White Paper

Iris® Diagnostics Division

Greiner Bio-One VACUETTE® Urine CCM Tube

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PURPOSE

The Greiner Bio-One VACUETTE® Urine CCM Tube, with a powder additive, has been shown to be an effective form of urine sample preservation. Using an iRICELL® Automated Urinalysis System, an evaluation of the Greiner Bio-One VACUETTE® Urine CCM Tube was performed to ensure specimen integrity was consistent throughout a 48-hour period.

OBJECTIVES

The objective of this study was to monitor the performance of the Greiner Bio-One VACUETTE® Urine CCM Tube, using UTI suspected urine samples, run on Iris Diagnostics' iRICELL Urinalysis System, composed of the iQ®200 Microscopy Analyzer and iChem®VELOCITY™ Chemistry Analyzer over a 48 hour period.

CONCLUSIONS

The Greiner Bio-One VACUETTE® Urine CCM Tube provided stability of both chemistry analytes (as determined by Iris Diagnostic's iChemVELOCITY) and microscopy particles (as determined by Iris Diagnostic's iQ200) for 48 hours and showed no statistically significant variation for any analyte or particle for the 48 hours tested.

INTRODUCTION

Urine collection and prompt processing is a major challenge for most hospitals. The 2-hour window allotted for accurate urinalysis is often not within the capacity of most facilities. To minimize cellular degradation and prevent bacterial overgrowth, alternative means of preserving the sample is required. Refrigeration will help with minimizing cell destruction and bacterial growth but it introduces crystals that may interfere with the recognition of other particles in the sample. Alternatively, samples collected in preservative tubes are better able to maintain the integrity of the sample without introducing changes to cellular morphology, bacterial growth or crystal formation. The Greiner Bio-One VACUETTE® Urine CCM Tube used for this study has been designed to maintain sample integrity for a period of 48 hours from the time of collection.

MATERIALS and METHODS

For this study, the Greiner Bio-One VACUETTE® Urine CCM Tube (10.5mL) was evaluated. A total of 498 UTI suspect urine samples were provided by a local reference laboratory, Western Health Sciences Medical Laboratory (Canoga Park, CA). Only abnormal urine specimens, those positive for bacteriuria, pyuria, and hematuria were included in our sample pool.

All manufacturers' instructions were followed with regards to system calibration and quality control methods. Procedures related to startup, maintenance and shutdown, as well as quality control procedures, were also performed as recommended by the manufacturer's guidelines.

Microscopic/Sediment system:

Microscopic/Sediment analysis was performed on the Iris Diagnostics iQ®200ELITE™. The microscopic results examined included:

- RBC
- Hyaline Casts
- WBC
- Bacteria
- Crystals
- Squamous Epithelial Cells
- Pathological Casts

Urine chemistry systems:

Iris Diagnostics' iChemVELOCITY Urine Chemistry Analyzer, with iChemVELOCITY strips, as well as the Arkray AX-4280 urine chemistry analyzer (Iris iQ200 Automated Urinalysis Workcell) with Aution 9EB strips, was used to run the urine chemistry results. The chemistry analytes measured for the two systems included:

- Glucose
- Ketone
- Protein
- Nitrite
- Bilirubin
- Leukocyte Esterase
- Urobilinogen
- Specific Gravity
- pH
- Blood

SPECIMENS

Urine samples used for the study were UTI suspected specimens submitted for urine culture only or reflexed for culture following the reference laboratory's urinalysis. Upon receipt into the reference laboratory, urine samples (with sufficient volume) were segregated into 2 categories as directed by physician instruction, urine culture only or urinalysis with reflex to culture. Each tube was filled to 10.5 ml and inverted 8 to 10 times as described by the manufacturer instructions. Samples were stored at room temperature (20-25°C) and protected from light throughout the duration of the study.

TESTING PROCEDURE

Study activities including, but not limited to, sample handling, record keeping and reporting were designed and carried out in compliance with standard HIPAA regulations. These regulations were followed to protect patient privacy; specimens were assigned a random identification code and all identifying information was removed.

Each sample aliquot was tested at the time intervals 0 hours, 24 hours, and 48 hours. Time interval 0 hours was tested immediately upon receipt with no more than 30 minutes between allocation into the Greiner Bio-One VACUETTE® Urine CCM Tube and urinalysis testing. All samples were run on the iQ200 and iChemVELOCITY while 72 specimens were UA/reflexed by the reference facility (Western Heath Sciences Medical Laboratory) using the Iris iQ200 Automated Urinalysis Workcell.

DATA ANALYSIS

Direct result comparison analysis for each analyte/particle and each time point was performed using the EP Evaluator 9 Software package. Agreements (direct agreements and agreements within ±1 grade for those reporting in levels or states with 80% or better agreement) were determined with 95% confidence levels using the Score Method. McNemar's test for symmetry was performed and Cohen's Kappa was calculated for each particle/analyte at each time point. The 0 hours' time point was used as the baseline with each subsequent time compared for indication of change.

RESULTS AND DISCUSSION

To determine the alignment of results between 0 hours, 24 hours, and 48 hours, EP Evaluator 9 (Data Innovations, South Burlington Vermont) was utilized for a direct time point to time point result analysis. For overall performance, there was good agreement within ±1 grade for all microscopy particles analyzed on the iQ200 (Tables 1 & 3). Sediment particles analyzed showed a 90% or higher ±1 grade agreement from 0 hour, 24 hours as well as the full 48 hours with the exception of WBCs. Low agreement between the WBC results of different systems (Table 3) could be explained by the fact that sediment results are subjective and can vary due to user editing and inconsistent sediment/microscopy interpretation. Specimens that were UA reflexed to culture then incubated for 48 hours were initially evaluated by the reference laboratory iQ200 instrument. Consequently, continuous microscopy readings varied due to several factors. First, multiple users were used to edit urine microscopies and interpretation as to cellular morphology and thus classification can vary among users.

Table 1. Urine Specimens: Urine Culture Only & UA/ Reflex Culture Microscopy/Sediment

iQ200	0 hrs vs. 24 hrs n=175		0 hrs vs. 48 hrs n=148	
Particle	Agreement	Agreement +/-1 grade	Agreement	Agreement +/-1 grade
RBC	88.0%	98.3%	83.8%	96.6%
WBC	73.7%	97.1%	58.8%	83.8%
SQEP	99.4%	100%	99.3%	100%
BACTERIA	59.4%	96.0%	68.9%	96.6%
HYALINE CAST	99.4%	100%	100%	100%
CRYSTALS	70.3%	99.4%	69.6%	100%
NSE	63.4%	99.4%	58.5%	100%
UNCLASSIFIED CASTS	84.0%	98.9%	88.5%	99.3%

Table 3. UA/Reflex Reference Laboratory vs. Iris Evaluation Microscopy/Sediment

iQ200	0 hrs vs. 0 hrs n=72		0 hrs vs. 24 hrs n=72		0 hrs vs. 48 hrs n=56	
Particle	Agreement	Agreement +/-1 grade	Agreement	Agreement +/-1 grade	Agreement	Agreement +/-1 grade
RBC	63.9%	91.7%	62.5%	91.7%	64.3%	91.1%
WBC	52.8%	84.7%	40.3%	100%	30.4%	57.1%
SQEP	56.9%	100%	56.9%	73.6%	60.7%	100%
BACTERIA	63.9%	97.2%	55.6%	93.1%	60.7%	96.4%
HYALINE CAST	93.1%	100%	93.1%	100%	91.1%	100%
CRYSTALS	66.7%	100%	70.8%	97.2%	71.4%	98.2%
NSE	44.4%	86.1%	36.1%	86.1%	39.3%	89.3%
UNCLASSIFIED CASTS	80.6%	98.6%	77.8%	94.4%	83.9%	96.4%

Second, as previously mentioned, our sample pool was composed of bacteriuria and pyuria specimens. Overtime, a significant population of neutrophils that had been exposed to such high levels of bacteria experienced their natural response of releasing their internal enzymes or respiratory burst which influenced the WBC image and thus the aggregate microscopic results at extended time points. Of particular interest and the primary focus of our study, there was no significant statistical change of bacteria levels throughout the measured time points. This would indicate that the preservative provided static integrity of the specimens and did not adversely reduce the bacteria concentrations or provide media for microbial growth.

Table 2. Urine Specimens: Urine Culture Only & UA/ Reflex Culture Chemistry

iChem VELOCITY	0 hrs vs. 24 hrs n=175		0 hrs vs. 48 hrs n=148	
Particle	Agreement	Agreement +/-1 grade	Agreement	Agreement +/-1 grade
Glucose	98.8%	100%	100%	100%
Blood	85.3%	99.4%	88.1%	99.0%
Protein	95.7%	100%	96.0%	100%
Bilirubin	99.4%	100%	100%	100%
Urobilinogen	96.9%	100%	96.0%	100%
Ketone	100%	100%	100%	100%
Leukocyte Esterase	77.9%	97.5%	84.2%	99.0%
Nitrite	91.4%	100%	84.2%	100%
pH	93.3%	100%	95.0%	100%
Specific Gravity	84.7%	98.2%	100%	100%

Performance of the preservative tube for urine chemistry analytes, as measured by the iChemVELOCITY, showed good agreement (see Tables 2 and 4) with the same urine analytes that were not exposed to the Greiner Bio-One VACUETTE® Urine CCM Tube measured with the Arkray AX-4280. All analytes had a better than 80% agreement at ±1 grade on both systems for the first 24 hours and was maintained throughout the entire 48 hours. Considerable variance was observed in the direct agreement between the AX4280 and iChemVELOCITY with regards to SG and pH readings. The difference in strip border metrics would be the primary cause for the non-concordant results. When statistics were adjusted to match clinical relevance metrics of within ±1 grade, pH results were in acceptable correlation agreement between the iChemVELOCITY and AX-4280. Similarly, regarding specific gravity results, there was low concordance regarding exact agreement but high concordance was observed within ±1 grade between the two systems. We observed that the Greiner Bio-One VACUETTE® Urine CCM Tube did not influence urine chemistry results.

CONCLUSIONS

The Greiner Bio-One VACUETTE® Urine CCM Tube demonstrated stability of both chemistry analytes (as determined by Iris Diagnostic's iChemVELOCITY) and microscopy particles (as determined by Iris Diagnostic's iQ200) for 48 hours, as claimed by the manufacturer. Aside from failed measurement symmetry for WBCs due to inconsistent sediment interpretation, analyses showed no statistically significant variation for chemical analytes or sediment particles over the 48 hours tested.

Table 4. UA/Reflex Reference Laboratory vs. Iris Evaluation Chemistry Arkray 4280 reference instrument at Time 0 hr.

AX4280 vs. iChemVELOCITY	0 hrs vs. 0 hrs n=72		0 hrs vs. 24 hrs n=68		0 hrs vs. 48 hrs n=37	
Particle	Agreement	Agreement +/-1 grade	Agreement	Agreement +/-1 grade	Agreement	Agreement +/-1 grade
Glucose	100%	100%	98.5%	100%	100%	100%
Blood	63.9%	88.9%	55.9%	86.8%	54.1%	89.2%
Protein	73.6%	86.1%	73.5%	88.2%	78.4%	91.9%
Bilirubin	93.1%	100%	92.6%	100%	96.4%	100%
Urobilinogen	88.9%	100%	88.2%	100%	91.9%	100%
Ketone	100%	100%	98.5%	100%	100%	100%
Leukocyte Esterase	54.2%	77.8%	50.0%	80.9%	62.2%	83.8%
Nitrite	87.5%	100%	77.9%	100%	73.0%	100%
pH	38.9%	97.2%	39.7%	95.6%	32.4%	100%
Specific Gravity	13.9%	65.3%	17.6%	69.1%	27.0%	91.9%

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