

Evaluation of **VACUETTE**[®] Urine CCM tube for Clinical Chemistry

Background

The **VACUETTE**[®] Urine CCM tube is for the collection, transport and storage of urine samples for urine culture and urinalysis in the laboratory. The evacuated tube contains a stabilizer to preserve the urine sample at room temperature (20 – 25°C) for up to 48 hours. **VACUETTE**[®] Urine CCM tubes are made of PET with a pre-defined vacuum for exact draw volumes. They are fitted with yellow **VACUETTE**[®] Safety Caps. The tube interior is sterile.

For patients with symptoms compatible with kidney diseases and/or descending urinary tract disorders, laboratory tests in blood and urine are an important basis for diagnosis, monitoring and therapy control. ^[1] For many urine components, a quantitative result is required for the diagnosis and follow-up of patients. ^[2] Generally it is considered to perform urinalysis within two hours of collection to rule out preanalytical bias. If testing is delayed, storage at 4°C is adequate for some chemical analytes, otherwise the chemical composition of urine changes, and formed elements begin to deteriorate. ^[3]

Study objective

A clinical evaluation of the **VACUETTE**[®] Urine CCM tube was carried out to evaluate the performance of quantitative determination of clinical-chemical parameters over a period of 48 hours stored at room temperature (20-25°C) compared to a **VACUETTE**[®] Urine No Additive tube stored at 4°C in the refrigerator. The quantitative chemical measurements were performed on the Cobas Integra[®] 800 instrument (Roche Diagnostics) and Olympus AU640 instrument (Olympus Medial Systems).

Materials and Methods

The following materials were used:

VACUETTE[®] Urine CCM tubes (item # 455052)

VACUETTE[®] Urine No Additive tubes (item # 455007)

Study design

Testing on the Roche Cobas Integra[®] 800 instrument

A total of 100 spiked, clinically normal and clinically abnormal (nitrite and leukocyte positive with dipstick urinalysis) urine samples were transferred into the two different urine collection tubes and measured on the Cobas Integra[®] 800 from Roche. The measurements were carried out within 4 hours, after 24 hours, after 48 hours and after 72 hours of urine collection.

The methods included photometry, potentiometry and turbidimetry. The following parameters were measured: Calcium, Magnesium, Potassium, Phosphate, Total Protein, Glucose, Uric acid, Urea, Albumin, Amylase, Creatinine and Chloride.

Testing on the Olympus AU640 instrument

A total of 15 clinically normal and spiked urine samples were transferred into the two different urine collection tubes and measured on the Olympus AU640. The measurements were carried out within 4 hours, after 24 hours, after 48 hours and after 72 hours of urine collection.

The methods included photometry, potentiometry and turbidimetry. The following parameters were measured: Calcium, Magnesium, Potassium, Phosphate, Total Protein, Glucose, Uric acid, Urea, Micro-Albumin, Amylase, Creatinine and Chloride.

Results

Assuming that preanalytics and systematic measuring deviations for an analyte remain constant, the significance of the deviation of a laboratory value versus the reference tube was estimated based on the "Reference Change Value" ($RCV = 2^{0.5} \cdot Z \cdot (CV_A^2 + CV_I^2)^{0.5}$). As the biological variation (CV_I) is negligible and at a significance level of $p < 0.05$ ($Z = Z$ score), the formula is simplified accordingly into: $RCV = 1.414 \cdot 1.96 \cdot CV_A$. The analytical variation coefficient (CV_A) was taken from validation data.

The analytical significance limit was determined to be 10%.^[4]

α -Amylase (IFCC method; Roche)

Reference range ($<10 \mu\text{kat/L}$)³

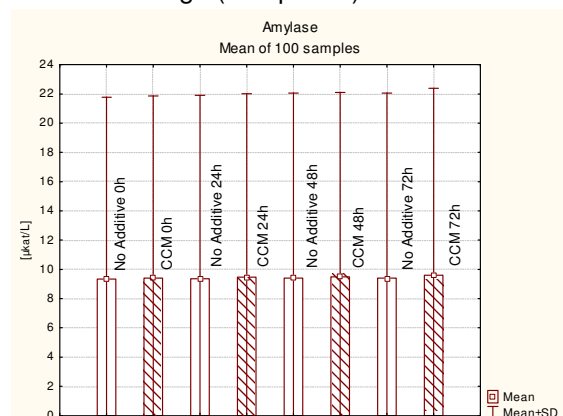


Figure 1: α -Amylase, Roche

Calcium (Calcium-Arsenazo III complex; Olympus)

Reference range ($<7.5 \text{ mmol/L}$)¹

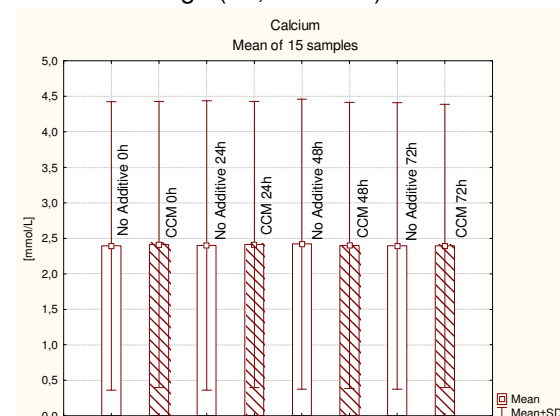


Figure 3: Calcium, Olympus

α -Amylase (IFCC method; Olympus)

Reference range ($<490 \text{ U/L}$)¹

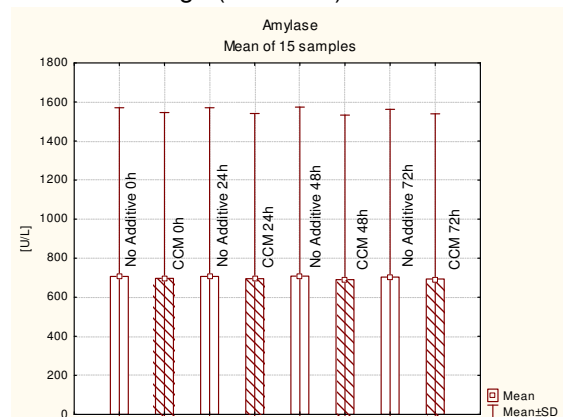


Figure 2: α -Amylase, Olympus

Creatinine (Jaffé reaction; Roche)

Reference range ($3.1-11.4 \text{ mmol/L}$)²

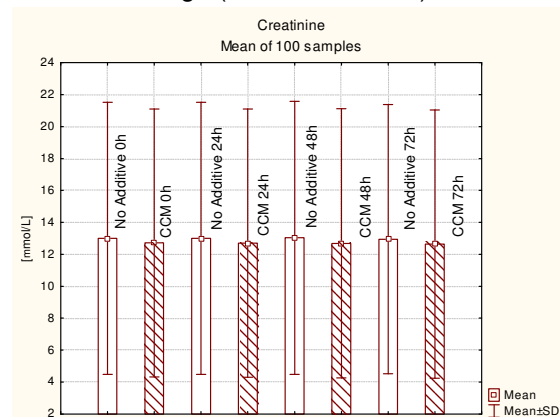


Figure 4: Creatinine, Roche

Creatinine (Realization with Creatinase and HMMPS; *Olympus*)

Reference range (3182-11492 $\mu\text{mol/L}$)²

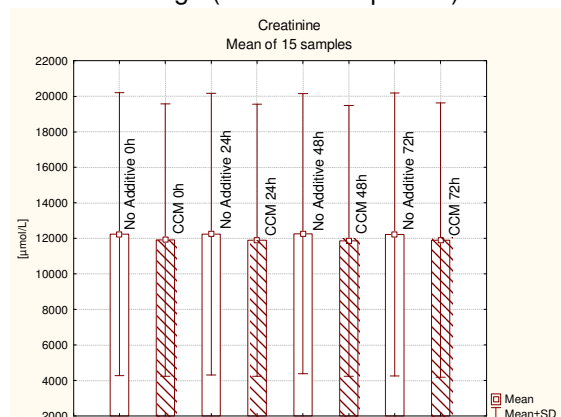


Figure 5: Creatinine, Olympus

Glucose (Hexokinase method; *Olympus*)

Reference range (1-15 mg/dL)¹

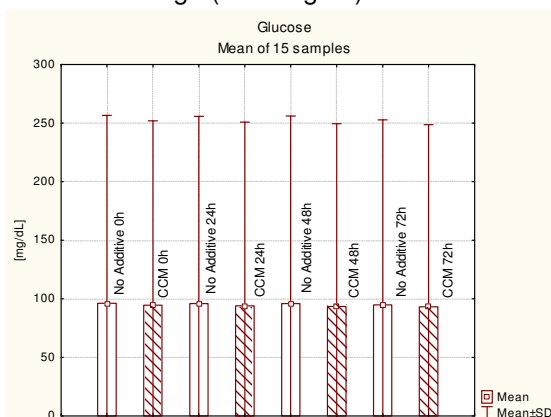


Figure 8: Glucose, Olympus

Total Protein (Turbidimetric method; *Roche*)

Reference range (<150 mg/L)³

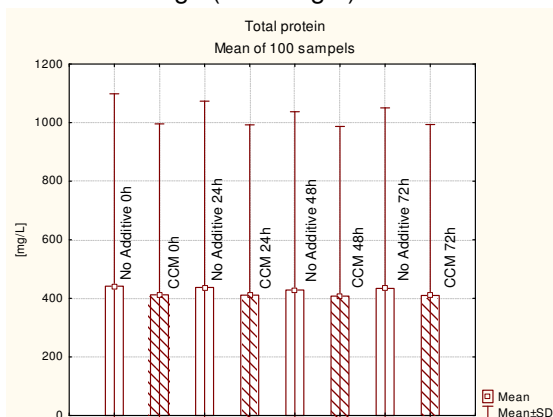


Figure 6: Total Protein, Roche

Uric Acid (split via Uricase in Allantoin and hydrogen peroxide; *Roche*)

Reference range (<5 mmol/L)³

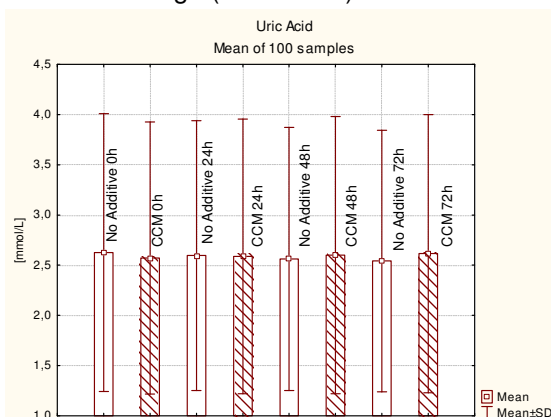


Figure 9: Uric Acid, Roche

Glucose (Hexokinase method; *Roche*)

Reference range (<1 mmol/L)³

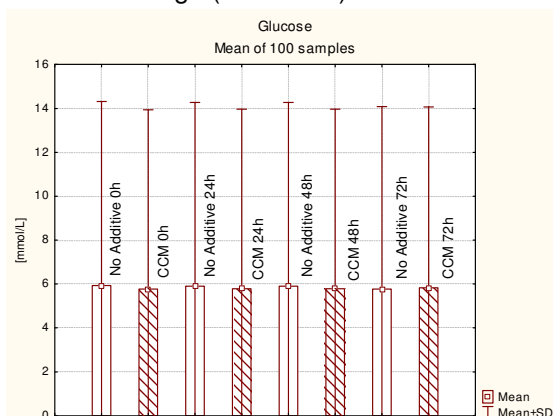


Figure 7: Glucose, Roche

Uric Acid (Trinder reaction; *Olympus*)

Reference range (<75 mg/dL)¹

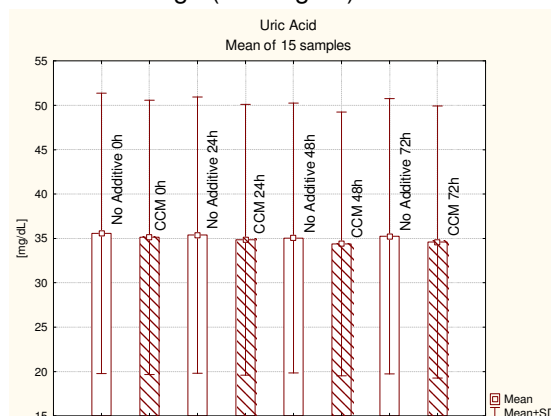


Figure 10: Uric Acid, Olympus

Urea (Kinetic test with Urease and glutamate dehydrogenase; *Roche*)

Reference range (150-350 mmol/L)³

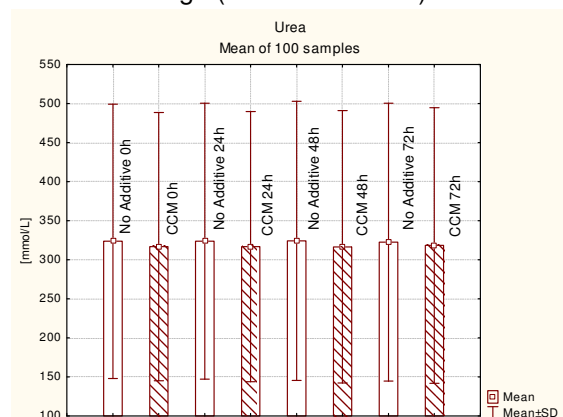


Figure 11: Urea, Roche

Magnesium (Colorimetric method with Chlorosphosphonazo III; *Roche*)

Reference range (3-5 mmol/L)¹

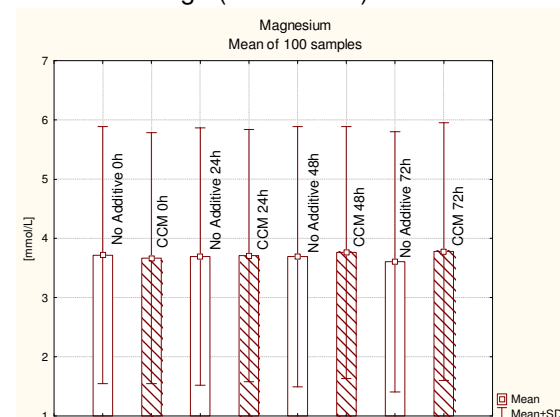


Figure 14: Magnesium, Roche

Potassium (Ion-selective electrode; *Roche*)

Reference range (25-125 mmol/L)¹

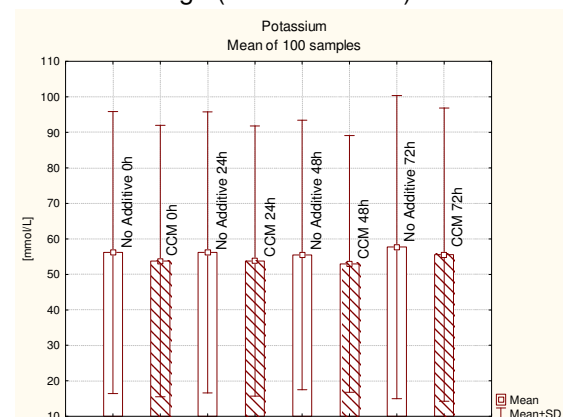


Figure 12: Potassium, Roche

Magnesium (Xylidylblau method; *Olympus*)

Reference range (3-5 mmol/L)¹

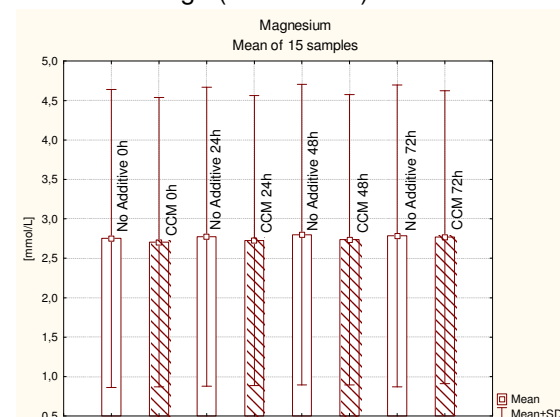


Figure 15: Magnesium, Olympus

Potassium (Ion-selective electrode; *Olympus*)

Reference range (25-125 mmol/L)¹

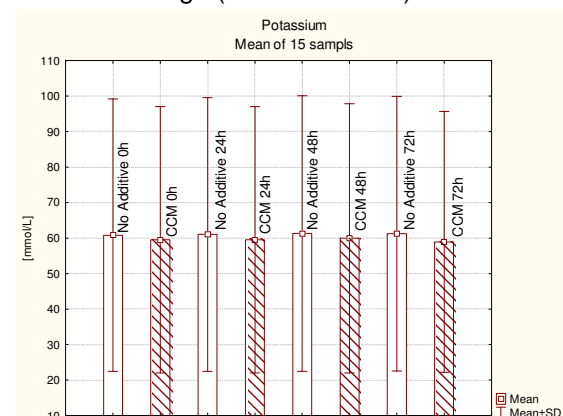


Figure 13: Potassium, Olympus

¹ On the basis of Olympus applications

Albumin (Immunological turbidity; *Roche*)

Reference range ($<30 \text{ mg/L}$)²

Linearity: 10-2000 mg/L

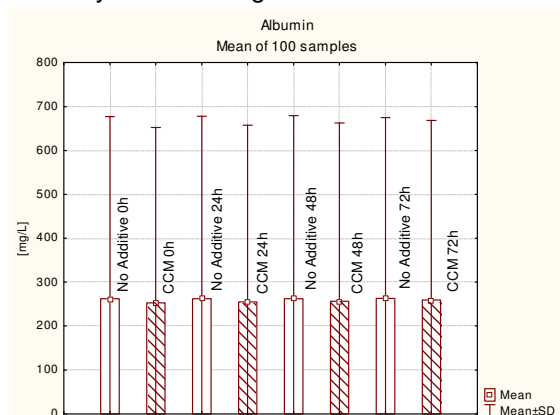


Figure 16: Albumin, Roche

Phosphate anorganic (Molybdate-Heteropolyacid-complex; *Olympus*)

Reference range ($12.9-42 \text{ mmol/L}$)¹

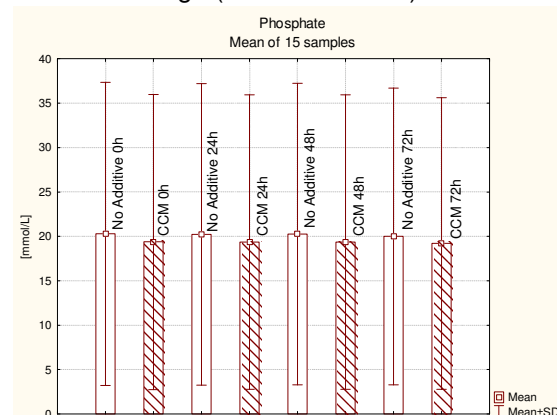


Figure 18: Phosphate anorganic, Olympus

(Micro) Albumin (Turbidimetric test; *Olympus*)

Reference range ($<30 \text{ mg/L}$)²

Linearity: 0.05-3 mg/L

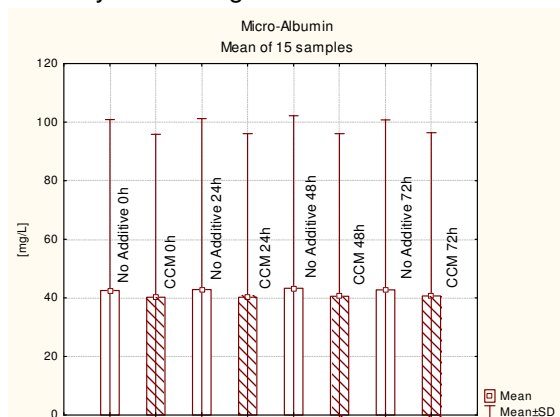


Figure 17: (Micro) Albumin, Olympus

² On the basis of „Thomas, L. Labor und Diagnose. 7th edition 2008. TH-Books“

³ On the basis of Roche applications and laboratory reference values

Discussion:

Roche Cobas Integra® 800 instrument

With the analyzer Cobas Integra® from Roche for the parameters Albumin (immunological turbidimetry), Glucose (Hexokinase method) and Total Protein (turbidimetric method) limitations were observed in the lower concentration range due to measurement close to the quantification limits.

Olympus AU640 instrument

With the analyzer Olympus AU640 for the parameters Glucose (Hexokinase method) and Inorganic Phosphate (Molybdate-Heteropolyacid-complex) there were limitations observed also in the lower concentration range due to measurement close to the quantification limit.

Conclusion

There are limitations close the quantification limit for Glucose, Albumin, inorganic Phosphate and Total Protein, but this is considered not to be clinically significant. Results may vary in addition within different test systems used for measurements.

Results of the **VACUETTE®** Urine CCM tube are comparable to the **VACUETTE®** Urine No Additive tube and show comparable results up to 48 hours.

References

- [1] Thomas, Lothar. (2005) Labor und Diagnose. TH-Books Verlagsgesellschaft mbH, Frankfurt.
- [2] European Urinalysis Guidelines. Scan J Clin Lab Invest 2000. 60: 1-96.
- [3] CLSI. Urinalysis and Collection, Transportation, and Preservation of Urine Specimens; Approved Guideline – Third Edition. GP16-A3. Vol 21 No 19.
- [4] Kouri, T. (2008) Limits of preservation of samples for urine strip tests and particle counting. Clin Chem Lab Med. 46 (5):703-713

Attachment – Summary of appropriate and inappropriate methods of analysis

Table 1: Information concerning appropriate methods of analysis for the clinical-chemical quantitative determinations of the VACUETTE® Urine CCM tubes

Parameter	Appropriate method of analysis			
	Roche		Olympus	
	Reagent	Method	Reagent	Method
α-Amylase	Roche cobas c pack AMYL2 03 183 742 122	Realization with Ethylen-G ₇ PNP (IFCC Method)	Olympus OSR6182	Realization with Ethylen-G ₇ PNP (IFCC Method)
Calcium			Olympus OSR60117	Calcium-Arsenazo III - complex
Creatinine	Roche cobas c pack CREJ2 04 810 716 190	Buffered kinetic Jaffé-Reaction without Deproteinization	Olympus OSR61204	Realization with Creatinase und HMMPS ⁵
Total protein	Roche cobas c pack TPU-C3 03 333 825 190	Turbidimetric Method, the sample is preincubated in an EDTA-containing, alkaline solution, which denatures the protein and eliminates interferences by magnesium ions. Benzethoniumchlorid is then added and leads to a clouding measured at 512 nm.		
Glucose	Roche cobas c pack GLUC2 20 767 131 322	Hexokinase Method	Olympus OSR6121	Hexokinase Method
Uric acid	Roche cobas c pack UA2 03 183 807 190	Uric acid is split via Uricase in Allantoin and hydrogen peroxide.	Olympus OSR6198	Realisation with Uricase and furthermore by Trinder-reaction
Urea	Roche cobas c pack UREAL 04 460 715 190	Kinetic Test with Urease and glutamate dehydrogenase		
Potassium	-	Ion-selective electrode		
Magnesium	Roche cobas c pack MG 20 737 593 322	Colorimetric Method with Chlorophosphonazo III	Olympus OSR 6189	Xylidylblau Method
Micro Albumin			Olympus OSR6167	Turbidimetric test with Antihuman-Albumin-Antibodies
Albumin	Roche cobas c pack ALBT2 04 469 658 190	Immunological turbidity test. Human albumin with a specific anti-serum forms a precipitate measured turbidimetrically at 340 nm.		
Inorganic Phosphate			Olympus OSR6122	Molybdate-Heteropolyacid-Complex

⁵ HMMPS = N-(3-Sulfopropyl)-3-methoxy-5-methylanilin

Table 2: Information concerning inappropriate methods of analysis for the clinical-chemical quantitative determinations of the VACUETTE® Urine CCM tubes

Parameter	Inappropriate method of analysis			
	Roche		Olympus	
	Reagent	Method	Reagent	Method
Calcium	Roche cobas c pack CA 20 763 128 322	Method after Schwarzenbach with o-cresolphthalein complexon		
Total protein			Olympus OSR6170	Pyrogallolrot-Molybdate-complex
Urea			Olympus OSR6134	Realization with Urease, 2-Oxoglutarate and NADH ⁶
Inorganic Phosphate	Roche cobas c pack PHOS2 03 183 793 122	Endpoint Method with sample blank value. Inorganic Phosphate with ammonium molybdate in sulphuric solution form a Ammonium-phospho-molybdate-Complex		
Chloride	-	Ion-selective Electrode	-	Ion-selective Electrode

⁶ NADH = NADH-Dehydrogenase