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

<table>
<thead>
<tr>
<th>Sample</th>
<th>RCF (x g)</th>
<th>Time</th>
<th>Temp [°C]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample A P-Gel</td>
<td>2200g</td>
<td>15 min</td>
<td>20 °C</td>
</tr>
<tr>
<td>Sample A P-Gel</td>
<td>1800g</td>
<td>10 min</td>
<td>20 °C</td>
</tr>
<tr>
<td>Sample B Gel</td>
<td>2200g</td>
<td>15 min</td>
<td>20 °C</td>
</tr>
<tr>
<td>Sample B Gel</td>
<td>1800g</td>
<td>10 min</td>
<td>20 °C</td>
</tr>
</tbody>
</table>

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 (RIILBÄK). 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:**


Alanine Aminotransferase (ALT, GPT)
Reference range: 0-45 U/l

Sample A 0h

Sample A 48h

Sample B 0h

Sample B 48h

Alkaline Phosphatase:
Reference range: 30-120 U/l

Sample A 0h

Sample A 48h

Sample B 0h

Sample B 48h
Aspartate Aminotransferase (AST, GOT)
Reference range: 0-35 U/l

Sample A 0h

Sample B 0h

Beta-HCG
Reference range: 0-0.1 mIE/ml

Sample A 0h

Sample B 0h

Sample A 48h

Sample B 48h

Sample A 48h
Blood Urea Nitrogen (BUN)
Reference range: 7-23 mg/dl

Sample A 0h

Sample B 0h

Sample A 48h

Sample B 48h

Calcium
Reference range: 2.1-2.7 mmol/l

Sample A 0h

Sample B 0h

Sample A 48h

Sample B 48h
Chloride
Reference range: 95-105 mmol/l

Sample A 0h

Sample A 48h

Sample B 0h

Sample B 48h

Cholesterol
Reference range: 100-200 mg/dl

Sample A 0h

Sample A 48h

Sample B 0h

Sample B 48h
Cholesterinesterase
Reference range: 4.5-11.5 kU/l

Sample A 0h

Sample B 0h

Cholesterinesterase 0h

Sample A 48h

Sample B 48h

Cholesterinesterase 48h

Creatinine
Reference range male: 0.55-1.17 mg/dl

Sample A 0h

Sample B 0h

Creatinine 0h

Sample A 48h

Sample B 48h

Creatinine 48h
Gamma Glutamyltransferase (GGT)
Reference range: 0-55 U/l

Sample A 0h

Sample B 0h

Glucose
Reference range: 60-110 mg/dl

Sample A 0h

Sample B 0h

Sample A 48h

Sample B 48h

Sample A 48h

Sample B 48h
Iron
Reference range: 14.3-32.2 µmol/l

Lactate Dehydrogenase
Reference range: 0-248 U/l
**Magnesium**
Reference range: 0.7-1.0 mmol/l

Sample A 0h

Sample B 0h

**Phosphate**
Reference range: 0.77-1.45 mmol/l

Sample A 0h

Sample B 0h

Sample A 48h

Sample B 48h
**Potassium**
Reference range: 3.5-5.3 mmol/l

Sample A 0h

Sample B 0h

Sample A 48h

Sample B 48h

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**Sodium**
Reference range: 135-150 mmol/l

Sample A 0h

Sample B 0h

Sample A 48h

Sample B 48h
Total Bilirubin
Reference range: 0.1-1.2 mg/dl

Total Protein
Reference range: 6-8 g/l
**Triglyceride**
Reference range: 25-180 mg/dl

Sample A 0h

Sample B 0h

Sample A 48h

Sample B 48h

**Troponin I**
Reference range: 0-0.028 ng/ml

Sample A 0h

Sample B 0h

Sample A 48h

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 B 0h

Vitamin B₁₂
Reference range male: 214-864 ng/l
Reference range female: 182-820 ng/l

Sample A 0h

Sample B 0h

Sample A 48h

Sample B 48h
Free Triiodthyronin (fT₃)
Reference range: 1.39-4.14 pg/ml

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