Sample B 48h



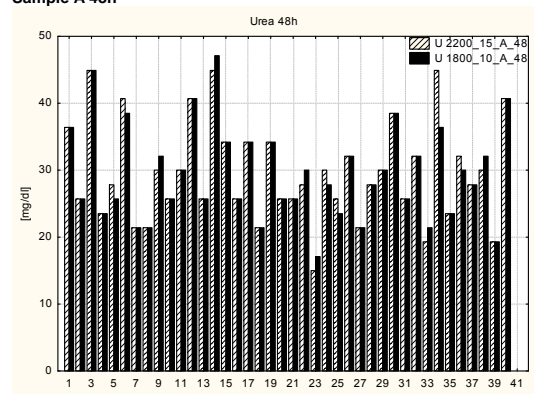
Urea

Reference range: 15-50 mg/dl

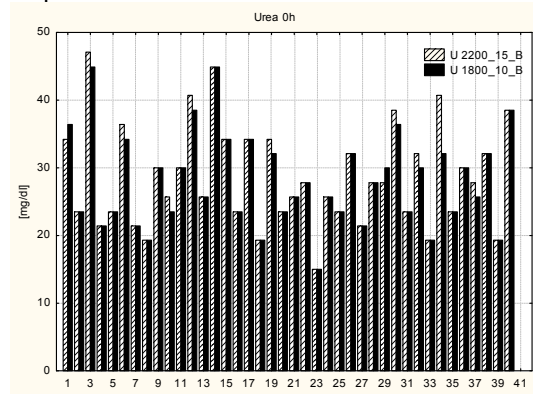
Sample A 0h



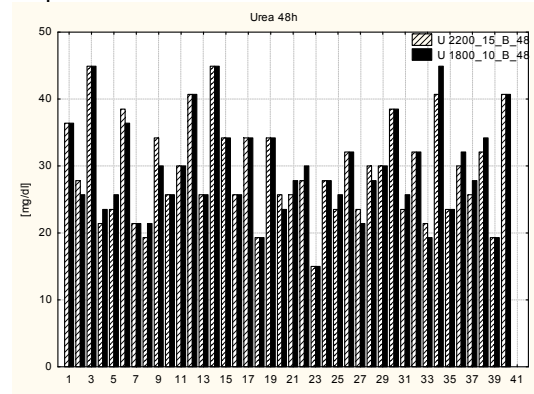
Sample A 48h



Sample B 0h



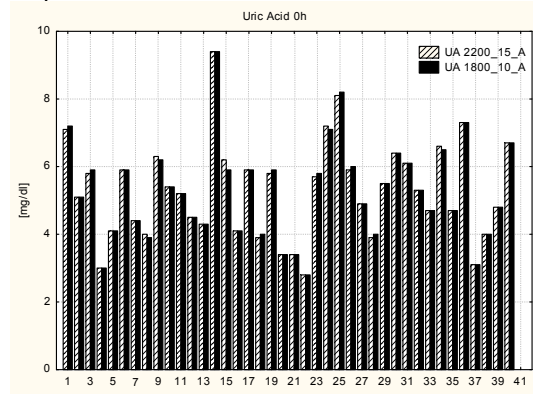
Sample B 48h



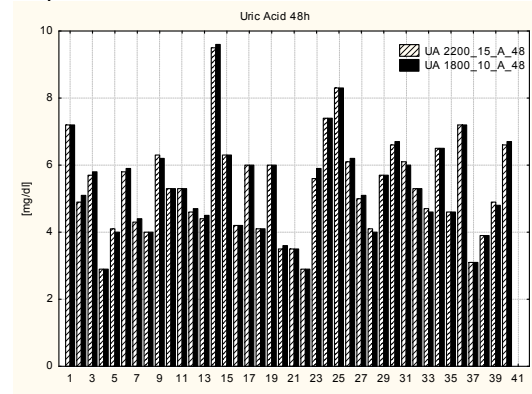
Uric acid

Reference range male: 3.5-7.0 mg/dl

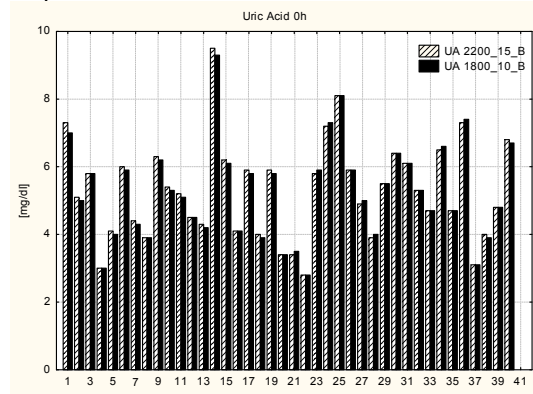
Sample A 0h



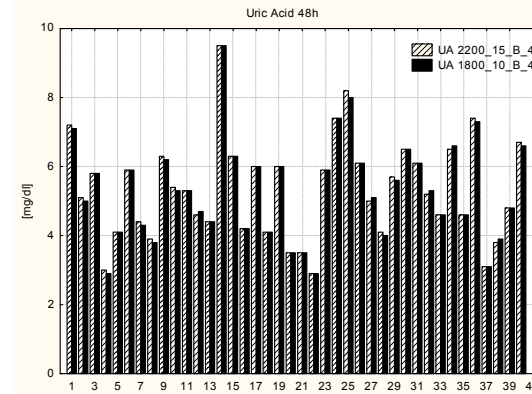
Sample A 48h



Sample B 0h



Sample B 48h

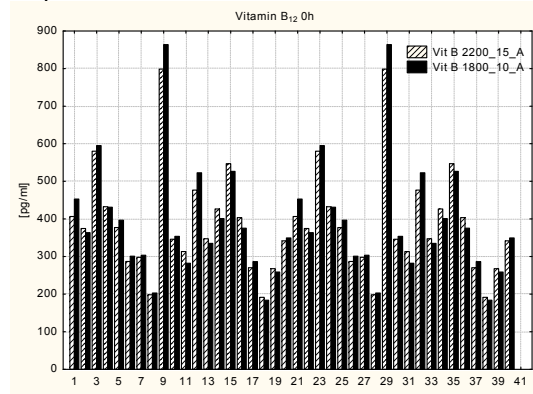


Vitamin B12

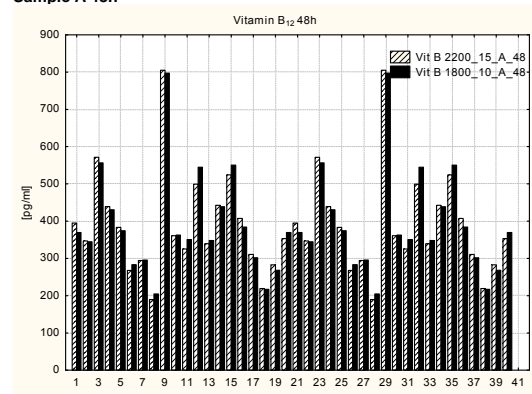
Reference range male: 214-864 ng/l

Reference range female: 182-820 ng/l

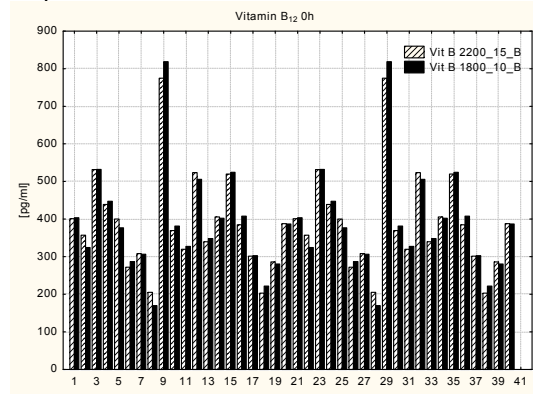
Sample A 0h



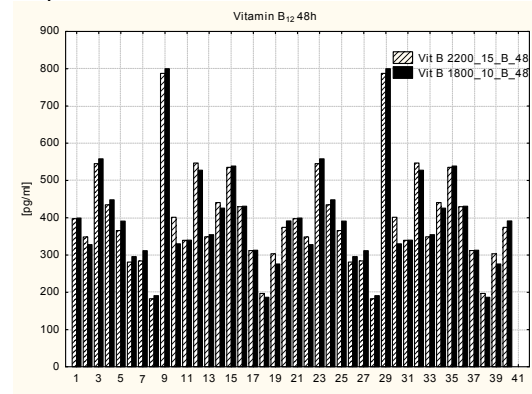
Sample A 48h



Sample B 0h

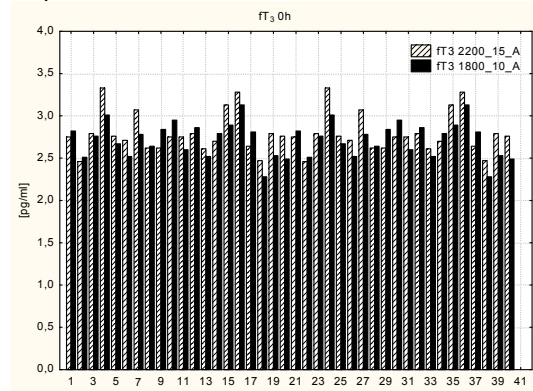


Sample B 48h

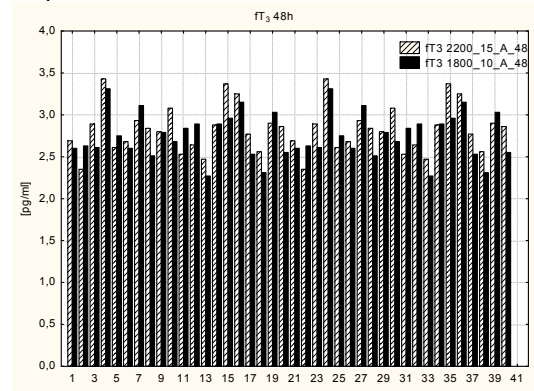


Free Triiodothyronin (fT₃)
 Reference range: 1.39-4.14 pg/ml

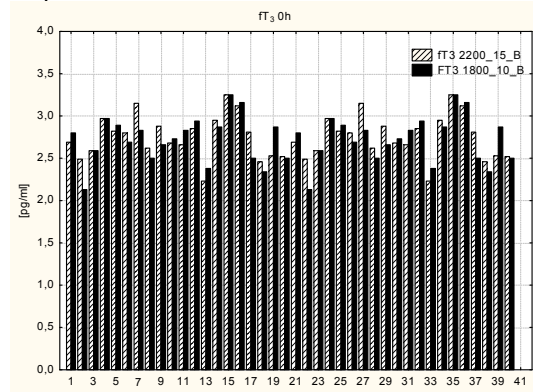
Sample A 0h



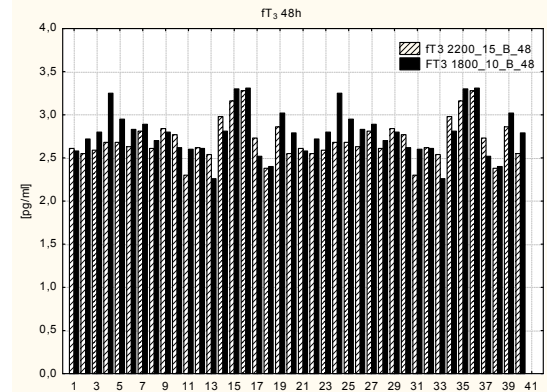
Sample A 48h



Sample B 0h

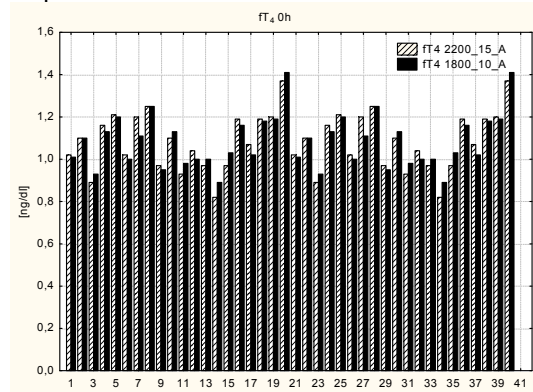


Sample B 48h

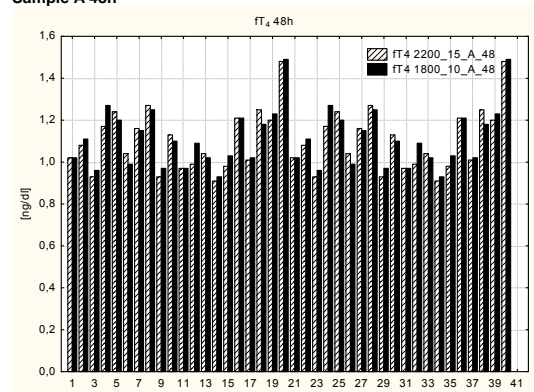


Free Thyroxin (fT₄)
 Reference range: 0.75-2.0 ng/dl

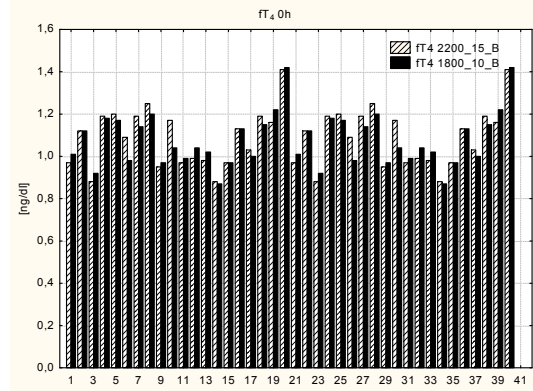
Sample A 0h



Sample A 48h



Sample B 0h



Sample B 48h

