WHITE PAPER

TECHNICAL NOTES & APPLICATIONS FOR LABORATORY WORK

EVALUATION OF VACUETTE® CAT SERUM FAST SEPARATOR BLOOD COLLECTION TUBE FOR ROUTINE CHEMISTRY ANALYTES IN COMPARISON TO SEKISUI INSEPAK® II -D RAPID COAGULATION TYPE TUBE

CONTENT

1/ 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 to further 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. According to the available study results, the tubes are suitable for the usage for routine chemistry analyses. Patients who are on heparin or other thrombin inhibitor therapy were not included in this study design.

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 room temperature (RT) for 24h and following at 4-8°C in the refrigerator up to 48h.

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2/ STUDY OBJECTIVE

The study has been carried out to demonstrate method comparison of modified VACUETTE® CAT Serum Fast Separator blood collection tubes to SEKISUI Insepak® II-D Rapid coagulation type blood collection tubes when centrifuged at 1800g for 10 min for routine chemistry analysis.

3/ STUDY DESIGN AND PROCEDURE

Venous blood was collected from 42 hospitalized patients aged 18-64 years by using a VACUSERA Tourniquet (Item #259001), a Vacutainer Eclipse blood collection needle (Item #368609), and a VACUSERA standard tube holder (Item #260001) into the following tubes:

Sample	Tube description	Item N°	Volume [ml]	Centrifugation
A	SEKISUI Insepak® II-D Rapid coagulation type	473548 Subgroup SMD750SQ	5	1800g / 10 min / 20°C
B	VACUETTE® CAT Serum Fast Separator	456309	5	1800g / 10 min / 20°C

One tube of each sample was drawn from each donor. All samples were gently inverted 5 times. After a minimum of 5 min. clotting time of the whole blood sample in an upright position, all samples were centrifuged within max. 2h according to the centrifugation setting in the table above at 20°C (NUVE 800 cooled centrifuge). Initial analysis was done after centrifugation on a Roche cobas® 8000. After initial measurement, the primary tube samples were recapped and stored avoiding light exposure at 4-8°C. Before the second measurement at 48 hours, all tubes were brought to room temperature again by removing the tubes from the refrigerator 30 min before the 48h-measurement was started.

4/ METHOD COMPARISON - ANALYTES ON ROCHE COBAS® 8000

Parameter	Abbreviation	Allowable Bias (Westgard)
Alanine Transaminase	ALT	11.48
Albumin	ALB	1.43
Alkaline Phosphatase	ALP	6.72
Calcium	Ca	0.82
Cholesterol	CHOL	4.10
Cortisol	Cort	10.26
Creatine Kinase	CK	11.50
CK-MB	CK-MB	14.88
Chloride	Cl	0.50
C-reactive Protein	CRP	21.80
Creatinine	Crea	10.003
Estradiol	E2	8.30
Iron	Fe	8.80
Folic Acid	FOL	19.20
Follicle stimulation hormone	hFSH	12.12
Free Triiodothyronine	fT_3	4.00
Gamma-Glutamyl Transferase	GGT	11.06
Glucose	Gluc	2.34
High Density Lipoprotein	HDL	5.61
Inorganic Phosphate	IP	3.38
Lactate-Dehydrogenase	LDH	4.30
Magnesium	Mg	1.80
Potassium	K	4.502
Sodium	Na	0.23
Thyroid-stimulating hormone	TSH	7.80
Total Bilirubin	TBil	8.95
Total Protein	TP	1.36
Triglyceride	TG	9.57
Troponin T	Trop T	23.70
Urea	Urea	6.05
Uric Acid	UA	4.87
Cobalamine (Vitamin B12)	$VitB_{12}$	
Lipemic Icteric Haemolysis Index	LIH	N.A.

Source: Desirable Biological Variation Database specifications (https://www.westgard.com/biodatabase1.htm).

Acceptance criteria provided in the table above were used for method comparison (bias estimations between sample A and B).

5/ RESULTS

None of the tubes was underfilled. After centrifugation, all samples were checked in view of any irregularities. All tubes were spun correctly without visible deviations.

In total, 5 "clotted samples" were detected on both clinical chemistry and immunology module of COBAS® 8000. Those samples were immediately checked by eye and none of the tubes was found with a clot. All samples were re-run as a cup on tube and then no further clot error was detected on the analyzer.

5.1/ METHOD COMPARISON – INITIAL AND 48H MEASUREMENT

Analyte	Sample	Centrifugation	Mean 0h	SD inital	Mean 24h	SD 24h
ALB [g/l]	A	1800g/10 min	45.100	3.189	45.519	3.143
	B	1800g/10 min	45.052	3.262	45.669	3.237
ALP [U/l]	A	1800g/10 min	76.548	25.914	75.524	26.073
	B	1800g/10 min	76.476	26.026	75.357	25.709
ALT [U/l]	A	1800g/10 min	17.214	8.12	16.742	7.799
	B	1800g/10 min	17.119	8.146	16.905	7.802
Ca [mmol/l]	A	1800g/10 min	2.391	0.083	2.412	0.093
	B	1800g/10 min	2.407	0.087	2.422	0.092
CHOL [mg/dl]	A	1800g/10 min	195.00	47.028	199.762	46.030
	B	1800g/10 min	194.452	46.166	200.310	46.041
CK [U/l]	A	1800g/10 min	93.881	46.053	93.810	46.638
	B	1800g/10 min	93.810	46.895	94.119	47.022
CK-MB [U/l]	A	1800g/10 min	1.968	1.487	1.882	1.449
	B	1800g/10 min	1.995	1.481	1.890	1.438
Cl [mmol/l]	A	1800g/10 min	100.952	2.326	101.452	2.421
	B	1800g/10 min	101.000	2.072	101.881	2.276
Cort [mg/dl]	A	1800g/10 min	11.943	5.731	12.002	5.698
	B	1800g/10 min	12.076	5.731	12.114	5.691
CRP [mg/l]	A	1800g/10 min	4.284	4.416	4.285	4.432
	B	1800g/10 min	4.289	4.519	4.357	4.570
Fe [µmol/l]	A	1800g/10 min	85.190	81.656	85.786	81.403
	B	1800g/10 min	85.357	81.625	86.190	82.174
FOL [ng/ml]	A	1800g/10 min	8.426	2.889	9.042	2.874
	B	1800g/10 min	8.432	3.111	9.076	2.986
fT_3 [pg/ml]	A	1800g/10 min	3.142	0.482	3.652	0.499
	B	1800g/10 min	3.153	0.478	3.650	0.497
GGT [U/l]	A	1800g/10 min	23.048	19.364	23.167	19.386
	B	1800g/10 min	23.190	19.347	23.357	19.581

Analyte	Sample	Centrifugation	Mean 0h	SD inital	Mean 24h	SD 24h
Gluc [mg/dl]	A	1800g/10 min	110.786	33.809	109.881	33.371
	B	1800g/10 min	110.976	33.752	110.738	33.510
HDL [mg/dl]	A	1800g/10 min	48.024	13.937	46.848	13.096
	B	1800g/10 min	48.071	13.774	46.964	13.268
hFSH [mIU/ml]	A	1800g/10 min	15.574	21.766	15.614	21.903
	B	1800g/10 min	15.531	21.530	15.512	21.711
IP [mmol/l]	A	1800g/10 min	1.100	0.166	1.101	0.162
	B	1800g/10 min	1.105	0.164	1.107	0.166
K [mmol/l]	A	1800g/10 min	4.281	0.413	4.360	0.417
	B	1800g/10 min	4.275	0.385	4.356	0.375
Crea [mg/dl]	A	1800g/10 min	0.752	0.261	0.692	0.245
	B	1800g/10 min	0.752	0.268	0.692	0.248
LDH [U/l]	A	1800g/10 min	166.357	45.177	156.452	42.120
	B	1800g/10 min	166.262	43.741	156.071	41.056
Mg [mmol/l]	A	1800g/10 min	0.804	0.062	0.810	0.062
	B	1800g/10 min	0.816	0.063	0.821	0.064
Na [mmol/l]	A	1800g/10 min	137.238	2.397	141.214	2.494
	B	1800g/10 min	137.405	2.270	141.881	2.098
E2 [pg/ml]	A	1800g/10 min	1239.285	4970.859	1443.686	5065.792
	B	1800g/10 min	1225.135	4857.999	1402.435	4881.469
TBili [mg/dl]	A	1800g/10 min	0.473	0.271	0.463	0.259
	B	1800g/10 min	0.465	0.267	0.468	0.263
TG [mg/dl]	A	1800g/10 min	175.662	102.622	175.326	101.334
	B	1800g/10 min	175.074	101.550	174.905	100.066
TP [g/l]	A	1800g/10 min	74.169	4.391	73.255	4.612
	B	1800g/10 min	74.588	4.335	74.067	4.511
TSH [µIU/ml]	A	1800g/10 min	1.881	1.304	2.019	1.404
	B	1800g/10 min	1.881	1.317	2.023	1.409
Trop T [µg/l]	A	1800g/10 min	7.019	4.408	7.171	4.275
	B	1800g/10 min	7.124	4.438	7.117	4.377
UA[mg/dl]	A	1800g/10 min	5.011	1.527	5.107	1.564
	B	1800g/10 min	5.033	1.550	5.122	1.555
Urea [mg/dl]	A	1800g/10 min	29.005	10.769	28.864	10.556
	B	1800g/10 min	29.050	10.713	29.076	10.553
Vit B12 [pg/ml]	A	1800g/10 min	408.585	224.335	380.550	204.313
	B	1800g/10 min	414.200	219.241	379.600	205.155

Measurement of LIH:

LIH was negative in all samples for icterus, lipemia and hemolysis.

5.2/ BIAS METHOD COMPARISON SAMPLE A TO B

Analyt	Bias [%] Initial A to B (1800g/10 min)
	Initial time point [0h]
ALB	-0.09
ALP	-0.09
ALT	0.10
TBİLI	-1.88
Ca	0.41
CHOL	-0.19
CK	-0.27
CI	0.06
CREA	0.01
CRP	-0.22
GGT	1.18
GLUC	0.20
HDL	0.18
Fe	-0.09
K	0.01
LDH	0.23
Mg	1.43
Na	0.13
IP	0.55
TP	0.58
TG	-0.16
UA	0.34
UREA	0.11
VitB12	-0.33
CORT	-1.70
CKMB	0.84
E	-0.34
FOL	-0.62
FSH	0.02
FT3	0.40
Trop T	2.08
TSH	0.16

5.3/ BIAS ESTIMATION STABILITY:

Analyt	Acceptance criteria (Westgard)	Bias estimation rel. difference	
		Bias A0-A48	Bias B0-B48
ALB	1.43	1.50	2.01
ALP	6.72	-1.53	-1.53
ALT	11.48	-1.74	-0.22
TBİL	8.95	-1.18	1.77
Ca	0.82	1.63	1.64
CHOL	4.10	2.74	3.24
CK	11.50	-0.30	0.27
CI	0.50	0.50	0.88
CREA	3.96	-8.10	-8.27
CRP	21.80	-0.97	1.91
GGT	11.06	0.87	0.76
GLUC	2.34	-0.80	-0.16
HDL	5.61	-2.19	-2.13
Fe	8.80	0.99	1.49
K	1.81	1.87	1.94
LDH	4.30	-5.62	-5.92
Mg	1.80	0.71	0.69
Na	0.23	2.91	3.27
IP	3.38	0.22	0.15
TP	1.36	-1.25	-0.70
TG	9.57	0.23	0.36
UA	4.87	1.84	1.85
UREA	6.05	-0.44	0.36
VitB12		-8.81	-8.52
CORT	10.26	0.82	1.33
CKMB	14.88	-5.56	-5.95
E2	8.30	9.05	8.29
FOL	19.20	8.24	9.76
FSH	12.12	0.30	-0.24
FT3	4.00	16.64	16.09
Trop T	23.70	-6.74	-5.68
TSH	7.80	8.23	8.06

6/ SUMMARY OF RESULTS - INITIAL MEASUREMENT:

Clinical acceptance criteria 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.

6.1/ METHOD COMPARISON

All of the parameters tested (23 parameters clinical chemistry and 9 parameters immunology) meet the acceptance criteria when comparing the tubes to each other as shown in the table above. The initial values of all tubes tested are comparable without clinically significances.

6.2/ STABILITY

The following parameters exceed the acceptance criteria comparing the time points T0 and T48: Alb, Ca, Cl, Crea, K, LDH, Na, fT_3, TSH in all tubes included in this study. The acceptance criterion for Estradiol is met only in sample B. Please note: Clinical acceptance criteria are used 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.

Well described in literature is the reduced stability of analytes influenced by active cell contact: "LDH and bicarbonate were the analytes with the lowest stability after centrifugation"; "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" [1]. Further studies cited in this publication indicate stability considering various pre-analytical conditions and point out to: "In practice, LDH, magnesium, phosphorus and potassium are the analytes most influenced by delayed centrifugation, as they are present in cells." [1].

"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 [2].

In literature, there is pointed out, that LC-MS is the "gold standard" for determination of estradiol: "Unfortunately, much of the current bioanalytical methodology employed for the analysis of plasma or serum estrogens has proved to be problematic. Major advances in risk assessment would be possible if more reliable methodology were readily available to quantify estradiol and its major metabolites in the plasma or serum…"; "It is almost impossible to overcome the inherent assay problems involved in using RIA-based methodology, particularly for multiple estrogens. For reliable measurements of multiple estrogens in plasma or serum, it is necessary to employ stable isotope dilution methodology in combination with LC-MS/MS or GC-MS/MS. These technologies represent the "gold standard" for the analysis of multiple estrogens when they are used under rigorously validated conditions [3].

Assessing the deviations found for the parameters ALB, Ca, Cl, CREA, K, LDH, Na, fT_3, TSH, it can be concluded that substantially clinically equivalent performance is provided in SEKISUI Insepak® II-D Rapid coagulation type blood collection tubes in comparison to VACUETTE® Serum Fast Separator tubes containing thrombin. The stability in VACUETTE® Serum Fast Separator tubes was found to be better for Estradiol compared to SEKISUI Insepak® II-D Rapid coagulation type tubes when centrifuged at 1800g/10 min.

7/ CONCLUSION

The substantially equivalent clinical performance of the modified VACUETTE® CAT Serum Fast Separator blood collection tube in comparison to the Sekisui Insepak® II-D Rapid coagulation type blood collection tube has been demonstrated for routine biochemical analytes on a Roche COBAS® 8000 analyzer at initial time and after 48 hours for hospitalized donors.

By providing a clear serum after centrifugation, the utilization of the modified 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 basis of the thrombin additive. Systematic differences to blood collection tubes without a clotting accelerator such as thrombin were found in studies and discussed with regard to the benefit in emergency situations but need to be taken in consideration by clinicians [4/5/6]. One study investigated the risk of hyperkalaemia in a thrombin-containing tube by measuring potassium values as well as LDH activity [7]. Another study presented stability data for a routine chemistry profile up to 4 days apart from bicarbonate, electrolytes and albumin [8].

8/ REFERENCES

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PRODUCT & ORDERING INFORMATION

- **Only 5 minutes[2] waiting time before centrifugation.**
- **Reduced turnaround time.**
- **Faster results.**
- **Faster diagnosis.**

Time is of the essence when it comes to accurate and fast test results for treating patients. Fast clotting following blood collection allows crucial minutes to be saved.

Heparinized plasma is often used as an emergency tube as there is no need to wait for clotting. Serum is sometimes indispensable in emergency departments and this is precisely where VACUETTE® CAT Serum Fast Tubes can save enormous amounts of time.[1]

The VACUETTE® CAT Serum Fast tube combines the speed of a plasma tube with the properties of serum. It allows clotting in the whole blood sample to be completed in just 5 minutes,[2] thus considerably shortening the preanalytical process. This means targeted treatment can be initiated quicker.
With a reduced centrifugation time of 5 minutes[2], the time from collection to analysis is 10 minutes instead of 40 minutes[3]. This makes it easy to effectively reduce turnaround time (TAT) by 30 minutes per sample.[1]

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

References and information:

1 Use of anticoagulants in diagnostic laboratory investigations. World Health Organization. WHO/DIL/LAB/99.1 Rev.2, 2002.
2 Serum Fast tubes are not intended for patients on thrombin inhibitor therapy or fibrinogen deficiency.
3 Depending on centrifugation conditions

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