TOTAL BILIRUBIN REAGENT KIT
MD-101109

Intended Use
For the quantitative determination of total bilirubin in urine.
NOT FOR USE IN UNPROFESSIONAL SETTINGS.

Summary and Principle
Urine bilirubin testing is a tool in detecting and monitoring liver dysfunction, biliary obstruction, and certain types of hemolytic disorders. Normally bilirubin is not present in urine in detectable amounts, so its presence often indicates an underlying pathology. When hepatic excretion into bile is impaired or the biliary tree is obstructed, direct (conjugated) bilirubin backs up into the blood and is excreted in urine (bilirubinuria).[1,2]
The method presented here was developed by Wahlefeld et al.[3] A detergent is used to accelerate the reaction and to avoid protein precipitation. The diazo reagent is 2,5-dichlorophenyldiazonium tetrafluoroborate (DPD) that reacts very rapidly in coupling with bilirubin under acidic conditions.

Reagents
Reagents provided as ready to use liquids.
R1 reagent: acid buffer 50 mmol/L, surfactant.
R2 reagent: acid buffer >30 mmol/L, >2.0 mmol/L DPD and stabilizers.

Reagent Stability and Storage
Packaged reagents are stored at 2-8°C. The reagents are stable until the expiration date on the label when stored as directed. Do not freeze reagents. Avoid exposure to direct sunlight. Do not use if reagents show evidence of contamination (turbidity). The R2 may develop very slight precipitation that does not affect performance and will re-dissolve if the R2 is warmed gently. R2 reagent containing a precipitate that does not re-dissolve and results in product discoloration should not be used. Do not use if reagent fails to achieve assigned assay values of fresh control sera.

Cautions
Reagents are toxic and corrosive. Do not pipette by mouth. Avoid contact with skin and clothing.

Instrumentation
MISSION Total Bilirubin Kit are 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
Collect fresh, random urine sample in a clean, dry, and opaque (amber-colored) plastic container, or wrap clear containers in foil to protect from light. Test should be performed immediately after collection or within 2 hours if refrigerated. Freezing the sample can destroy bilirubin.

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]:

MD-101213 – Total Bilirubin Calibrator

Specificity, Limitations, and Interferences
Samples with values above 10 mg/dl must be diluted 1:1 with deionized water, reanalyzed and the result multiplied by two.

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

Linearity
The following results were obtained on an Affirm C200 and Beckman AU680 Analyzers using the MISSION Total Bilirubin Kit on samples containing 0.25, 0.50, 1.00, 2.50 and 5.00 mg/dL bilirubin. The table below includes mean, standard deviation (SD) and Coefficient of Variation (CV) for each value.

Mean (mg/dL)	SD	CV%
0.2406	0.0046	1.9
0.5208	0.0092	1.8
1.0640	0.0211	2.0
2.6914	0.0508	1.9
4.9904	0.0807	1.6

[1] Also known as Zybio EXC200 Analyzer

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Manufactured by: Diamond Diagnostics Inc., 333 Fiske St., Holliston, MA 01746
Technical Assistance Call: 1.508.429.0450
Email: support@diamonddiagnostics.com

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%**
0.1978	0.01	5.0	0.2006	0.0171	8.5
22.84	0.25	1.1	21.8025	1.1063	5.1

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 bilirubin 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	660
Sample volume	2.0
R1 volume	140
R2 volume	35
Blank rxn read (cycles)	21 - 22
Sample rxn read (cycles)	38 - 41

Calibration Settings

Calibration Type	Linear
Reagent Blank required	No
Calibrator 1	1
Calibrator 2	5

Analytical Measuring Range (AMR)

Range: (low)	0
Range: (high)	5

BIBLIOGRAPHY

1. Rifai N., Horvath A. R., Wittwer C. T. (Eds.). *Tietz Textbook of Clinical Chemistry and Molecular Diagnostics* (6th ed.). Elsevier, 2017.
2. McPherson R. A., Pincus M. R. (Eds.). *Henry's Clinical Diagnosis and Management by Laboratory Methods* (24th ed.). Elsevier, 2022.
3. Wahlefeld AW, et al. Scand J Clin Lab Invest. 29 Supplement 126(1972).
4. National Laboratory Certification Program. General Laboratory Inspection Checklist / Report. OMB No. 0930-0158. November, 2002.

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