UROBILINOGEN REAGENT KIT
MD-101103

Intended Use
A urinary urobilinogen test provides a non-invasive way of assessing liver function. This method allows the automation of the test for its use on a chemistry laboratory analyzer. For in vitro quantitative measurement of urobilinogen in urine.
NOT FOR USE IN UNPROFESSIONAL SETTINGS.

Summary and Principle
Urobilinogen is a bile pigment formed by the degradation of hemoglobin.[1] Although it is normal to have around 1 mg/dL of urobilinogen in the urine, both high levels over 3 mg/dL and very low levels, less than 0.5 mg/dL, can indicate disease. Measurements of urobilinogen can assist in the determination of liver diseases such as cirrhosis, hepatitis and liver cancer.
One of the original tests for urobilinogen was the Ehrlich test based on the Ehrlich Aldehyde Reaction[2]. The reagent works by binding to the C2 position of two indole moieties in the urobilinogen molecule to produce a pink color whose intensity is related to the amount of urobilinogen present in the sample. This method however suffers from various interferences, one of these being high levels of bilirubin in a urine sample. The method employed here uses a two-reagent system, Reagent 1 designed to eliminate potential bilirubin interference and Reagent 2 to react with the urobilinogen to form a colored complex. The intensity of this color then being directly related to the level of urobilinogen in the sample. The method is based on the work of Carter and Smith[3].

Reagents
Both R1 and R2 reagents are provided ready to use. No preparation is required.
Urobilinogen R1 reagent contains: Buffer, Ethylene diamine tetraacetic acid >2 g/L, nonreactive ingredients and stabilizer.
Urobilinogen R2 Reagent contains: Buffer, 4-Methoxy benzenediazonium tetrafluoro borate >1 g/L, 2,3 Butanedione monoxime >1 g/L, surfactant and stabilizers

Reagent Stability and Storage
Store MISSION Urobilinogen reagents at 2-8°C. When stored as directed, the reagent is stable until the expiration date stated on the label. The Urobilinogen Reagents have been tested to reflect shipping conditions and is stable for the lifespan of the product if frozen up to 5 times or reaching temperatures up to 40°C for up to one week. Manufacturer studies have shown that reagents are stable for 30 days once placed in the refrigerated reagent carousel (2-10°C), however reagent stability may vary based on individual laboratory conditions.

Cautions
Avoid contact of specimens with skin and eyes. Should contact occur, wash affected area with plenty of water.
DO NOT PIPETTE SPECIMENS BY MOUTH.

Instrumentation
Urobilinogen Reagent Kit is for use on Mission Diagnostics Affirm C200[1] and Beckman AU680 Analyzers. Refer to instrument procedure instructions in the instrument manual provided with the specific analyzer.

Specimen Collection and Handling
Test specimens should be fresh urine samples. Handle all urine samples as if potentially infectious. Proper collection and storage of urine are essential for accurate testing, as urobilinogen can degrade over time or under improper conditions. Collection: use a dark (amber), clean, dry, sterile container (plastic or glass). Storage: Room temperature (20–25°C) stable for <2 hours. Refrigerated at 2–8°C samples are stable for up to 24 hours.

Quality control
Standard practice for Quality Control should be applied to this procedure. Store and handle reagents properly before and during use. Every laboratory should establish its own test requirements using controls and calibrators. Mission Diagnostics provides Calibrators to meet your program needs and which conform to NLCP Guidelines[4]:

Cat. No. MD-101203 – Urobilinogen Calibrator

Typical Performance Characteristics
The following performance data was obtained using the Affirm C200 and Beckman AU680 Analyzers. Other instruments may yield different performance data.

[1] Also known as Zybio EXC200 Analyzer

Distributed by: Mission Diagnostics LLC, 1 Burton Dr., Meridith, NH 03246
Manufactured by: Diamond Diagnostics Inc., 333 Fiske St., Holliston, MA 01746
Technical Assistance Call: 1.508.429.0450
Email: support@diamonddiagnostics.com

Linearity
The following results were obtained on an Affirm C200 and Beckman AU680 Analyzers using the MISSION Urobilinogen Reagent Kit on samples containing approximately 0, 1, 4, 6, and 10 mg/dL urobilinogen. The table below includes mean, standard deviation (SD) and Coefficient of Variation (CV) for each value.

Mean (mg/dL)	SD	CV%
0.000	0.000	0.0
1.070	0.019	1.7
4.244	0.034	0.8
6.392	0.085	1.3
9.556	0.080	0.8

Precision
Studies performed on Affirm C200 and Beckman AU680 Analyzers. The precision of the assay was evaluated following a modification of NCCLS protocol EPT-T2. The within-run precision data was obtained by running two samples in replicates of 20 on the same day. The run-to-run data was obtained by running two samples in replicates over a five-day period.

Within-Run			Run-to-Run		
Mean (mg/dL)	**SD**	**CV%**	**Mean** (mg/dL)	**SD**	**CV%**
1.962	0.011	0.6	1.836	0.173	9.4
7.993	0.051	0.6	8.062	0.131	1.6

Analytical Specificity
Cross contamination studies have not been performed on Affirm C200 and Beckman AU680 Analyzers. Certain reagent/ instrument combinations used in sequence with this assay may interfere with reagent performance and test results. The existence of, or effects of, any potential cross contamination issues are unknown.

Test Conditions
For the data presented in this insert, studies using this reagent were performed on Affirm C200 and Beckman AU680 Analyzers using the parameters listed below.

Limitations
A sample with a urobilinogen concentration exceeding the analytical measuring range should be diluted with deionized water and reanalyzed incorporating the dilution factor in the calculation of the value.

Calibration
Calibration material should be used to calibrate the procedure. The frequency of calibration using an automated system is dependent on the system and the parameters used. If control results are found to be out of range, the test may need to be re-calibrated. Under typical operating conditions manufacturer calibration stability studies have shown the calibration curve will be stable for at least 14 days

Method Parameters

Analyzer Specific Settings

Method type:	Endpoint
Slope:	positive
Units	mg/dL
DOM wavelength	546
SUB wavelength	600
Sample volume	5
R1 volume	100
R2 volume	100
Blank rxn read (cycles)	16 - 20
Sample rxn read (cycles)	40 - 44

Calibration Settings

Calibration Type	Linear
Reagent Blank required	No
Calibrator 1 (deionized water)	0
Calibrator 2	9.50

Analytical Measuring Range (AMR)

Range: (low)	0
Range: (high)	10

BIBLIOGRAPHY

1. Fundamentals of Clinical Chemistry. Edited by Norbert Teitz. WB Saunders, Philadelphia (1976).
2. Clinical Chemistry: Principles and Technics. Richard Henry, page 611.
3. J. Carter & J. Smith US Patent 5,736,408.
4. National Laboratory Certification Program. General Laboratory Inspection Checklist / Report. OMB No. 0930-0158. November, 2002.

Distributed by: Mission Diagnostics LLC, 1 Burton Dr., Meridith, NH 03246
Manufactured by: Diamond Diagnostics Inc., 333 Fiske St., Holliston, MA 01746
Technical Assistance Call: 1.508.429.0450
Email: support@diamonddiagnostics.com