Evaluation of **VACUETTE[®]** Urine CCM Tubes for Urine Strip Testing

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 $(20 - 25 \,^{\circ}\text{C})$ 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.

Dipstick urinalysis is one of the most important tests used in clinical laboratories for the diagnosis and follow-up of urinary tract infections (UTI). ^[1] The semi-quantitative dipstick method is rapid and practical for screening purposes. ^[2, 3, 4] The longer the sample transport, the more critical is the requirement for preservation. ^[5, 6]

The chemical analysis of urine is performed using one of many different urine dipsticks commercially available from several manufacturers. These dipsticks consist of a plastic strip that contains several chemically impregnated reaction pads. A color reaction develops upon contact of the urine with the reagent pads. Dipsticks for urinalysis are a simple and fast approach for semi-quantitative urine testing. Visual reading of the reagent strip is subject to some degree of variability due to different color interpretations by individuals.^[9]

Due to special formulation, the pH value cannot be measured with the **VACUETTE**[®] Urine CCM tubes, as the pH gets buffered by the preservative. Furthermore the specific gravity cannot be determined because the additive changes the physical character of the urine sample. ^[8]

Study objective

A clinical evaluation of the **VACUETTE**[®] Urine CCM tubes was carried out to assess the performance and suitability for the determination of the semi-quantitative parameters in dipstick urinalysis. The aim was to show stabilization of the parameters or particles determined over a period for up to 48 hours at room temperature (20- 25 °C) compared to a **VACUETTE**[®] Urine No Additive tube stored at 4 °C in the refrigerator. Urinalysis was carried out using dipsticks from three different manufacturers: Uryxxon[®] 10, Combur¹⁰ urine strip test[®] and Multistix[®] 10 SG urine strip test.

Material and Methods

The following tube types were used:

- VACUETTE[®] Urine CCM tubes (item # 455052)
- **VACUETTE**[®] Urine No Additive tubes (item # 455007)

Dipstick urinalysis was done using the following strips:

- Uryxxon[®] 10 from Machery-Nagel on the Uryxxon 500 instrument (item # 93068)
- Combur¹⁰ urine strip test[®] from Roche Diagnostics GmbH (item # 04510062171)
- Multistix[®] 10 SG urine strip test from Siemens Healthcare Diagnostics Ltd. (item # 0536597)

Study design and evaluation

The dipsticks had reagent pads for semi-quantitative assessment of Leukocytes, Nitrite, Protein, Glucose, Ketone, Urobilinogen, Bilirubin and Blood/Hemoglobin. When analyzing the urine status by means of strip testing, test fields are measured by reflection photometry or manually and the results are mostly presented in several semi-quantitative units. Each unit corresponds to a certain

concentration range of the respective analyte. This is also why an analyte may, at the decision limit to the next unit, alternatively yield the higher and the lower unit as result.

For the study, clinically normal, abnormal and spiked samples were used. Between the measurements, the **VACUETTE**^{\circ} Urine No Additive tubes were stored in the refrigerator at 4 °C-8 °C; the **VACUETTE**^{\circ} Urine CCM tubes were stored at room temperature at 20-25 °C (which were exposed to day light). The tubes were filled from the same urine beaker. The parameters were measured within 4-6 hours after specimen collection, after 24 hours, after 48 hours and after 72 hours.

Result comparison for each analyte and time point was performed. Agreement within ± 1 grade (two units significance level) was used as significance level. For nitrite there existed only two units as measured values in terms of a positive or negative result, therefore direct agreement was used (one unit significance level). As mentioned in the following tables Agree/ Not Agree relates to the grading.

The following acceptance criteria were chosen:

- Good agreement: Agreement between 90-100 %
- Acceptable agreement: Agreement between 80-90 %
- Slight disagreement: Agreement of < 80 %

Uryxxon[®] 10 from Machery-Nagel

A total of 100 urine samples were tested, consisting of clinically normal, clinically abnormal and spiked samples. The dipsticks were read by reflection photometry on the instrument Uryxxon 500 from Machery-Nagel.

Combur¹⁰ urine strip test[®] from Roche and Multistix[®] 10 SG urine strip test from Siemens

A total of 24 clinically normal and spiked urine samples were tested for each dipstick. The results were read manually by comparing the test strips with the provided colour scale.

Results

Uryxxon[®] 10 from Machery-Nagel

The baseline values of all parameters of the urine strip test obtained from the **VACUETTE**[®] Urine CCM tube did not differ from the corresponding baseline values of the **VACUETTE**[®] Urine No Additive tube.

Results from Combur¹⁰ urine strip test[®] from Roche

The values obtained from the **VACUETTE**[®] Urine CCM tubes are comparable to the values obtained from the **VACUETTE**[®] Z Urine No Additive tubes.

Results Multistix[®] 10 SG urine strip test from Siemens

For leukocyte testing using the Multistix[®] 10 SG urine strip test, atypical colour reactions occurred that were classified as significant deviations. The manufacturers' data for the Bio-Rad quality controls also mentioned atypical colour reactions. ^[9]

Discussion

For the three tested urine dipsticks there were no clinically significant differences observed, as there were no false positive or false negative results. Some analytes show limitations when tested after 72 hours.

For leukocyte testing using the Multistix[®] 10 SG urine strip test, atypical colour reactions occurred, which were also mentioned in the manufacturer's data for the Bio-Rad quality control. ^[9]

For Multistix[®] 10 SG urine strip test potential small deviations occurred due to inconsistent colour interpretation of the user.

Conclusion

The **VACUETTE**[®] Urine CCM tubes are comparable and showed no clinically significant difference in comparison to the **VACUETTE**[®] Urine No Additive tubes regarding dipstick urinalysis testing using Uryxxon[®] 10 from Machery-Nagel, Combur¹⁰ urine strip test[®] from Roche Diagnostics and Multistix[®] 10 SG urine strip test from Siemens Healthcare Diagnostics.

References

- [1] Conrad dos Santos, Juliana et al. (2007) Evaluation of Urinalysis Parameters to Predict Urinary-Tract Infection. The Brazilian Journal of Infectious Diseases. 11 (5):479-481.
- [2] Bonini, Pierangelo et al. (1988) Automation in urinalysis: evaluation of three urine test strip analysers. Journal of Automatic Chemistry. Vol. 10, No. 3, pp. 121-129.
- [3] European Urinalysis Guidelines. Scan J Clin Lab Invest 2000. 60: 1-96.
- [4] St John, Andrew. (2006) The Use of Urinary Dipstick Tests to Exclude UTI. Am J Clin Pathol. 126:428-436.
- [5] Kouri, Timo. (2007) Evaluation of commercial tubes for their capability to preserve urine strip tests and particle counts. (I58)
- [6] Kouri, Timo. (2008) Limits of reservation of samples for urine strip tests and particle counting. Clin Chem Lab Med. 46 (5):703-713.
- [7] CLSI. Urinalysis and Collection, Transportation, and Preservation of Urine Specimens; Approved Guideline Third Edition. GP16-A3. Vol 21 No 19.
- [8] Instructions for Use. Urine CCM Tube. Greiner Bio-One.
- [9] Bio Rad Liquicheck™ Urinalysis Quality Controls

Results in Detail Results from Uryxxon[®] 10 from Machery-Nagel

Table 1: Simultaneous comparison with the VACUETTE® Urine No Additive tube at the starting time

CCM tube								
Parameter	2-6 hours		24 hours		48 hours		72 hours	
	Agree	Not Agree						
	Absolute/ %							
Glucose	99/100	1/100	99/100	1/100	98/100	2/100	97/100	3/100
	(99 %)	(1 %)	(99 %)	(1 %)	(98 %)	(2 %)	(97 %)	(3 %)
Bilirubin	100/100	0/100	100/100	0/100	96/100	4/100	95/100	5/100
	(100 %)	(0 %)	(100 %)	(0 %)	(96 %)	(4 %)	(95 %)	(5 %)
Ketone	97/100	3/100	97/100	3/100	96/100	4/100	98/100	2/100
	(97 %)	(3 %)	(97 %)	(3 %)	(96 %)	(4 %)	(98 %)	(2 %)
Haemglobin	93/100	7/100	93/100	7/100	89/100	11/100	90/100	10/100
-	(93 %)	(7 %)	(93 %)	(7 %)	(89 %)	(11 %)	(90 %)	(10 %)
Protein	85/100	15/100	85/100	15/100	83/100	17/100	79/100	21/100
	(85 %)	(15 %)	(85 %)	(15 %)	(83 %)	(17 %)	(79 %)	(21 %)
Urobilinogen	100/100	0/100	100/100	0/100	97/100	3/100	95/100	5/100
-	(100 %)	(0 %)	(100 %)	(0 %)	(97 %)	(3 %)	(95 %)	(5 %)
Nitrite	98/100	2/100	98/100	2/100	99/100	1/100	99/100	1/100
	(98 %)	(2 %)	(98 %)	(2 %)	(99 %)	(1 %)	(99 %)	(1 %)
Leukocytes	86/100	(14/100)	86/100	14/100	81/100	19/100	76/100	24/100
	(86 %)	(14 %)	(86 %)	(14 %)	(81 %)	(19 %)	(76 %)	(24 %)

Results from Combur¹⁰ urine strip test[®] from Roche

Table 2: Comparison with VACUETTE° Urine No Additive tube at the starting time

CCM tube								
Parameter	2-6 hours		24 hours		48 hours		72 hours	
	Agree	Not Agree						
	Absolute/ %	Absolute /%						
Glucose	24/24	0/24	24/24	0/24	24/24	0/24	24/24	0/24
	(100 %)	(0 %)	(100 %)	(0 %)	(100 %)	(0 %)	(100 %)	(0 %)
Bilirubin	24/24	0/24	24/24	0/24	24/24	0/24	24/24	0/24
	(100 %)	(0 %)	(100 %)	(0 %)	(100 %)	(0 %)	(100 %)	(0 %)
Ketone	24/24	0/24	24/24	0/24	24/24	0/24	24/24	0/24
	(100 %)	(0 %)	(100 %)	(0 %)	(100 %)	(0 %)	(100 %)	(0 %)
Haemglobin	23/24	1/24	21/24	3/24	23/24	1/24	23/24	1/24
	(96 %)	(4 %)	(88 %)	(12 %)	(96 %)	(4 %)	(96 %)	(4 %)
Protein	24/24	0/24	24/24	0/24	24/24	0/24	24/24	0/24
	(100 %)	(0 %)	(100 %)	(0 %)	(100 %)	(0 %)	(100 %)	(0 %)
Urobilinogen	24/24	0/24	24/24	0/24	24/24	0/24	24/24	0/24
	(100 %)	(0 %)	(100 %)	(0 %)	(100 %)	(0 %)	(100 %)	(0 %)
Nitrite	24/24	0/24	24/24	0/24	24/24	0/24	24/24	0/24
	(100 %)	(0 %)	(100 %)	(0 %)	(100 %)	(0 %)	(100 %)	(0 %)
Leukocytes	24/24	0/24	24/24	0/24	24/24	0/24	24/24	0/24
	(100 %)	(0 %)	(100 %)	(0 %)	(100 %)	(0 %)	(100 %)	(0 %)

Results from Multistix[®] 10 SG urine strip test from Siemens

Table 3: Comparison with VACUETTE® Urine No Additive tube at the starting time

CCM tube								
Parameter	2-6 hours		24 hours		48 hours		72 hours	
	Agree	Not Agree	Agree	Not Agree	Agree	Not Agree	Agree	Not Agree
	Absolute/ %	Absolute/ %	Absolute/ %	Absolute/ %	Absolute/ %	Absolute/ %	Absolute/ %	Absolute/ %
Glucose	24/24	0/24	21/24	3/24	22/24	2/24	22/24	2/24
	(100 %)	(0 %)	(88 %)	(12 %)	(92 %)	(8 %)	(92 %)	(8 %)
Bilirubin	24/24	0/24	24/24	0/24	23/24	1/24	21/24	3/24
	(100 %)	(0 %)	(100 %)	(0 %)	(96 %)	(4 %)	(88 %)	(12 %)
Ketone	24/24	0/24	24/24	0/24	23/24	1/24	22/24	2/24
	(100 %)	(0 %)	(100 %)	(0 %)	(96 %)	(4 %)	(92 %)	(8 %)
Haemglobin	24/24	0/24	24/24	0/24	24/24	0/24	24/24	0/24
-	(100 %)	(0 %)	(100 %)	(0 %)	(100 %)	(0 %)	(100 %)	(0 %)
Protein	24/24	0/24	21/24	3/24	21/24	3/24	20/4	4/24
	(100 %)	(0 %)	(88%)	(12 %)	(88 %)	(12 %)	(83 %)	(17 %)
Urobilinogen	24/24	0/24	24/24	0/24	19/24	5/24	19/24	5/24
-	(100 %)	(0 %)	(100 %)	(0 %)	(79 %)	(21 %)	(79 %)	(21 %)
Nitrite	24/24	0/24	24/24	0/24	18/25	6/24	21/24	3/24
	(100 %)	(0 %)	(100 %)	(0 %)	(75 %)	(25 %)	(88 %)	(12 %)
Leukocytes	23/24	1/24	18/25	6/24	18/25	6/24	18/25	6/24
•	(96 %)	(4 %)	(75 %)	(25 %) ¹	(75 %)	(25 %) ¹	(75 %)	(25 %) ¹

¹ due to atypical colour reactions

Attachment: Significance limits for the different dipsticks used for urinalysis

Parameter	Significant change from
Leukocytes	negative to positive, v.v. 2 units
Nitrite	negative to positive, v.v.
Protein	negative to positive, v.v., 2 units
Glucose	negative to positive, v.v., 2 units
Ketone	negative to positive, v.v., 2 units
Urobilinogen	negative to positive, v.v., 2 units
Bilirubin	negative to positive, v.v., 2 units
Blood/ Hemoglobin	negative to positive, v.v., 2 units

Table 4: Significance limits f	or Uryxxon [®] 10) strip test from	Machery-Nage
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Key: v.v. = vice versa

Table 5: Significance limits for Combur¹⁰ urine strip test[®] from Roche

Parameter	Significant change from
Leukocytes	negative to positive, v.v., 2 units
Nitrite	negative to positive, v.v.
Protein	negative to positive, v.v., 2 units
Glucose	negative to positive, v.v., 2 units
Ketone	negative to positive, v.v., 2 units
Urobilinogen	negative to positive, v.v., 2 units
Bilirubin	negative to positive, v.v., 2 units
Blood/ Hemoglobin	negative to positive, v.v., 2 units

Key: v.v. = vice versa

Table 6: Significance limits for the Multistix[®] 10 SG urine strip test from Siemens

Parameter	Significant change from
Leukocytes	negative to 1+, v.v. 2 units
Nitrite	negative to positive, v.v.
Protein	negative to 1+, v.v., 2 units
Glucose	negative to 1+ v.v., 2 units
Ketone	negative to 1+, v.v., 2 units
Urobilinogen	normal 0,2 mg/dl to 1+ (= 2 mg/dl), v.v., or
	normal 1mg/dl to $2+$ (= 4 mg/dl), v.v., 2 units ¹
Bilirubin	negative to 2+, v.v., 2 units
Erythrocytes	1 unit
Hemoglobin	negative to 1+, v.v., 2 units

Key: v.v. = vice versa

¹ according to IFU from manufacturer