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Total Bile Acids Assay Kit

Configuration

The Diazyme Total Bile Acids reagent is provided in bulk and the following kit configurations:

Instrument	Catalog No.	Kit Size
Beckman AU	DZ042A-KY1	R1: 2 x 60 mL R2: 2 x 20 mL
Half Kit	DZ042A-K01	R1: 2 x 30 mL R2: 2 x 10 mL

Note: Calibrators and Controls Sold Separately

Intended Use

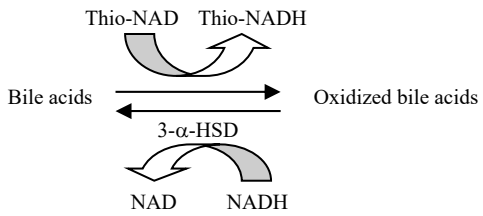
Diazyme Total Bile Acids Assay Kit is intended for the *in vitro* quantitative determination of total bile acids (TBA) in human serum samples. Total bile acids are metabolized in the liver and serve as a marker for normal and abnormal liver function. Serum total bile acids are increased in patients with liver disease.

Clinical Significance [1,2]

Total bile acids are metabolized in the liver and, hence, serve as a marker for normal liver function. Serum total bile acids are increased in patients with acute hepatitis, chronic hepatitis, liver sclerosis and liver cancer.

Assay Principle

The reagents of the assay kit are in a stable liquid formulation that allows for ease of use coupled with enhanced performance characteristics. In the presence of Thio-NAD, the enzyme 3-α-hydroxysteroid dehydrogenase (3- α-HSD) converts bile acids to 3-keto steroids and Thio-NADH. The reaction is reversible and 3-α-HSD can convert 3-keto steroids and Thio-NADH to bile acids and Thio-NAD. In the presence of excess NADH, the enzyme cycling occurs efficiently and the rate of formation of Thio-NADH is determined by measuring specific change of absorbance at 405 nm.



Materials Required But Not Provided

An analyzer capable of dispensing two reagents and of measuring absorbance at 405 nm with temperature control (37°C).
Calibrator for the Diazyme Total Bile Acids assay (DZ042A-CAL).
0.9% Saline is used for diluting samples and as Calibrator 0.
Controls for validating the performance of the Diazyme Total Bile Acids assay (DZ042A-CON).

Reagent Composition

Reagent	Composition
R1	Thio-NAD >0.1mM, Buffer
R2	3-α-HSD >2kU/L, NADH >0.1 mM, Buffer
Calibrator	Conjugated cholic acids, Buffer

Reagent Preparation

Diazyme Total Bile Acids Assay Reagents are ready-to-use, liquid reagents. The intrinsic yellow to yellow-brown color of the TBA reagent does not interfere with the test.

Reagent Stability and Storage

Unopened reagents are stable until the expiration date printed on the label. Reagents are light sensitive and should be stored at 2-8°C. Reagents from different lots must not be interchanged.

Specimen Collection and Handling[4]

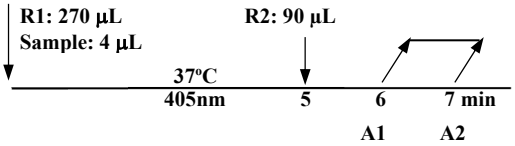
Use fresh patient serum samples. TBA concentration is increased after meals; hence, samples should be collected under fasting conditions*. EDTA treated plasma or Lithium heparin plasma samples are suitable for use. Serum or plasma samples are stable for a week at 4 °C, or for 3 months at –20 °C. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine specific stability criteria for its laboratory.[5]
Specimens from patients, who are on Ursodeoxycholic Acid (UDCA) treatment, are not suitable for use with TBA Assay (DZ042A).

*This does not apply to women with intrahepatic cholestasis of pregnancy who will need peak bile acid testing and samples should therefore be taken post-prandially.

Precautions

1. For *in vitro* diagnostic use. For prescription use only. For professional use only.
2. Assay calibration frequency is dependent on instrument validation data. Additionally, the assay should be recalibrated, and controls run with each new lot of reagents.
3. Specimens containing human sourced materials should be handled as if potentially infectious using safe laboratory procedures, such as those outlined in Biosafety in Microbiological and Biomedical Laboratories (USA CDC.gov).
4. Do not ingestion and allow contact with skin and eyes. Additional safety information is provided within the Safety Data Sheet (SDS) for this product. To obtain an SDS, please contact our Diazyme technical support at support@diazyme.com or +1 858-455-4768.
5. Exercise standard precautions required for handling of all laboratory reagents. Infectious or microbial waste: Handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures. Environmental hazards: Apply all relevant local disposal regulations to determine the safe disposal.

Assay Scheme for Chemistry Analyzers



Application sheets for use of Diazyme Total Bile Acids Enzymatic Cycling Assay on automated clinical chemistry analyzers are available upon request. Please call +1 858-455-4768 or email: support@diazyme.com.

Calibration

A bile acids calibrator (DZ042A-CAL), along with 0.9% saline as a zero reference, should be used as directed to calibrate the procedure. Calibration frequency may vary and is dependent on instrument application. For more information, please call 858-455-4768 or email: support@diazyme.com.

Quality Control

We recommend that each laboratory use bile acid controls to validate the performance of bile acid reagents. A set of normal and abnormal range bile acid controls is available from Diazyme Laboratories (DZ042A-CON). If the results from the controls fall outside the acceptable limits, as determined by the manufacturer, the test should not be performed. We recommend that your quality control testing follows federal, state, and local guidelines or accreditation requirements.

Results

Bile acids concentration is expressed as µmol/L (µEq/L).

Reference Range [3]

Serum or plasma containing 0-10 µmol/L bile acids is considered normal range. We suggest that each laboratory establish a range of normal values for the population in their region.

Limitations

- A sample with a bile acids level exceeding the linearity limit should be diluted with 0.9% saline and reassayed incorporating the dilution factor in the calculation of the value.
- Specimens from patients, who are on Ursodeoxycholic Acid (UDCA) treatment, are not suitable for use with TBA Assay (DZ042A).
- Although verification and validation studies have examined the effect of likely interferents in this assay, there is a potential for interference from unknown substances Therefore, as with any diagnostic test, is possible that technical, procedural errors as well as substances and factors not listed may interfere with the proper functioning of the test kit.
- Assay results should be utilized in conjunction with other clinical and laboratory data to assist the clinician in making individual patient management decisions.

Performance Characteristics

These performance characteristics were determined at Diazyme Laboratories using automated procedures on a Hitachi 717.

Accuracy

The performance of this assay was compared with the performance of a similar total bile acids assay on a Hitachi 717 analyzer using serum samples.

Fifty-two (52) serum samples ranging from 0.47 – 131.25 µmol/L gave a correlation coefficient of 0.9918. Linear regression analysis gave the following equation:

This method = 1.1536 (reference method) – 0.8567 µmol/L

A matched set of serum and lithium heparin plasma samples ranging from 0.14 – 21.18 µmol/L gave a correlation coefficient of 0.9805. Linear regression analysis gave the following equation:

Lithium heparin = 0.9972 (serum) + 0.1178 µmol/L

Precision Studies

The intra-assay precision and inter-assay precision were evaluated in samples containing two different bile acid levels (8 µM and 23 µM). The inter-assay precision was evaluated by testing these two-level specimens (low = 8 µM and high = 23 µM) in 20 runs. All tests were done using the Hitachi 717 Auto-analyzer instrument. Precision data is summarized in the table below:

Intra-Assay Precision

	Level 1 (8 µM)	Level 2 (23 µM)
Number of Replicates	20	20
Mean	7.93	23.5
SD	0.31	0.3
CV%	3.9%	1.3%

Inter-Assay Precision

	Level 1 (8 µM)	Level 2 (23 µM)
Number of Replicates	20	20
Mean	8.12	23.0
SD	0.24	0.61
CV%	2.9%	2.6%

Linearity

Linearity studies using a Hitachi 717 analyzer showed that Diazyme Total Bile Acids assay has a linear range from 1 to 180 µM.

Interference

Interference for the Diazyme Total Bile Acids Assay was evaluated on a Hitachi 717 analyzer. The following substances normally present in serum produced less than 10% deviation at the listed concentrations: Triglycerides at 750 mg/dL, Ascorbic acid at 50 mg/dL, Bilirubin at 50 mg/dL and Hemoglobin at 500 mg/dL.

Serious incident

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the EU Member State in which the user and/or the patient is established.

Symbols

The following symbols and signs in addition to those listed in the ISO 15223-1 standard are used.

REF	Catalog Number
CONTENT	Contents of kit
GTIN	Global Trade Item Number

REAGENT 1	= R1	Reagent 1
REAGENT 2	= R 2	Reagent 2
CALIBRATOR		Calibrator
CONTROL		Control

References

1. LaRusso, N.F. et al., Dynamics of Enterohepatic Circulation of Bile Acids, New Engl J M, 291, 689-692, (1974).
2. Skrede S. et al: Bile acids measured in serum during fasting as a test for liver disease, Clin Chem 24: 1095-1099, 1978
3. Wu, Alan H. B. Tietz Clinical Guide to Laboratory Tests. 4th ed. St. Louis, MO: Saunders/Elsevier, 2006. 170-171.
4. Ovadia C, Seed P, Sklavounos A, et al. "Association of adverse perinatal outcomes of intrahepatic cholestasis of pregnancy with biochemical markers: results of aggregate and individual patient data meta-analyses." *The Lancet,* Elsevier Inc., 14 Feb 2019, dx.doi.org/10.1016/S0140-6736(18)31877-4.
5. CLSI, Procedures for the Handling and Processing of Blood Specimens for Common Laboratory Tests; Approved Guideline, H18-A4, Vol.30 No. 10.

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