



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 VACUETTE® CAT SERUM SEPARATOR TUBE

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.

CONTENT

1. Background
2. Study Objective
3. Study design and procedure
4. Analytes for stability testing on Roche cobas® 8000
5. Results
6. Results - Summary
7. Conclusion
8. References

2/ STUDY OBJECTIVE

The study has been carried out to demonstrate method comparison of modified VACUETTE® CAT Serum Fast Separator blood collection tubes to the VACUETTE® CAT Serum Separator Blood Collection tubes when centrifuged at 1800g for 10 min for routine chemistry analysis. The objective of the study was to show representative biochemical analytes in VACUETTE® CAT Serum Fast Separator blood collection tubes when centrifuged at 1800g/10 min as well as at 3000g/5 min.

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	Prod. No.	Volume [ml]	Centrifugation
A	VACUETTE® CAT Serum Separator tube	456073	5	1800g / 10 min / 20°C
B	VACUETTE® CAT Serum Fast Separator tube	456309	5	1800g / 10 min / 20°C
C	VACUETTE® CAT Serum Fast Separator tube	456309	5	3000g / 5 min / 20°C

One tube of each sample was drawn from each donor. All samples were gently inverted 5 times and sample A was allowed to clot for 30 min. After a minimum of 5 min. clotting time (sample B and C) 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 (centrifuge: NUVE 800 cooled centrifuge for Sample A and B; CENCE MEDIKAL 4110 rpm/2500 rcf for Sample C).

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	10.00
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	4.50

Parameter	Abbreviation	Allowable Bias (Westgard)
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 B ₁₂)	VitB ₁₂	
Lipemic Icteric Haemolysis Index	LIH	N.A.

Statistics were performed using Excel for method comparison.

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

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, 7 clotted samples were detected on both clinical chemistry and immunology module of cobas® 8000. Those samples were immediately checked by eye and only one tube (sample C) was found with a clot. All tubes were rerun 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 initial	SD initial	Mean 48h	SD 48h
ALB [g/l]	A	1800g/10 min	44.719	2.969	45.564	2.987
	B	1800g/10 min	45.052	3.262	45.669	3.237
	C	3000g/5 min	45.242	3.189	45.638	3.178
ALP [U/l]	A	1800g/10 min	76.238	25.914	75.429	25.868
	B	1800g/10 min	76.476	26.026	75.357	25.709
	C	3000g/5 min	76.381	26.023	75.095	25.640
ALT [U/l]	A	1800g/10 min	17.095	8.328	16.786	7.773
	B	1800g/10 min	17.119	8.146	16.905	7.802
	C	3000g/5 min	17.524	8.370	16.714	7.731
Ca [mmol/l]	A	1800g/10 min	2.403	0.092	2.420	0.101
	B	1800g/10 min	2.407	0.087	2.422	0.092
	C	3000g/5 min	2.405	0.088	2.423	0.084
CHOL [mg/dl]	A	1800g/10 min	194.191	47.672	199.952	47.355
	B	1800g/10 min	194.452	46.166	200.310	46.041
	C	3000g/5 min	194.310	46.983	200.071	46.374

Analyte	Sample	Centrifugation	Mean initial	SD initial	Mean 48h	SD 48h
CK [U/l]	A	1800g/10 min	93.143	46.570	93.452	46.617
	B	1800g/10 min	93.810	46.895	94.119	47.022
	C	3000g/5 min	94.000	46.670	94.119	47.085
CK-MB [U/l]	A	1800g/10 min	1.996	1.491	1.888	1.446
	B	1800g/10 min	1.995	1.481	1.890	1.438
	C	3000g/5 min	1.996	1.499	1.872	1.438
Cl [mmol/l]	A	1800g/10 min	100.881	2.297	102.262	2.049
	B	1800g/10 min	101.000	2.072	101.881	2.276
	C	3000g/5 min	100.929	2.267	102.167	2.294
Cort [mg/dl]	A	1800g/10 min	12.015	5.790	12.120	5.746
	B	1800g/10 min	12.076	5.731	12.114	5.691
	C	3000g/5 min	11.936	5.771	11.995	5.782
CRP [mg/l]	A	1800g/10 min	4.319	4.532	4.327	4.547
	B	1800g/10 min	4.289	4.519	4.357	4.570
	C	3000g/5 min	4.316	4.538	4.330	4.581
Fe [µmol/l]	A	1800g/10 min	85.119	81.416	85.667	81.175
	B	1800g/10 min	85.357	81.625	86.190	82.174
	C	3000g/5 min	85.690	82.463	86.429	81.919
FOL [ng/ml]	A	1800g/10 min	8.039	2.779	8.513	2.949
	B	1800g/10 min	8.432	3.111	9.076	2.986
	C	3000g/5 min	8.605	2.962	9.217	3.177
fT3 [pg/ml]	A	1800g/10 min	3.166	0.485	3.640	0.507
	B	1800g/10 min	3.153	0.478	3.650	0.497
	C	3000g/5 min	3.176	0.494	3.644	0.510
GGT [U/l]	A	1800g/10 min	23.167	19.423	23.286	19.549
	B	1800g/10 min	23.190	19.347	23.357	19.581
	C	3000g/5 min	23.000	19.536	23.238	19.616
Gluc [mg/dl]	A	1800g/10 min	109.286	33.413	108.833	33.129
	B	1800g/10 min	110.976	33.752	110.738	33.510
	C	3000g/5 min	111.571	34.242	111.071	33.577
HDL [mg/dl]	A	1800g/10 min	47.817	13.783	47.005	13.308
	B	1800g/10 min	48.071	13.774	46.964	13.268
	C	3000g/5 min	48.010	13.912	47.193	13.241
hFSH [mIU/ml]	A	1800g/10 min	15.586	21.946	15.636	21.958
	B	1800g/10 min	15.531	21.530	15.512	21.711
	C	3000g/5 min	15.499	21.578	15.594	21.831
IP [mmol/l]	A	1800g/10 min	1.097	0.165	1.106	0.168
	B	1800g/10 min	1.105	0.164	1.107	0.166
	C	3000g/5 min	1.107	0.164	1.113	0.166
K [mmol/l]	A	1800g/10 min	4.341	0.390	4.449	0.387
	B	1800g/10 min	4.275	0.385	4.356	0.375

Analyte	Sample	Centrifugation	Mean initial	SD initial	Mean 48h	SD 48h
Crea [mg/dl]	C	3000g/5 min	4.200	0.388	4.389	0.400
	A	1800g/10 min	0.758	0.272	0.686	0.241
	B	1800g/10 min	0.752	0.268	0.692	0.248
LDH [U/l]	C	3000g/5 min	0.752	0.260	0.696	0.252
	A	1800g/10 min	164.262	43.860	155.357	41.549
	B	1800g/10 min	166.262	43.741	156.071	41.056
Mg [mmol/l]	C	3000g/5 min	167.000	42.432	158.488	41.613
	A	1800g/10 min	0.808	0.062	0.815	0.065
	B	1800g/10 min	0.816	0.063	0.821	0.064
Na [mmol/l]	C	3000g/5 min	0.821	0.076	0.821	0.065
	A	1800g/10 min	137.262	2.557	142.095	2.487
	B	1800g/10 min	137.405	2.270	141.881	2.098
E2 [pg/ml]	C	3000g/5 min	137.238	2.694	142.071	2.858
	A	1800g/10 min	1237.095	4917.854	1443.486	5007.448
	B	1800g/10 min	1225.135	4857.999	1402.435	4881.469
TBili [mg/dl]	C	3000g/5 min	1225.280	4858.626	1463.501	5106.642
	A	1800g/10 min	0.469	0.263	0.525	0.259
	B	1800g/10 min	0.465	0.267	0.468	0.263
TG [mg/dl]	C	3000g/5 min	0.473	0.266	0.461	0.260
	A	1800g/10 min	174.631	102.311	174.679	100.549
	B	1800g/10 min	175.074	101.550	174.905	100.066
TP [g/l]	C	3000g/5 min	174.774	101.753	175.138	100.736
	A	1800g/10 min	74.160	4.133	73.745	4.233
	B	1800g/10 min	74.588	4.335	74.067	4.511
TSH [µIU/ml]	C	3000g/5 min	74.543	4.402	74.038	4.203
	A	1800g/10 min	1.882	1.315	2.027	1.422
	B	1800g/10 min	1.881	1.317	2.023	1.409
Trop T [µg/l]	C	3000g/5 min	1.874	1.298	2.020	1.418
	A	1800g/10 min	6.871	4.470	7.182	4.426
	B	1800g/10 min	6.871	4.470	7.182	4.426
UA [mg/dl]	B	1800g/10 min	7.124	4.438	7.117	4.377
	C	3000g/5 min	6.916	4.413	7.420	4.492
	A	1800g/10 min	5.026	1.540	5.124	1.555
Urea [mg/dl]	B	1800g/10 min	5.033	1.550	5.122	1.555
	C	3000g/5 min	5.013	1.537	5.119	1.559
	A	1800g/10 min	28.952	10.744	29.014	10.499
Vit B12 [pg/ml]	B	1800g/10 min	29.050	10.713	29.076	10.553
	C	3000g/5 min	29.212	10.706	29.026	10.591
	A	1800g/10 min	404.073	220.237	375.675	202.959
	B	1800g/10 min	414.200	219.241	379.600	205.155
	C	3000g/5 min	403.268	219.013	376.425	206.154

Measurement of LIH:

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

5.2/ BIAS METHOD COMPARISON (SAMPLE A TO B AND SAMPLE B TO C)

Analyt	Bias[%] Sample A to B (1800g/10 min)	Bias[%] Sample B (1800g/10 min) to C (3000g/5min)
	Initial time point [0h]	Initial time point [0h]
ALB	0.72	-0.16
ALP	0.32	0.12
ALT	1.40	-1.33
TBILI	-0.94	-1.95
Ca	0.09	-0.14
CHOL	0.31	0.17
CK	0.83	-0.27
Cl	0.13	0.09
CREA	-0.44	0.01
CRP	0.00	0.03
GGT	0.29	1.34
GLUC	1.59	-0.48
HDL	0.54	0.19
Fe	0.26	-0.31
K	-1.45	-0.47
LDH	1.40	-0.52
Mg	0.95	-0.48
Na	0.11	0.13
IP	0.84	-0.17
TP	0.58	0.07
TG	0.48	0.19
UA	0.13	0.39
UREA	0.35	-0.56
VitB12	0.73	0.88
CORT	0.20	-1.62
CKMB	0.14	0.01
E	0.28	0.09
FOL	4.80	-2.54
FSH	0.14	0.04
FT ₃	-0.38	-0.63
Trop T	1.54	2.68
TSH	-0.43	0.72

5.3/ BIAS ESTIMATION STABILITY:

Analyt	Acceptance criteria (Westgard)	Bias estimation rel. difference		
		Bias A0-A48	Bias B0-B48	Bias C0-C48
ALB	1.43	2.45	2.01	1.77
ALP	6.72	-1.17	-1.53	-1.70
ALT	11.48	0.10	-0.22	-3.22
TBIL	8.95	-1.31	1.77	-1.82
Ca	0.82	1.64	1.64	1.53
CHOL	4.10	3.23	3.24	3.21
CK	11.50	0.42	0.27	-0.02
Cl	0.50	1.38	0.88	1.24
CREA	3.96	-9.27	-8.27	-7.73
CRP	21.80	-0.18	1.91	-1.30
GGT	11.06	0.35	0.76	1.50
GLUC	2.34	-0.38	-0.16	-0.35
HDL	5.61	-1.55	-2.13	-1.46
Fe	8.80	1.07	1.49	1.26
K	1.81	2.50	1.94	2.10
LDH	4.30	-5.20	-5.92	-5.34
Mg	1.80	0.83	0.69	0.17
Na	0.23	3.53	3.27	3.53
IP	3.38	0.85	0.15	0.47
TP	1.36	-0.56	-0.70	-0.66
TG	9.57	0.59	0.36	0.64
UA	4.87	1.94	1.85	2.18
UREA	6.05	0.45	0.36	-0.55
VitB12		-9.03	-8.52	-8.85
CORT	10.26	1.42	1.33	0.84
CKMB	14.88	-5.90	-5.95	-6.89
E2	8.30	10.66	8.29	8.21
FOL	19.20	6.27	9.76	7.50
FSH	12.12	0.51	-0.24	0.08
FT ₃	4.00	15.26	16.09	15.13
Trop T	23.70	-6.33	-5.68	-2.81
TSH	7.80	7.71	8.06	8.83

6/ SUMMARY OF RESULTS - INITIAL MEASUREMENT

Clinical acceptance criteria are intended to support the identification of deviations which should be discussed in view of clinical 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, fT₃ in all tubes included in this study. The acceptance criterion for TSH is only met in sample A and for Estradiol only in samples B and C.

Please note: Clinical acceptance criteria are used to support the identification of deviations which should be discussed in view of clinical 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, fT₃, it can be concluded that substantially clinically equivalent performance is provided in VACUETTE® Serum Fast Separator tubes containing thrombin in comparison to VACUETTE® Serum Separator tubes and centrifuged at various centrifugation conditions. The stability in VACUETTE® Serum Fast Separator tubes was found to be better for Estradiol compared to VACUETTE® Serum Separator tubes when centrifuged at 1800g/10 min. However, stability found for TSH was better in VACUETTE® Serum Separator tubes in comparison to the other tubes tested.

7/ CONCLUSION:

The substantially equivalent clinical performance of the modified 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 Roche cobas® 8000 analyzer at initial time and after 48 hours for hospitalized donors when centrifuged at 1800g/10 min or at 3000g/5 min.

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 hyperkalemia 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 minutes² 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 minutes², the time from collection to analysis is 10 minutes instead of 40 minutes³. 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

Legal text. All tests described in this document were performed applying good practice and maximum care. Sit explicunt, Soloriatem niant eostiatum facid untus, utatum quuntem is minullaut prestiorrum re voluptatem rem unt ommolesequam harciat volorume remposam, sed que officil igendunt magnamus derissi Imus illiquiam quid mo veni.



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