Evaluation of the **VACUETTE[®]** Urine CCM tubes for Urine Sediment testing on the Sysmex UF-1000i flow cytometer

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.

Urinalysis is a fundamental laboratory examination that provides valuable diagnostic information for variety of diseases. The longer the transport, the more critical is the requirement for preservation. ^[1,2,3,4,5,6] The Sysmex UF-1000i instrument is a urine analyzer based on fluorescence flow cytometry and cluster analysis. ^[7]

Study objective

A clinical evaluation of the **VACUETTE**[®] Urine CCM tube was carried out by assessing the measurement characteristics of particles in urine sediment. Urine samples were collected in parallel with different collection tubes and storage conditions, and results of the measurements where then compared according to agreement.

Materials and Methods

The following materials were used:

- **VACUETTE[®]** Urine CCM tube (item # 455052)
- VACUETTE[®] Urine No Additive tube (item # 455007)
- BD Vacutainer[®] Plus Urinalysis Preservative tube (item # 364992)
- BD Vacutainer[®] Plus C&S Preservative tube (item # 364955)

The analysis was performed on a Sysmex UF-1000i flow cytometer instrument.

Study design

For the study, clinically normal and abnormal urine samples were used. A total of 99 urine samples were collected in a urine beaker with integrated transfer device (Greiner urine beaker, item # 724311) and then transferred into the four collection tubes. The tubes were measured on the UF-1000i flow cytometer within 2-4 hours after specimen preparation, further after 24 hours, 48 hours and 72 hours. Between the measurements, the **VACUETTE**[®] Urine No Additive tubes were stored in the refrigerator at 4-8 °C, the three different preservative tubes were stored at room temperature (20-25 °C). The following particles and cells were measured on the instrument: erythrocytes, leukocytes, epithelial cells, casts, bacteria, crystals, yeast cells, small round cells and pathological casts.

For data analysis the statistical analysis was performed using Cohen's Kappa. Cohen's Kappa was calculated for each particle/cell at each time point. The initial time point was defined as the baseline value, and each subsequent time point value obtained was compared against the baseline.

The following criteria were set: $\kappa \leq 0.1$ no agreement κ 0.1 - κ ≤ 0.4 poor agreement κ 0.4 - $κ \le$ 0.6 moderate agreement κ 0.6 - $κ \le$ 0.8 good agreement κ 0.8 - $\kappa \le$ 1.0 complete agreement

Graphs show means and standard deviation for each parameter measured.

Results

Erythrocytes (RBC)





Leukocytes (WBC)





Epithelial Cells (EC)

Reference range: < 20/µl¹



Casts (CASTS)

Reference range: <5/µl¹



Bacteria (BACT) Reference range: < 100/µl¹



Crystals (X'TALS)



Figure 6: Crystals (X'TALS)





CT) nae: < 100/ul¹

Small Round Cells (SRC)



Figure 8: Small Round Cells (SRC)

Pathological Casts (Path. CASTS)



 $^{\rm 1}$ On the basis and derivation of "Thomas, L. Labor und Diagnose. $7^{\rm th}$ edition 2008. TH-Books."

Discussion

Regarding the statistical analysis using Cohen's Kappa, for the **VACUETTE**[®] Urine CCM tube erythrocytes, leukocytes, epithelial cells and bacteria showed complete agreement or good agreement at all time points, with Kappa values of >0.6 respectively. Casts showed up to 24 hours good agreement, 48h and 72h results showed moderate agreement. Crystals, small round cells and pathological casts showed mostly good or moderate agreement. Only yeast cells showed no agreement, as most samples were negative and Kappa results were not interpretable. Also, results were comparable to BD Vacutainer[®] Plus Urinalysis Preservative tube and BD Vacutainer[®] Plus C&S Preservative tube. Outlier testing was performed and wherever applicable considered.

Graphs also show stabilization of particles and cells.

Conclusion

For the **VACUETTE**[®] Urine CCM tube, erythrocytes, leukocytes, epithelial cells and bacteria showed very good to good agreement at all time points compared to the **VACUETTE**[®] Urine No Additive tubes at the baseline time point. For the other particles like casts, crystals, small round cells and pathological casts acceptable agreement was achieved. The **VACUETTE**[®] Urine CCM tube is comparable to the BD Vacutainer[®] Plus C&S Preservative tube and the BD Vacutainer[®] Plus Urinalysis Preservative tube at all time points tested. Results did not show any clinically significant differences.

References

[1] European Urinalysis Guidelines. Scand J Clin Lab Invest 2000 ; 60:1-96.

- [2] CLSI. Urinalysis and Collection, Transportation, and Preservation of Urine Specimens; Approved Guidelines Third Edition. GP 16-A3. Vol 21 No 19.
- [3] Influence of Vacuette Urine System on the Evaluation of Urine Sediment. R. H. J. Bruijns en J. W. Smit, Dutch Biochemistry Magazine 2003; 28:159-160.
- [4] Limits of preservation of samples for urine strip tests and particle counting. Clin Chem Lab Med 2008; 46:703-713. Timo Kouri
- [5] Preservation of Urine for Flow Cytometry and Visual Microscopic Testing. Timo Kouri. Clinical Chemistry 2002; 48: 900-905

[6] Automated Urinalysis. Evaluation of the Sysmex UF-50. Hiroshi Okada. Am J Clin Pathol 2001; 115:605-610.

[7] www.sysmex.at

Kappa Results in comparison to VACUETTE[®] Urine No Additive tube at the starting time

VACUETTE[®] Urine CCM Tube BD Vacutainer[®] Plus BD Vacutainer[®] Plus C&S at 0h Urinalysis Preservative Tube Preservative Tube at 0h at 0h Parameter Kappa 95% Confidence Kappa 95% Confidence Kappa 95% Confidence interval interval interval 0.954 RBC 0.890-1.000 0.632-0.903 0.907 0.818-0.996 0.767 WBC 1.000 1.000-1.000 1.000 1.000 1.000-1.000 1.000-1.000 EC 0.880 0.787-0.973 0.800 0.682-0.918 0.880 0.787-0.918 CASTS 0.652 0.502-0.803 0.618 0.463-0.773 0.614 0.457-0.770 BACT 0.960 0.905-1.000 1.000 1.000-1.000 0.960 0.905-1.000 X'TALS 0.322-0.666 0.451-0.767 0.580 0.415-0.745 0.494 0.609 YLC SRC 0.000 0.000-1.000 0.490 0.000-1.000 -0.014 0.489-0.790 0.512-0.807 0.560 0.398-0.722 0.659 0.639 Path. CASTS 0.584 0.421-0.747 0.592 0.432-0.752 0.570 0.407-0.734

Table 1: Kappa for time point 0h

Table 2: Kappa for time point 24h

	VACUETTE [®] Urine CCM Tube at 24h		BD Vacutainer [®] Plus Urinalysis Preservative Tube at 24h		BD Vacutainer [®] Plus C&S Preservative Tube at 24h	
Parameter	Карра	95% Confidence interval	Карра	95% Confidence interval	Карра	95% Confidence interval
RBC	0.860	0.753-0.968	0.488	0.304-0.673	0.860	0.753-0.968
WBC	0.880	0.787-0.973	0.920	0.843-0.997	0.920	0.843-0.997
EC	0.800	0.682-0.918	0.800	0.682-0.918	0.680	0.536-0.824
CASTS	0.677	0.532-0.822	0.590	0.429-0.751	0.473	0.298-0.647
BACT	0.920	0.843-0.997	0.840	0.734-0.946	0.960	0.905-1.000
X'TALS	0.664	0.513-0.815	0.556	0.393-0.720	0.553	0.388-0.718
YLC	0.000	0.000-1.000	0.485	0.000-0.979	0.000	0.000-1.000
SRC	0.478	0.306-0.651	0.620	0.467-0.774	0.560	0.398-0.722
Path. CASTS	0.578	0.413-0.744	0.504	0.331-0.677	0.395	0.209-0.580

Table 3: Kappa for time point 48h

	VACUETTE [®] Urine CCM Tube at 48h		BD Vacutainer [®] Plus Urinalysis Preservative Tube at 48h		BD Vacutainer [®] Plus C&S Preservative Tube at 48h	
Parameter	Kappa	95% Confidence interval	Kappa	95% Confidence interval	Карра	95% Confidence interval
RBC	0.814	0.691-0.937	0.535	0.356-0.713	0.744	0.603-0.885
WBC	0.920	0.843-0.997	0.840	0.734-0.946	0.880	0.787-0.973
EC	0.720	0.584-0.856	0.680	0.536-0.824	0.680	0.536-0.024
CASTS	0.460	0.286-0.634	0.556	0.393-0.720	0.652	0.502-0.803
BACT	0.920	0.843-0.997	0.760	0.633-0.887	0.880	0.787-0.973
X'TALS	0.664	0.513-0.815	0.556	0.393-0.720	0.733	0.597-0.868
YLC	0.000	0.000-1.000	0.154	0.000-0.681	0.000	0.000-1.000
SRC	0.600	0.443-0.757	0.280	0.092-0.468	0.599	0.442-0.756
Path. CASTS	0.395	0.209-0.580	0.458	0.284-0.633	0.568	0.403-0.732

Table 4: Kappa for time point 72h

	VACUETTE [®] Urine CCM Tube at 72h		BD Vacutainer [®] Plus Urinalysis Preservative Tube at 72h		BD Vacutainer [®] Plus C&S Preservative Tube at 72h	
Parameter	Kappa	95% Confidence interval	Kappa	95% Confidence interval	Kappa	95% Confidence interval
RBC	0.674	0.518-0.830	0.558	0.382-0.733	0.581	0.409-0.753
WBC	0.880	0.787-0.973	0.800	0.682-0.918	0.840	0.734-0.946
EC	0.680	0.536-0.824	0.640	0.489-0.791	0.720	0.787-0.973
CASTS	0.460	0.286-0.634	0.420	0.242-0.598	0.537	0.372-0.703
BACT	0.920	0.843-0.997	0.720	0.584-0.856	0.840	0.734-0.946
X'TALS	0.440	0.264-0.616	0.571	0.409-0.734	0.612	0.455-0.769
YLC	-0.020	-	0.197	0.000-0.771	-0.014	-
SRC	0.460	0.285-0.634	0.339	0.155-0.524	0.520	0.353-0.687
Path. CASTS	0.411	0.230-0.591	0.495	0.324-0.666	0.356	0.172-0.540