WHITE PAPER

TECHNICAL NOTES & APPLICATIONS FOR LABORATORY WORK

CONTENT

COMPARISON OF VACUETTE® CAT SERUM FAST SEPARATOR TUBES TO VACUETTE® LITHIUM HEPARIN SEPARATOR TUBES ON ABBOTT ALINITY INSTRUMENT

1/ SCOPE OF THE STUDY

The aim of the study was to demonstrate data on clinical chemistry analytes in VACUETTE® CAT Serum Fast Separator tubes in comparison to VACUETTE® Lithium Heparin Separator tubes.

In total 20 subjects (male and female) without therapy on anticoagulants in the range of 18 – 64 years have been included in the study. According to CLSI GP34-A a sample size of 20 subjects was determined. Venous blood specimens were drawn using a VACUETTE® EVOPROTECT SAFETY Blood Collection Set with Holder (Item No. 450122) into three tubes of each tube type and before blood collection of the first sample, a discard tube was used to avoid e.g. underfilling. All tubes were gently inverted 5 times after blood collection.

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TABLE 1/ SAMPLE TUBES

Item. No.	Sample	Product Name	Dimension	Volume	Lot. No.	Expiry date	Serum clotting time:	Centrifugation condition
456087	A	VACUETTE® Lithium Heparin Separator	13x100	5 ml	A23042BD	2024-08-05		1800 g / 10 min
456309	B	VACUETTE® CAT Serum Fast Sep Clot Activator	13x100	5 ml	A23023EN	2024-02-01	5 min	1800 g / 10 min

For serum samples (B) whole blood was allowed to clot for 5 minutes at room temperature (20 - 25°C). All serum and plasma samples were centrifuged at 1800g for 10 min at 20°C (Hettich Rotanta 460R acceleration/deceleration level 9) within 15-20 min after blood collection. All samples were transported and analyzed within 2 hours post centrifugation at an external laboratory to obtain the "0 hours = T0" values.

After the analysis the primary tube samples were transferred to the refrigerator (4-8°C) to be stored up to the 24h and 48h analysis. Thirty minutes prior to the analysis, the samples are transferred to room temperature (20-25°C). After the time interval of 24 and 48 hours post centrifugation, the samples were analyzed to obtain the "twenty-four hours = T24" values and "forty-eight hours = T48" values, respectively. All analyses were done on Abbott Alinity Instrument: Clinical Chemistry Analyzer and Immunology Analyzer.

2/ STATISTICS

2.1/ TUBE COMPARISON:

For the evaluation of clinical significance (tube comparison), bias estimation was performed between the predicate and candidate samples and deviations were summarized.

2.2/ ANALYTE STABILITY:

For analyte stability, the differences between the time points T0 to T24 and T0 to T48 were calculated.

In addition, extreme values / outliers were identified by extreme studentized deviate (ESD) test or Grubbs test (outliers were excluded from further calculations if a valid reason can be found).

The statistical evaluation was performed using GLIMS for Tube Comparison and Analyte Stability.

TABLE 2/ BIOCHEMICAL ANALYTES

Experiment	Analytes (30)	Parameter measured out of tube number (three tubes per tube type in total)
Tube comparison and analyte stability	Alanine aminotransferase (ALT)	1
	Alkaline phosphatase (ALP)	1
	Complement 3 (C3)	1
	Calcium total (Ca)	1
	Cholesterol (CHOL)	1
	Cortisol (Cort)	1
	Creatine kinase total (CK)	1
	Chloride (Cl)	1
	C-reactive Protein (CRP)	1
	Creatinine enzymatic (CREA)	1
	Iron (Fe)	2
	Folic acid (FOL)	2
	Follicle stimulation hormone (hFSH)	2
	Free Triiodothyronine (fT_3)	2
	Gamma glutamyl transferase (GGT)	2
	Glucose (GLUC)	2
	High-density lipoproteine (HDL)	2
	Immunglobuline G (IgG)	2
	Phosphate inorganic (IP)	2
	Lactate dehydrogenase (LDH)	2
	Magnesium (Mg)	3
	Potassium (K)	3
	Sodium (Na)	3
	Thyroid-stimulating hormone (TSH)	3
	Total Bilirubin (TBIL)	3
	Total Protein (TP)	3
	Triglycerides (TG)	3
	Urea (UREA)	3
	Uric acid (UA)	3
	Vitamin B_{12}	3

3/ ACCEPTANCE CRITERIA

Acceptance criteria for bias estimations between samples A and B: the overall mean estimated bias including 95% Confidence Interval (CI) for each parameter is within the clinical acceptance limit (CAL).

Acceptance criteria for analyte stability: the differences between tested time points for each parameter is lower than the Total Change Limit (TCL).

TABLE 3/ ACCEPTANCE CRITERIA BIOCHEMICAL PARAMETERS

Parameter	Abbreviation	TCL AC for analyte stability [%]	CAL AC for method comparison [%]	Assay CV(A) [%]
Alanine aminotransferase	ALT	5.76	11.50[R]	1.00
Alkaline phosphatase	ALP	7.16	11.00[R]	2.40
Complement 3	C3	4.27	8.40*	1.30
Calcium total	Ca	2.91	3.45[R]	1.00
Cholesterol	CHOL	3.45	7.00[R]	0.80
Cortisol	Cort	11.06	16.00[R]	2.70
Creatine kinase total	CKtot	7.90	11.00[R]	0.90
Chloride	Cl	2.02	4.50[R]	0.70
C-reactive Protein	CRP	29.92	13.5[R]	1.90
Creatinine enzymatic	CREA	3.57	10.00[C]	1.00
Iron	Fe	10.79	15.00[C]	1.10
Folic acid	FOL	14.55	25.00[R]	4.80
Follicle stimulation hormone	hFSH	8.13	14.00[R]	1.90
Free Triiodothyronine	fT_3	10.55	13.00[R]	3.70
Gamma glutamyl transferase	GGT	6.96	11.50[R]	1.90
Glucose	Gluc	3.73	8.00[C]	1.00
High-density lipoprotein	HDL	6.50	13.00[R]	2.10
Immunglobuline G	IgG	2.61	10.00[R]	0.70
Phosphate inorganic	IP	6.33	9.00[R]	1.80
Lactate dehydrogenase	LDH	6.12	9.00[R]	2.00
Magnesium	Mg	4.14	7.50[R]	1.40
Potassium	K	3.02	4.50[R]	0.80
Sodium	Na	1.68	3.00[R]	0.60
Thyroid-stimulating hormone	TSH	9.78	13.50[R]	1.50
Total Bilirubin	TBil	10.31	20.00[C]	0.90
Total Protein	TP	3.57	6.00[R]	1.20
Triglycerides	TG	12.32	9.00[R]	2.60
Urea	Urea	8.55	9.00[C]	1.80
Uric acid	UA	5.32	7.00[R]	1.20
Vitamin B_{12}	Vit B_{12}	18.48	25.00[R]	4.90

[R] Rili-BÄK: Richtlinie der Bundesärztekammer zur Qualitätssicherung laboratoriumsmedizinischer Untersuchungen, https://www.bundesaerztekammer.de/aerzte/qualitaetssicherung/richtlinien-leitlinien-empfehlungen-stellungnahmen/richtlinien-leitlinien-empfehlungen-zur-qualitaetssicherung/labor

[C] CLIA: Clinical Laboratory Improvement Amendments (CLIA) Federal Register / Vol 84, No. 23 /Proposed Rules, Feb.4, 2019
* other

4/ RESULTS

4.1/ OUTLIER SECTION

The outlier section is available in the GLIMS-Report.

4.2/ SUMMARY RESULTS TUBE COMPARISON AND STABILITY TESTING

Comparison of samples is listed in the following table indicating the mean ± standard deviation. For analyte stability the differences between the time points T0 to T24 and T0 to T48 were calculated using GLIMS.

Acceptance criteria for bias estimation of analyte stability: the differences of the mean between tested time points for each parameter is lower than the Total Change Limit (TCL).

TABLE 4/ OVERVIEW RESULTS AT INITIAL TIME POINT

		Initial time point T0 VACUETTE® Lithium Heparin Separator		Initial time point T0 VACUETTE® CAT Serum Fast Separator	
Parameter	*Unit*	*Mean value*	*SD*	*Mean value*	*SD*
Alanine aminotransferase	U/l	20.05	7.41	20.45	7.71
Alkaline phosphatase	U/l	56.70	13.85	58.00	14.95
Complement 3	mg/dl	116.25	19.77	120.00	19.48
Calcium total	mmol/l	2.38	0.086	2.40	0.082
Cholesterol	mg/dl	196.45	40.26	200.10	41.14
Cortisol	µg/dl	10.46	3.31	10.46	3.22
Creatine kinase total	U/l	135.70	101.08	138.80	100.59
Chloride	mmol/l	106.05	1.50	106.65	1.84
C-reactive Protein	mg/l	0.22	0.24	0.22	0.24
Creatinine enzymatic	mg/dl	0.78	0.097	0.78	0.096
Iron	µmol/l	17.38	6.67	17.53	6.67
Folic acid	ng/ml	9.74	6.29	9.38	6.37
Follicle stimulation hormone	mIU/ml	18.29	35.49	16.58	32.17
Free Triiodothyronine	pg/ml	3.31	0.33	3.12	0.29
Gamma glutamyl transferase	U/l	23.10	16.70	24.15	16.94
Glucose	mg/dl	96.45	30.90	96.45	31.29
	mmol/l	5.35	1.72	5.35	1.74
High-density lipoprotein	mg/dl	63.90	14.92	63.30	14.62
Immunglobuline G	mg/dl	1127.50	263.91	1137.75	278.28
Phosphate inorganic	mmol/l	0.98	0.20	1.05	0.21
Lactate dehydrogenase	U/l	183.20	27.87	165.95	28.13

TABLE 4/ OVERVIEW RESULTS AT INITIAL TIME POINT

		Initial time point T0 VACUETTE® Lithium Heparin Separator		Initial time point T0 VACUETTE® CAT Serum Fast Separator	
Parameter	*Unit*	*Mean value*	*SD*	*Mean value*	*SD*
Magnesium	mmol/l	0.94	0.059	0.97	0.075
Potassium	mmol/l	3.96	0.23	4.15	0.25
Sodium	mmol/l	140.25	1.62	140.95	1.23
Thyroid-stimulating hormone	µlU/ml	1.54	0.48	1.45	0.43
Total Bilirubin	mg/dl	0.70	0.38	0.68	0.36
Total Protein	g/l	72.20	3.62	70.65	3.63
Triglycerides	mg/dl	88.95	72.73	92.70	74.46
Urea	mg/dl	25.25	7.07	25.47	7.14
Uric acid	mg/dl	4.92	0.97	4.94	0.96
Vitamin B_{12}	pg/ml	400.11	129.11	370.78	128.31

TABLE 5/ OVERVIEW RESULTS AT TIME POINT 24H

		Time point T24 VACUETTE® Lithium Heparin Separator		Time point T24 VACUETTE® CAT Serum Fast Separator	
Parameter	*Unit*	*Mean value*	*SD*	*Mean value*	*SD*
Alanine aminotransferase	U/l	20.55	7.47	20.70	7.68
Alkaline phosphatase	U/l	55.65	13.17	58.80	15.24
Complement 3	mg/dl	117.55	19.39	122.20	19.58
Calcium total	mmol/l	2.38	0.078	2.39	0.077
Cholesterol	mg/dl	199.30	41.21	203.60	41.76
Cortisol	µg/dl	10.37	3.27	10.30	3.24
Creatine kinase total	U/l	134.95	100.49	139.75	102.82
Chloride	mmol/l	107.40	1.57	107.55	1.85
C-reactive Protein	mg/l	0.23	0.25	0.23	0.25
Creatinine enzymatic	mg/dl	0.79	0.10	0.80	0.098
Iron	µmol/l	17.54	6.46	16.70	6.72
Folic acid	ng/ml	10.08	6.24	8.16	4.07
Follicle stimulation hormone	mlU/ml	18.65	36.38	18.02	35.79
Free Triiodothyronine	pg/ml	3.34	0.30	3.24	0.30
Gamma glutamyl transferase	U/l	23.55	17.01	24.05	17.18
Glucose	mg/dl	93.00	32.22	96.35	32.14
	mmol/l	5.16	1.79	5.35	1.78
High-density lipoprotein	mg/dl	66.00	14.58	64.85	14.91
Immunglobuline G	mg/dl	1120.30	263.66	1142.30	271.90
Phosphate inorganic	mmol/l	1.01	0.20	1.06	0.20
Lactate dehydrogenase	U/l	189.65	31.65	161.70	26.73

TABLE 5/ OVERVIEW RESULTS AT TIME POINT 24H

		Time point T24 VACUETTE® Lithium Heparin Separator		Time point T24 VACUETTE® CAT Serum Fast Separator	
Parameter	*Unit*	*Mean value*	*SD*	*Mean value*	*SD*
Magnesium	mmol/l	0.94	0.068	0.97	0.075
Potassium	mmol/l	4.06	0.23	4.15	0.26
Sodium	mmol/l	140.95	0.94	140.95	1.19
Thyroid-stimulating hormone	µlU/ml	1.55	0.48	1.49	0.45
Total Bilirubin	mg/dl	0.68	0.37	0.68	0.36
Total Protein	g/l	73.00	3.74	71.60	3.56
Triglycerides	mg/dl	92.60	83.68	93.90	75.02
Urea	mg/dl	25.57	6.43	25.58	6.49
Uric acid	mg/dl	4.96	0.97	4.94	0.97
Vitamin B_{12}	pg/ml	350.08	127.47	349.38	123.97

TABLE 6/ OVERVIEW RESULTS AT TIME POINT 48H

		Time point T48 VACUETTE® Lithium Heparin Separator		Time point T48 VACUETTE® CAT Serum Fast Separator	
Parameter	*Unit*	*Mean value*	*SD*	*Mean value*	*SD*
Alanine aminotransferase	U/l	18.95	7.57	19.30	7.83
Alkaline phosphatase	U/l	57.85	14.17	60.75	15.58
Complement 3	mg/dl	119.50	20.02	123.40	19.83
Calcium total	mmol/l	2.37	0.084	2.38	0.084
Cholesterol	mg/dl	199.05	41.26	203.60	41.16
Cortisol	µg/dl	10.65	3.45	10.40	3.20
Creatine kinase total	U/l	135.75	101.65	139.60	102.25
Chloride	mmol/l	106.80	1.77	107.05	1.64
C-reactive Protein	mg/l	0.22	0.24	0.22	0.25
Creatinine enzymatic	mg/dl	0.79	0.10	0.79	0.099
Iron	µmol/l	16.41	6.77	16.43	6.83
Folic acid	ng/ml	9.96	5.87	9.09	5.97
Follicle stimulation hormone	mlU/ml	19.02	37.46	18.29	36.67
Free Triiodothyronine	pg/ml	3.39	0.33	3.35	0.34
Gamma glutamyl transferase	U/l	24.40	17.01	24.30	17.28
Glucose	mg/dl	86.90	30.01	97.60	30.94
	mmol/l	4.82	1.67	5.42	1.72
High-density lipoprotein	mg/dl	66.30	14.53	65.50	14.86
Immunglobuline G	mg/dl	1144.20	284.23	1150.00	286.61
Phosphate inorganic	mmol/l	1.06	0.19	1.07	0.20
Lactate dehydrogenase	U/l	211.80	31.19	160.10	28.05

TABLE 6/ OVERVIEW RESULTS AT TIME POINT 48H

		Time point T48 VACUETTE® Lithium Heparin Separator		Time point T48 VACUETTE® CAT Serum Fast Separator	
Parameter	*Unit*	*Mean value*	*SD*	*Mean value*	*SD*
Magnesium	mmol/l	0.90	0.065	0.92	0.085
Potassium	mmol/l	4.23	0.28	4.18	0.26
Sodium	mmol/l	141.70	1.30	141.40	1.23
Thyroid-stimulating hormone	µIU/ml	1.58	0.49	1.53	0.48
Total Bilirubin	mg/dl	0.66	0.36	0.67	0.36
Total Protein	g/l	73.05	3.72	71.40	3.84
Triglycerides	mg/dl	92.20	77.10	95.35	75.76
Urea	mg/dl	25.79	7.06	25.79	6.82
Uric acid	mg/dl	4.96	0.98	4.94	0.99
Vitamin B_{12}	pg/ml	349.31	132.71	335.62	125.76

TABLE 7/ BIAS ESTIMATION BETWEEN SAMPLE A (VACUETTE® LITHIUM HEPARIN SEPARATOR) AND B (VACUETTE® CAT SERUM FAST SEPARATOR)

Parameter	Acceptance criteria CAL [%]	Bias [%]- Time point 0h	Bias [%]- Time point 24h	Bias [%]-Time point 48h
Alanine aminotransferase	11.50[R]	2.75	2.95	2.94
Alkaline phosphatase	11.00[R]	3.37	6.39	4.69
Complement 3	8.40*	3.55	4.14	3.65
Calcium total	3.45[R]	1.37	1.43	1.29
Cholesterol	7.00[R]	2.29	2.31	2.82
Cortisol	16.00[R]	2.55	2.55	3.63
Creatine kinase total	11.00[R]	3.87	4.21	3.87
Chloride	4.50[R]	0.75	0.70	0.42
C-reactive Protein	13.5[R]	6.83	4.45	7.07
Creatinine enzymatic	10.00[C]	1.82	1.54	1.75
Iron	15.00[C]	5.34	6.15	1.41
Folic acid	25.00[R]	17.82	18.58	13.84
Follicle stimulation hormone	14.00[R]	10.26	6.63	5.24
Free Triiodothyronine	13.00[R]	6.37	3.66	3.45
Gamma glutamyl transferase	11.50[R]	7.07	3.02	2.50
Glucose	8.00[C]	3.04	5.52	13.19
High-density lipoprotein	13.00[R]	2.29	2.69	2.39
Immunglobuline G	10.00[R]	1.67	2.31	1.77
Phosphate inorganic	9.00[R]	6.73	5.39	2.22
Lactate dehydrogenase	9.00[R]	9.54	14.42	24.43
Magnesium	7.50[R]	3.22	2.72	7.33
Potassium	4.50[R]	4.96	2.59	2.55

TABLE 7/ BIAS ESTIMATION BETWEEN SAMPLE A (VACUETTE® LITHIUM HEPARIN SEPARATOR) AND B (VACUETTE® CAT SERUM FAST SEPARATOR)

Parameter	Acceptance criteria CAL [%]	Bias [%]- Time point 0h	Bias [%]- Time point 24h	Bias [%]-Time point 48h
Sodium	3.00[R]	0.79	0.36	0.85
Thyroid-stimulating hormone	13.50[R]	5.59	3.74	3.73
Total Bilirubin	20.00[C]	2.79	2.71	3.97
Total Protein	6.00[R]	2.70	1.90	2.39
Triglycerides	9.00[R]	4.80	5.78	5.51
Urea	9.00[C]	2.80	4.73	1.36
Uric acid	7.00[R]	0.05	0.77	1.22
Vitamin B_{12}	25.00[R]	9.60	8.84	11.56

TABLE 8/ TOTAL CHANGE LIMIT (TCL) BETWEEN TIME POINTS TESTED FOR SAMPLE A (VACUETTE® LITHIUM HEPARIN SEPERATOR) AND B (VACUETTE® CAT SERUM FAST SEPARATOR)

Parameter	Acceptance criteria TCL [%]	Sample A 0h/24h	Sample A 0h/48h	Sample B 0h/24h	Sample B 0h/48h
Alanine aminotransferase	5.76	4.68	6.27	2.03	6.61
Alkaline phosphatase	7.16	3.15	3.17	2.96	4.86
Complement 3	4.27	1.60	2.84	2.10	2.94
Calcium total	2.91	1.34	1.52	0.83	1.54
Cholesterol	3.45	1.45	1.72	1.76	1.83
Cortisol	11.06	1.92	2.91	2.67	2.13
Creatine kinase total	7.90	1.62	1.81	1.66	1.57
Chloride	2.02	1.27	0.71	0.85	0.56
C-reactive Protein	29.92	5.78	7.89	5.48	4.02
Creatinine enzymatic	3.57	1.78	2.35	1.40	1.94
Iron	10.79	6.07	7.02	6.14	7.66
Folic acid	14.55	13.39	19.88	15.72	12.71
Follicle stimulation hormone	8.13	1.83	3.78	5.73	9.11
Free Triiodothyronine	10.55	3.88	5.26	3.98	7.75
Gamma glutamyl transferase	6.96	4.64	7.71	3.20	2.16
Glucose	3.73	4.15	11.06	2.14	1.86
High-density lipoprotein	6.50	3.73	4.06	2.49	3.57
Immunglobuline G	2.61	1.55	1.94	1.67	1.69
Phosphate inorganic	6.33	4.01	8.84	1.85	2.95
Lactate dehydrogenase	6.12	5.04	15.88	3.29	3.86
Magnesium	4.14	0.50	3.68	0.00	4.12
Potassium	3.02	2.80	6.94	0.23	0.96
Sodium	1.68	0.86	1.04	0.21	0.39

TABLE 8/ TOTAL CHANGE LIMIT (TCL) BETWEEN TIME POINTS TESTED FOR SAMPLE A (VACUETTE® LITHIUM HEPARIN SEPERATOR) AND B (VACUETTE® CAT SERUM FAST SEPARATOR)

Parameter	Acceptance criteria TCL [%]	Sample A 0h/24h	Sample A 0h/48h	Sample B 0h/24h	Sample B 0h/48h
Thyroid-stimulating hormone	9.78	2.72	4.06	2.80	5.30
Total Bilirubin	10.31	1.67	4.71	2.50	1.25
Total Protein	3.57	1.12	1.19	1.36	1.05
Triglycerides	12.32	2.20	3.09	2.09	3.61
Urea	8.55	5.32	2.26	3.10	2.82
Uric acid	5.32	0.75	0.82	0.31	0.84
Vitamin B_{12}	18.48	15.12	14.53	8.62	11.23

5/ DISCUSSION

PLEASE NOTE:

Clinical acceptance criteria that are used by GBO are intended to support the identification of deviations which should be discussed in view of clinically relevance. Those criteria might be different from one study to another as perspectives from various clinical experts are considered in each study. Each laboratory should generate their own acceptance criteria based on validation of the tubes.

Assessment of the deviations found for comparison of samples tested and stability testing exceeding the acceptance criteria:

5.1/ GLUCOSE

Table 9

Mean and Median at each time point for sample A (VACUETTE® Lithium Heparin Separator) and sample B (VACUETTE® CAT Serum Fast Separator) for glucose in mg/dl and mmol/l:

			Mean	Median
A_0h	mg/dl	20	96.45	93.50
B_0h	mg/dl	20	96.45	93.50
A_24h	mg/dl	20	93.00	89.50
B_24h	mg/dl	20	96.35	94.00
A_48h	mg/dl	20	86.90	81.50
B_48h	mg/dl	20	97.60	94.00

			Mean	Median
A_0h	mmol/l	20	5.35	5.19
B_0h	mmol/l	20	5.35	5.19
A_24h	mmol/l	20	5.16	4.97
B_24h	mmol/l	20	5.35	5.22
A_48h	mmol/l	20	4.82	4.52
B_48h	mmol/l	20	5.42	5.22

No significant deviation was found for glucose at the initial time point of analysis and at 24h when comparing VACUETTE® CAT Serum Fast Separator tubes (sample B) to the VACUETTE® Lithium Heparin Separator tubes (sample A). From literature **[1-3]**, it is well known that other additives are recommended for stabilizing glucose over time (up to 24h and 48h).

5.2/ LDH

Table 10

Mean and Median at each time point for sample A (VACUETTE® Lithium Heparin Separator) and sample B (VACUETTE® CAT Serum Fast Separator) for LDH in U/l

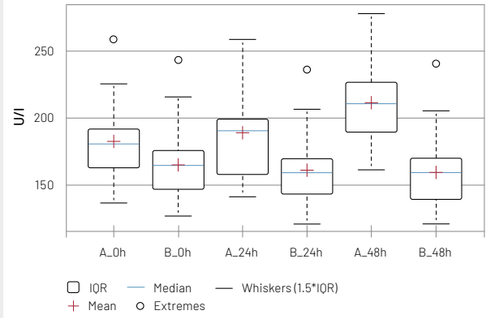
			Mean	Median
A_0h	U/l	20	183.20	181.00
B_0h	U/l	20	165.95	165.00
A_24h	U/l	20	189.65	191.00
B_24h	U/l	20	161.70	160.00
A_48h	U/l	20	211.80	211.00
B_48h	U/l	20	160.10	160.00

The bias of 9.54% is slightly above the acceptance criterion of 9%. It may result from platelets remaining on top of the gel barrier after centrifugation or from a slight hemolysis in the plasma as samples were only visually examined in view of hemolysis.

LDH was stable at all tested timepoints in the VACUETTE® CAT Serum Fast Separator (sample B) since the concentration changes over time were below the acceptance criterion (TCL 6.12%). The exceedance of the acceptance criterion in the VACUETTE® Lithium Heparin Separator tubes (sample A) over time (48h) might be due to the prolonged contact with the cells. The following box plot shows the slight increase over time in the VACUETTE® Lithium Heparin Separator tube (sample A) which is in accordance with literature. Well described is the reduced stability of analytes influenced by active cell contact: "LDH and bicarbonate were the analytes with the lowest stability after centrifugation" **[4]**.

Boxplot 1

Mean and Median at each time point for sample A (VACUETTE® Lithium Heparin Separator) and sample B (VACUETTE® CAT Serum Fast Separator) for LDH



5.3/ POTASSIUM

Table 11

Mean and Median at each time point for sample A (VACUETTE® Lithium Heparin Separator) and sample B (VACUETTE® CAT Serum Fast Separator) for potassium in mmol/l

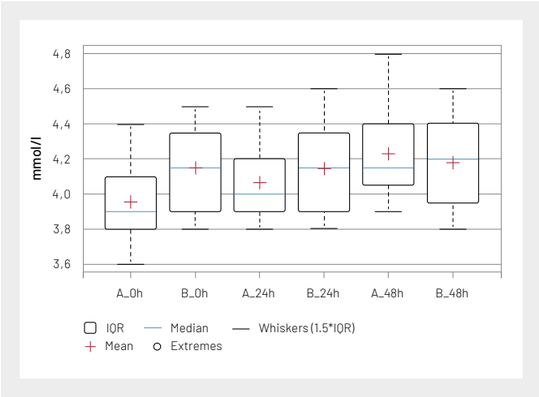
			Mean	Median
A_0h	mmol/l	20	3.96	3.90
B_0h	mmol/l	20	4.15	4.15
A_24h	mmol/l	20	4.06	4.00
B_24h	mmol/l	20	4.15	4.15
A_48h	mmol/l	20	4.23	4.15
B_48h	mmol/l	20	4.18	4.20

The acceptance criterion of 4.5 % is slightly exceeded by the bias found of 4.96% only at the initial time point of analysis comparing both samples.

Usually, the potassium value found in plasma samples is 0.1-0.3 mmol lower than in serum samples. "The coagulation process leads to an artificial increase of the potassium concentration of approximately 0.3 mmol/L in serum samples compared to plasma" **[5]**.

Boxplot 2

Mean and Median at each time point for sample A (VACUETTE® Lithium Heparin Separator) and sample B (VACUETTE® CAT Serum Fast Separator) for potassium



The change in potassium concentration from the initial time point and the 48h analysis in the VACUETTE® Lithium Heparin Separator tube (sample A) resulted in 6.94% (acceptance criterion = 3.02%).

In addition to "Phosphorus and potassium may be additionally requested up to 12 h after centrifugation if the sample is stored at 4 °C and if the delay in transporting the blood is minimal"; "plasma potassium was found to be stable up to 12 h when the sample was centrifuged and stored at 4 °C" **[4]** it is reported that "most tested analytes remained stable up to 24 h at all storage conditions prior to centrifugation, using our statistical approach. However, some important analytes were significantly affected because of:

- **prolonged contact of plasma and serum with cells and leakage of intracellular constituents such as potassium, inorganic phosphorus, magnesium, LD [6].**

However, it is well described in literature **[9]** that serum does not reflect the physiological situation of the patient due to the release of intracellular potassium from platelets, leukocytes (and erythrocytes in case of a hemolysis) into the extracellular space. Falsely elevated potassium is reported due to the release of potassium from the platelets during the clotting process that means during the formation of serum.

5.4/ ALT

Table 12

Mean and Median at each time point for sample A (VACUETTE® Lithium Heparin Separator) and sample B (VACUETTE® CAT Serum Fast Separator) for ALT in U/l

			Mean	Median
A_0h	U/l	20	20.05	19.50
B_0h	U/l	20	20.45	19.00
A_24h	U/l	20	20.55	20.00
B_24h	U/l	20	20.70	20.00
A_48h	U/l	20	18.95	18.50
B_48h	U/l	20	19.30	18.50

The decline of 6.27% in ALT concentration over 48h in the VACUETTE® CAT Serum Fast Separator tube (sample B) is slightly above the acceptance criterion of 5.76% but in accordance with literature "Mean activity loss in separated serum at 2 days was 6 percent..." **[7]**

5.5/ FOLIC ACID

Table 13

Mean and Median at each time point for sample A (VACUETTE® Lithium Heparin Separator) and sample B (VACUETTE® CAT Serum Fast Separator) for folic acid in ng/ml

			Mean	Median
A_0h	ng/ml	19	9.74	9.11
B_0h	ng/ml	19	9.38	7.66
A_24h	ng/ml	19	10.08	9.25
B_24h	ng/ml	19	8.16	7.24
A_48h	ng/ml	19	9.96	7.57
B_48h	ng/ml	20	9.09	8.00

The change from the initial value T0 to the time point T48 for sample B (VACUETTE Lithium Heparin Separator: bias 19.88%) exceeds the acceptance criterion of 14.55% as well as between T0 and T24 for sample A (VACUETTE CAT Serum Fast Separator: bias 15.72%).

According to a paper from 1997 **[8]**, "folate is a more robust analyte. In refrigerated serum specimens, folate was stable up to 7 days of storage." Oddoze **[6]** found folic acid to be stable up to 72h in serum at 4°C. As all values found in this study lie above the lower limit of the reference range of 2.5 ng/ml, the slightly higher bias found in this trial is not clinically relevant.

5.6/ hFSH

Table 14

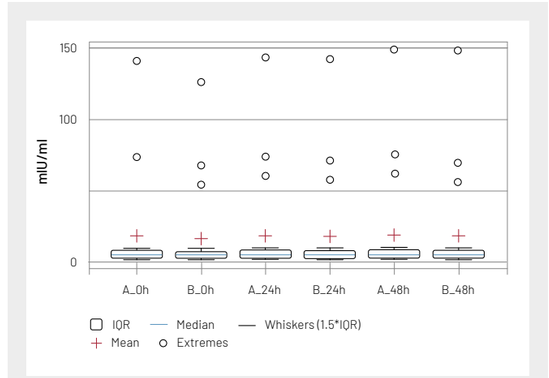
Mean and Median at each time point for sample A (VACUETTE® Lithium Heparin Separator) and sample B (VACUETTE® CAT Serum Fast Separator) for hFSH in mIU/ml

			Mean	Median
A_0h	mIU/ml	19	18.29	4.80
B_0h	mIU/ml	19	16.58	4.40
A_24h	mIU/ml	19	18.65	4.90
B_24h	mIU/ml	19	18.02	4.50
A_48h	mIU/ml	19	19.02	4.90
B_48h	mIU/ml	19	18.29	4.70

The acceptance criterion is 8.13% and the change found from T0 and T48 in the VACUETTE® CAT Serum Fast Separator tube (sample B) is 9.11 %. According to **[6]** the analyte is stable up to 72h at 4C in serum. The boxplot below identifies extreme values in the patient collective involved in this trial as the range for the reference values is very high for that analyte depending on the female hormone status. Therefore, the bias found is not considered to be clinically significant.

Boxplot 3

Mean and Median at each time point for sample A (VACUETTE® Lithium Heparin Separator) and sample B (VACUETTE® CAT Serum Fast Separator) for hFSH



5.7/ GGT

Table 15

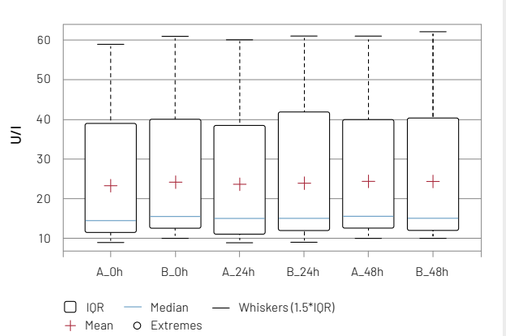
Mean and Median at each time point for sample A (VACUETTE® Lithium Heparin Separator) and sample B (VACUETTE® CAT Serum Fast Separator) for GGT in U/l

			Mean	Median
A_0h	U/l	20	23.10	14.50
B_0h	U/l	20	24.15	15.50
A_24h	U/l	20	23.55	15.00
B_24h	U/l	20	24.05	15.00
A_48h	U/l	20	24.40	15.50
B_48h	U/l	20	24.30	15.00

The acceptance criterion is 6.96% and the change in GGT concentration found is 7.71% in VACUETTE Lithium Heparin Separator tubes (sample A) between T0 and T48h. The boxplot below indicates a high standard deviation in relation the absolute values and the median is with 6.46% within the acceptance criterion. The bias found in this trial is not considered to be clinically significant.

Boxplot 4

Mean and Median at each time point for sample A (VACUETTE® Lithium Heparin Separator) and sample B (VACUETTE® CAT Serum Fast Separator) for hFSH



5.8/ INORGANIC PHOSPHATE

Table 16

Mean and Median at each time point for sample A (VACUETTE® Lithium Heparin Separator) and sample B (VACUETTE® CAT Serum Fast Separator) for inorganic phosphate in mmol/l

			Mean	Median
A_0h	mmol/l	20	0.98	0.95
B_0h	mmol/l	20	1.05	1.02
A_24h	mmol/l	20	1.01	0.96
B_24h	mmol/l	20	1.06	1.04
A_48h	mmol/l	20	1.06	1.02
B_48h	mmol/l	20	1.07	1.04

The acceptance criterion for stability testing of inorganic phosphate is 6.33%. The change found (8.84%) between T0 and T48 in the VACUETTE® Lithium Heparin Separator tube (sample A) exceeds the acceptance criterion. In papers **[4/6]** is described that "Phosphorus and potassium may be additionally requested up to 12 h after centrifugation if the sample is stored at 4 °C and if the delay in transporting the blood is minimal" and that the stability of the analytes is influenced by the "prolonged contact of plasma and serum with cells and leakage of intracellular constituents such as potassium, inorganic phosphorus, magnesium, LD".

6/ CONCLUSION:

6.1/ TUBE COMPARISON (ACCEPTANCE CRITERIA: CLINICAL ACCEPTANCE LIMIT (CAL))

Analytical testing demonstrated clinically equivalent results comparing VACUETTE® CAT Serum Fast Separator Clot Activator tubes to VACUETTE® Lithium Heparin Separator tubes for the following parameters:

ALT, ALP, Ca, Cl, Chol, C3, Cort, CRP, CREA, CKtot, fT3, GGT, Gluc, HDL, hFSH, IgG, Fe, FOL, LDH, Mg, K, IP, Na, TSH, TBIL, TP, TG, UA, UREA, VitB12.

The bias found for glucose at T48, for K at T0 as well as for LDH at T0, T24 and T48 was discussed and found to be clinically acceptable in accordance with literature.

6.2/ ANALYTE STABILITY (ACCEPTANCE CRITERIA: TOTAL CHANGE LIMIT (TCL))

Analyte stability tested for 24 and 48 hours (storage refrigerated):

In this trial, no clinically significant deviations were found in VACUETTE® CAT Serum Fast Separator Clot Activator and VACUETTE® Lithium Heparin Separator tubes when comparing the initial measurement (T0) to the measurement at 24 and 48 hours after centrifugation (T24/T48) out of the same tube (primary tube) for the following parameters:

ALT, ALP, Ca, Cl, Chol, C3, Cort, CRP, CREA, CKtot, fT3, GGT, Gluc, HDL, hFSH, IgG, Fe, FOL, LDH, Mg, K, IP, Na, TSH, TBIL, TP, TG, UA, UREA, VitB12.

The change in stability measurements for ALT, Gluc, hFSH, FOL, K, IP, GGT as well as for LDH in this trial was discussed and found to be clinically acceptable in accordance with literature.

7/ REFERENCES

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8/ PRODUCT OVERVIEW

VACUETTE® CAT Serum Fast Separator Tube

Item No.	Nominal volume	Cap colour	Ring colour	Thread type	Tube size	Label	Barcode	Inner / Outer [Qty.]
454592	3.5 ml	orange	yellow	PREMIUM	13 x 75	Paper	no	50/1,200
454593	3.5 ml	orange	yellow	non-ridged	13 x 75	Paper	no	50/1,200
456309	5 ml	orange	yellow	PREMIUM	13 x 100	Paper	no	50/1,200
456313	5 ml	orange	yellow	non-ridged	13 x 100	Paper	no	50/1,200
486509	5 ml	orange	yellow	PREMIUM	13 x 100	Paper	yes	50/1,200

23CS_056 [Rev. 00, 03.2024]

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