

Evaluation of **VACUETTE[®] SECONDARY** Tubes

Background

VACUETTE[®] SECONDARY Tubes are used as a secondary container for aliquoting, storing and transporting blood, blood components and urine from the primary tube in the clinical laboratory and for the analysis on laboratory analysers. **VACUETTE[®] SECONDARY** Tubes have a dimension of 13x75mm, are made of inert plastic (PET or PP), are non-sterile and not evacuated. They are for single use and have a maximum capacity of 5 ml. Some items are available with a safety cap made of plastic and latex-free, synthetic rubber. ^[1]

Study objective

A clinical evaluation of the **VACUETTE[®] SECONDARY** PET Tubes and the **VACUETTE[®] SECONDARY** PP Tubes was carried out to assess the performance of heat and cold resistance, centrifugation performance and biochemistry parameters.

Materials and Methods

The following tube types were used:

- **VACUETTE[®] SECONDARY** Tube MULTIPLEX PP with white Screw Cap (item # 459000)
- **VACUETTE[®] SECONDARY** Tube MULTIPLEX PET without cap (item # 459011)
- **VACUETTE[®]** Screw Cap (item # 371208L)
- **VACUETTE[®]** Lithium Heparin Gel tubes, 8 ml (item # 455083)

The following instruments were used

- Eppendorf Centrifuge 5804R
- Hettich Rotana 460R Centrifuge
- Olympus AU640 analyzer
- Abbott AxSYM[®] analyzer

Study design

Biochemistry testing

Venous blood collection was performed from 25 presumably healthy donors to fill **VACUETTE[®]** Lithium Heparin Gel tubes using a **VACUETTE[®]** Safety Blood Collection Set with Holder (item # 450085). A discard tube was used to guarantee appropriate filling of the tubes. Directly after blood collection, the tubes were carefully inverted 8 times to allow proper mixing. Tubes were centrifuged at 1800 g for 10 minutes in a cooled Hettich Rotana 460R centrifuge at 20°C. From each donor, 3 ml of plasma was transferred into a **VACUETTE[®] SECONDARY** PET tube and a **VACUETTE[®] SECONDARY** PP tube. The aliquoted samples were analyzed on the Olympus AU640 analyzer and Abbott AxSYM[®] analyzer directly after blood collection and after 48 hours. Accompanying reagents were used from instrument manufacturers. Between the measurements, the samples were stored in the refrigerator at 4-8°C.

Centrifugation

For each **SECONDARY** tube type, 24 tubes were filled with 5 ml Lithium Heparin Whole Blood and centrifuged at 4400g for 10 minutes. Furthermore 80 samples for **VACUETTE[®] SECONDARY** PET tubes and 80 samples for **VACUETTE[®] SECONDARY** PP tubes were filled with 5 ml of a NaCl solution (density 1.080 g/cm³). Those tubes were centrifuged at 4400g for 10 minutes and at 4400g for 30 minutes in a cooled Eppendorf Centrifuge 5804R. Afterwards all samples were visually checked for damage to tube material.

Heat and Cold resistance

For the freezing tests for each **SECONDARY** tube type, 200 tubes were filled with 1-3 ml of Lithium Heparin Plasma. All tubes were placed into a metal rack (from manufacturer Lactan) and samples were frozen first at -20°C for 2 hours. More gentle freezing process can be achieved by keeping the samples in the refrigerator for at least 2 hours before freezing. Afterwards all samples were placed in a freezer at -80 °C for two weeks and for three months. After two weeks half of the samples and after three months the other half of samples were removed to be thawed in the refrigerator (4 °C – 8 °C) overnight. Before the visual examination of the tube material for any breakage, the samples were taken out of the refrigerator to reach room temperature.

For the heat resistance testing, 100 empty **SECONDARY** PP tubes were heated up to 121°C for 20 minutes. PET tubes were constantly stored at 50°C in a warming cabinet for three months.

Results

Results for Biochemistry testing

Comparison analysis of the **VACUETTE® SECONDARY** PET tubes and the **VACUETTE® SECONDARY** PP tubes was performed at the initial time point and at 48 hours.

Statistical evaluation was performed with the T-test ($\alpha = 0.05$) using StatSoft Software, Version 9. Any statistically significant differences were evaluated if clinically significant. Moreover the highest deviations for each comparison and parameter were evaluated for clinically significant differences. Clinical evaluation was based on the allowed recommendation by the German Medical Association (RILIBÄK).^[4] No clinical significant differences could be observed between the **VACUETTE® SECONDARY** PP and **VACUETTE® SECONDARY** PET tubes for the initial time point and after 48 hours.

For the parameters on the instrument Abbott AxSYM®, for fT₃, fT₄, TSH, β -HCG, Troponin-I and Vitamin B₁₂ only a few values for the **VACUETTE® SECONDARY** PET tubes were available due to instrumentation errors during measurement. Outlier tests were performed and where applicable considered.

The following tables 1-2 show mean values and standard deviation for each tube type and time point.

Table 1: SECONDARY PP Tubes at initial time point and at time point 48h

Parameter	Valid N	Mean	Std. Dev.	Valid N	Mean	Std. Dev.	Reference Range [2,3]
	Initial time point			Time point 48h			
Albumin	25	43.94	2.50	25	44.34	2.54	35-53 g/l
Alkaline Phosphatase	25	56.00	17.77	25	53.68	17.03	♂: 40-130 U/L, ♀: 55-105 U/L
Aspartat-Amino-transferase	25	23.86	11.36	24	24.43	11.49	♂: < 45 U/L, ♀: < 34 U/L
Calcium Arsenazo	25	2.44	0.09	25	2.42	0.10	2.20-2.65 mmol/L
Cholesterol	25	207.81	43.36	25	206.80	43.08	< 200 mg/dl
Creatinine Kinase	25	163.70	271.62	25	160.42	266.84	♂: < 190 U/L, ♀: < 167 U/L
Chloride	24	105.37	2.26	25	105.53	2.15	95-105 mmol/L
Creatinine	25	73.58	14.54	25	74.01	14.74	♂: 49-97 µmol/L, ♀: 42-80 µmol/L
Gamma-GT	25	19.00	18.29	25	18.78	18.64	♂: < 55 U/L, ♀: < 38 U/L
Glucose	25	87.61	9.51	25	88.82	9.64	74-109 mg/dl
Inorganic Phosphate	25	1.07	0.13	25	1.06	0.13	0.84-1.45 mmol/L
Iron	25	18.08	7.19	25	18.13	7.28	25 yrs: ♂: 7.2-27.7 µmol/L, ♀: 6.6-29.5 µmol/L; 40 yrs: ♂: 6.3-30.1 µmol/L, ♀: 4.1-24.0 µmol/L; 60 yrs: ♂: 7.2-21.5 µmol/L, ♀: 7.0-26.7 µmol/L
Potassium	24	3.84	0.18	25	3.85	0.19	3.4-4.5 mmol/L
Lactatdehydro-genase	25	147.97	25.75	25	140.87	24.23	♂: < 248 U/L, ♀: < 247 U/L
Magnesium	25	0.83	0.06	25	0.83	0.06	0.75-1.10 mmol/L
Sodium	24	140.03	1.62	25	140.17	1.44	135-145 mmol/L
Total Bilirubin	25	0.96	0.61	25	0.93	0.60	0.1-1.2 mg/dL
Total Protein	25	73.04	4.45	25	73.00	4.29	66-83 g/L
Triglyceride	25	100.72	42.19	25	98.88	42.24	< 150 mg/dL
Uric acid	25	5.03	1.43	25	4.98	1.40	♂: 3.6-7.0 mg/dL, ♀: 2.3-6.1 mg/dL
Urea	25	26.12	7.17	25	26.35	7.24	♂ 18-55 mg/dL, ♀ 15-43 mg/dL
Free Triiodothyronine	25	2.50	0.25	25	2.54	0.25	1.45-3.48 pg/mL
Free Thyroxine	25	1.03	0.16	25	1.03	0.17	0.71-1.85 ng/dL
Thyroid-stimulating hormone	25	2.20	1.58	25	2.09	1.53	0.49-4.67 µU/mL
Beta-HCG	25	0.00	0.00	25	0.01	0.03	<5 mU/L
Troponin I	25	0.00	0.00	25	0.00	0.00	0.27-4.00 ng/mL
Vitamin B12	25	321.69	165.93	25	351.45	180.83	98.5- >1200 pg/ml

Table 2: SECONDARY PET Tubes at initial time point and at time point 48h

Parameter	Valid N	Mean	Std. Dev.	Valid N	Mean	Std. Dev.	Reference Range [2,3]
	Initial time point			Time point 48h			
Albumin	25	44.10	2.64	24	44.49	2.75	35-53 g/l
Alkaline Phosphatase	25	56.23	17.81	24	54.78	16.48	♂: 40-130 U/L, ♀: 55-105 U/L
Aspartat-Amino-transferase	25	23.85	11.22	23	23.90	11.72	♂: < 45 U/L, ♀: < 34 U/L
Calcium Arsenazo	25	2.47	0.09	25	2.47	0.09	2.20-2.65 mmol/L
Cholesterol	25	210.30	44.25	24	210.19	44.50	< 200 mg/dl
Creatinine Kinase	25	164.40	271.57	25	161.01	268.22	♂: < 190 U/L, ♀: < 167 U/L
Chloride	25	105.32	2.26	23	105.85	2.40	95-105 mmol/L
Creatinine	25	73.28	14.36	25	73.86	14.80	♂: 49-97 µmol/L, ♀: 42-80 µmol/L
Gamma-GT	25	18.86	18.05	25	18.88	18.45	♂: < 55 U/L, ♀: < 38 U/L
Glucose	25	88.14	9.78	25	88.14	9.71	74-109 mg/dl
Inorganic Phosphate	25	1.07	0.13	24	1.14	0.15	0.84-1.45 mmol/L
Iron	25	18.01	7.19	24	17.70	7.03	25 yrs: ♂: 7.2-27.7 µmol/L, ♀: 6.6-29.5 µmol/L; 40 yrs: ♂: 6.3-30.1 µmol/L, ♀: 4.1-24.0 µmol/L; 60 yrs: ♂: 7.2-21.5 µmol/L, ♀: 7.0-26.7 µmol/L
Potassium	25	3.84	0.17	23	3.86	0.19	3.4-4.5 mmol/L
Lactatdehydro-genase	25	148.78	26.01	24	139.72	25.66	♂: < 248 U/L, ♀: < 247 U/L
Magnesium	25	0.84	0.06	25	0.83	0.06	0.75-1.10 mmol/L
Sodium	25	140.09	1.49	23	141.01	1.67	135-145 mmol/L
Total Bilirubin	25	0.96	0.62	25	0.84	0.55	0.1-1.2 mg/dL
Total Protein	25	73.07	4.60	25	72.95	4.70	66-83 g/L
Triglyceride	25	100.59	42.37	23	100.82	41.68	< 150 mg/dL
Uric acid	25	5.03	1.42	25	5.02	1.43	♂: 3.6-7.0 mg/dL, ♀: 2.3-6.1 mg/dL
Urea	25	26.38	7.26	23	26.31	7.40	♂ 18-55 mg/dL, ♀ 15-43 mg/dL
Free Triiodothyronine	7	2.28	0.19	7	2.48	0.22	1.45-3.48 pg/mL
Free Thyroxine	7	0.96	0.09	7	0.96	0.09	0.71-1.85 ng/dL
Thyroid-stimulating hormone	6	2.03	0.77	7	2.45	1.55	0.49-4.67 µU/mL
Beta-HCG	7	0.00	0.00	7	0.00	0.00	<5 mU/L
Troponin I	7	0.00	0.00	7	0.00	0.00	0.27-4.00 ng/mL
Vitamin B12	7	309.91	178.30	7	358.30	170.58	98.5- >1200 pg/ml

Results for centrifugation

All tubes were visually examined after centrifugation. None of the tubes showed any breakage or leakage.

Results for Heat and Cold resistance

After two weeks and three months freezing at -80°C, the tubes were thawed according to the protocol and none of the tubes showed any breakage or leakage stored in the metal rack.

None of the **VACUETTE® SECONDARY** PP tubes showed any breakage of tubes after heat application (121°C for 20 min). For **VACUETTE® SECONDARY** PET tubes heat application of 50 °C for three months showed no influence on tube material.

Conclusion

VACUETTE® SECONDARY PET tubes and **VACUETTE® SECONDARY** PP tubes were tested for their performance of heat and cold resistance, centrifugation and biochemistry parameters. The PET and PP tubes showed comparable results for Biochemistry parameters at the initial time point and at 48 hours. Results did not show any clinically significant deviations. Also both tube types did not show any breakage after centrifugation up to 4400g for 30 minutes. None of the tested **VACUETTE® SECONDARY** PET tubes and **VACUETTE® SECONDARY** PP tubes showed any cracks in the tube material nor consecutive leaking of sample after freezing at -80°C for two weeks and three months. Moreover for the **VACUETTE® SECONDARY** PET tubes regarding storage at 50°C for three months and for **VACUETTE® SECONDARY** PP tubes heat application of 121°C for 20 minutes, no cracks were found in the tube material.

References

- [1] Instructions for Use **VACUETTE® SECONDARY** tubes. 980254. Rev 00.
- [2] Olympus product insert.
- [3] AxSYM® product insert.
- [4] Richtlinie der Bundesärztekammer zur Qualitätssicherung laboratoriumsmedizinischer Untersuchungen. Deutsches Ärzteblatt. Jg. 105, Heft 7. 2008.