respons®910

Total bile acids 21 FS*

(in human stool)

Order Information

Cat. No.	Kit size
1 2238 99 10 921	Σ 200 (4 x 50)
1 2238 99 10 920	Σ 800 (4 x 200)

Intended Use

Diagnostic reagent for quantitative in vitro determination of total bile acids in extracted human stool samples on automated respons®910.

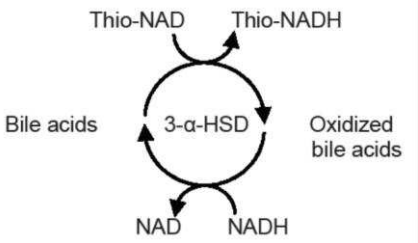
Summary

Bile acids (BA) are water soluble, amphipathic final products of the cholesterol catabolism which are synthesized in the liver, stored in the gall bladder and secreted in the intestine during digestion [1,2]. Throughout this metabolism, BA change from primary via secondary to tertiary BA and conjugates. Total bile acids (TBA) refer to the sum of all these forms. Approximately 95% of the intestinal bile acids are reabsorbed and transported back to the liver [1,2]. In bile acid malabsorption, the enterohepatic circulation is disturbed and an elevated amount of bile acids is excreted by stool. The main symptom of bile acid malabsorption is diarrhea [3]. Furthermore, TBA determination in stool plays a major role in the diagnosis of gastrointestinal tract disorders, like irritable bowel syndrome with diarrhea (IBS-D), bile acid diarrhea (BAD) or Crohn's disease [4-7]. Approximately 25 – 50% of patients with IBS-D display increased total fecal bile acids [5, 8-9]. BAD is characterized by elevated concentrations of bile acids within the colon, resulting in increased colonic motility and secretion. BAD patients typically present with chronic diarrhea and abdominal cramping [10]. In addition, BAD is a common cause of chronic unexplained diarrhea, with loose, watery feces or even incontinence. Hence, measuring TBA levels in stool provides a helpful tool to diagnose patients with BAD who were suspected to have IBS-D or remain undiagnosed [8].

Method

Enzymatic cycling method

Two reactions are combined in the new generation enzymatic cycling method. In the presence of Thio-NAD, the enzyme 3-α-hydroxysteroid dehydrogenase (3-α-HSD) converts bile acids to 3-ketosteroids and Thio-NADH. The reaction is reversible and 3-α-HSD can convert 3-ketosteroids and NADH to bile acids and NAD. In the presence of excess NADH, the enzyme cycling occurs efficiently and the rate of formation of Thio-NADH is determined by measuring the specific change of absorbance at 405 nm. This cycling reaction leads to significant signal amplification [11].



Reagents

Components and Concentrations

R1:	Buffer	
	Thio-NAD	> 0.1 mmol/L
R2:	Buffer	
	3-α-HSD	> 2 kU/L

Storage and Stability

Reagents are stable up to the date of expiry indicated on the kit, if stored at 2 – 8°C and contamination is avoided. Do not freeze and protect from light.

Reagents are temperature-sensitive. Avoid discontinuity of the cold chain.

The in-use stability of the reagent is 15 months.

Warnings and Precautions

1. Reagent 2 contains sodium azide (0.95 g/L) as preservative. Do not swallow! Avoid contact with skin and mucous membranes.
2. Reagent 2 contains material of biological origin. Handle the product as potentially infectious according to universal precautions and good clinical laboratory practice.
3. Daily food intake, esp. fatty nutrition, influences the daily bile acid excretion. Therefore, also repeated stool sampling might be necessary [12].
4. In case of product malfunction or altered appearance that could affect the performance, contact the manufacturer.
5. Any serious incident related to the product must be reported to the manufacturer and the competent authority of the Member State where the user and/or patient is located.
6. Please refer to the safety data sheets (SDS) and take the necessary precautions for the use of laboratory reagents. For diagnostic purposes, the results should always be assessed with the patient's medical history, clinical examinations and other findings.
7. For professional use only.

Waste Management

Refer to local legal requirements for chemical disposal regulations as stated in the relevant SDS to determine the safe disposal.

Warning: Handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Reagent Preparation

The reagents are ready to use. The bottles are placed directly into the reagent rotor.

Materials Required

General laboratory equipment

Dedicated stool preparation system

respons®910 rotor adaptors for stool sample preparation tubes Cat. No. 950580.

Specimen

Human stool

Due to the high amount of bacteria in raw stool samples, it is highly recommended to extract samples within three days.

Stability in stool extracts (1:100)

Data valid for IDK Extract®. Values might vary with other stool preparation systems:

Stability:

8 day	at	20 – 25°C
10 days	at	2 – 8°C
15 days	at	–20°C

Only freeze once. Discard contaminated specimens.

Sample Preparation

For collection and preparation of specimen material, use only dedicated stool specimen tubes, e.g. IDK Extract®; always follow the manufacturer's instructions.

Extraction of stool sample (exemplary shown using IDK Extract®):
Caution: Before starting the sample extraction, bring stool specimen and the extraction buffer within the collection tube to room temperature.
Carefully unscrew the tube and remove connected dipstick.
Insert dipstick into three different sections of stool sample and make sure that notches are completely covered with sample material. By inserting dipstick with stool sample back into preparation tube the defined amount of sample material (approx. 15 mg) is transferred into the extraction matrix whereas the excess stool is removed at the narrow opening of the conical insert. Screw tightly and vortex the preparation tube until no stool residues remain in the notches and maximally homogenize the stool suspension. For more solid specimen, incubate stool sample within extraction buffer for

approximately 10 min. to improve homogenization. **Resulting dilution 1:100.**
After successful homogenization, let sediments settle within preparation tube for 10 min. Remove complete cap including the dipstick. Place sample tubes directly into the respons®910 analyzer by using the dedicated adaptors for stool sample preparation tubes; avoid re-suspending the sediment!

Calibrators and Controls

DiaSys TruCal TBA is recommended for calibration. Calibrator values have been made traceable to a commercially available measurement procedure. Use DiaSys TruLab N and P for internal quality control. Quality control must be performed after calibration. Control intervals and limits have to be adapted to the individual requirements of each laboratory. Results must be within the defined ranges. Follow the relevant legal requirements and guidelines. Each laboratory should establish corrective action in case of deviations in control recovery. Dilution from sample preparation needs to be considered (e.g. when using IDK Extract® the dilution is 1:100). If other dilutions are utilized, the individual dilution factor has to be considered.

	Cat. No.	Kit size
TruCal TBA	1 2240 99 10 037	3 x 1 mL
TruLab N	5 9000 99 10 061	6 x 5 mL
	5 9000 99 10 062	20 x 5 mL
TruLab P	5 9050 99 10 061	6 x 5 mL
	5 9050 99 10 062	20 x 5 mL

Performance Characteristics

Exemplary data mentioned below were collected using stool preparation systems from IDK. The use of other extraction systems and deviating measurement conditions may lead to deviating results.

Measuring range up to 130 µmol/L. When values exceed this range samples should be diluted 1+3 with dedicated stool extraction buffer and the result multiplied by 4.	
Limit of detection**	1 µmol/L
Limit of quantification***	3.5 µmol/L
Onboard stability	14 days with chimneys
Calibration stability	7 days with chimneys

Interfering substance	Interferences in stool sample extract (dilution 1:100) ≤ 10% up to	Analyte concentration [µmol/L]
Ascorbic acid	1.20 mg/dL	30.5
	1.20 mg/dL	86.6
Bilirubin (conjugated)	0.74 mg/dL	31.8
	0.74 mg/dL	91.8
Bilirubin (unconjugated)	0.68 mg/dL	31.1
	0.68 mg/dL	90.3
Hemoglobin	120 mg/L	30.8
	120 mg/L	88.9
Immunoglobulin A	30 mg/L	30.7
	30 mg/L	87.6
Lipemia (triglycerides)	240 mg/L	30.1
	240 mg/L	84.7
For further information on interfering substances, refer to Young DS [13,14].		

Precision			
Within run (n=20)	**Sample 1**	**Sample 2**	**Sample 3**
Mean [µmol/L]	14.7	70.8	115
CV [%]	1.50	1.09	2.06
Total precision CLSI (n=80)	**Sample 1**	**Sample 2**	**Sample 3**
Mean [µmol/L]	14.8	72.5	120
CV [%]	4.09	3.51	3.44
Reproducibility (n=75)	**Sample 1**	**Sample 2**	**Sample 3**
Mean [µmol/L]	15.0	74.2	125
CV [%]	4.08	3.01	3.35

Method comparison (n=122)	
Test x	Competitor bile acids
Test y	DiaSys Total bile acids 21 FS
Slope	1.22
Intercept	1.89 µmol/L
Coefficient of correlation	0.988

** according to CLSI document EP17-A2, Vol. 32, No. 8

*** according to CLSI document EP5-A3, Vol. 34, No. 13

Reference Range

Based on internal data of a routine laboratory (women n=35849/men n=16114) the following values were estimated in human stool sample extracts (dilution: 1:100):

Women: 4.5 – 70.3 µmol/L
Men: 4.3 – 83.8 µmol/L

Each laboratory should check if the reference ranges are transferable to its own patient population and determine own reference ranges if necessary.

Literature

1. Stamp D and Jenkins G. An overview of bile- acid synthesis, chemistry and function. In: Jenkins GJ, Hardie L, editors. Bile Acids: Toxicology and Bioactivity. Royal Society of Chemistry; 2008. p. 1-13.
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aaccweb/aacc/, accessed in May 2022. Published by AACC Press and John Wiley and Sons, Inc.

Additions and/or changes in the document are highlighted in grey. For deletions, please refer to the customer information for the corresponding edition number of the package inserts.

IVD CE

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* Fluid Stable

respons®910

Total bile acids 21 FS

Application for stool samples

This application was set up and evaluated by DiaSys. It is based on the standard equipment at that time and does not apply to any equipment modifications undertaken by unqualified personnel.

Identification	
This method is usable for analysis:	Yes
Twin reaction:	No
Name:	TBAst
Shortcut:	
Reagent barcode reference:	071
Host reference:	075

Technic	
Type:	Linear kinetic
First reagent:[µL]	180
Blank reagent	Yes
Sensitive to light	
Second reagent:[µL]	60
Blank reagent	No
Sensitive to light	
Main wavelength:[nm]	405
Secondary wavelength:[nm]	600
Polychromatic factor:	1.0000
1 st reading time [min:sec]	05:48
Last reading time [min:sec]	08:00
Reaction way:	Increasing
Linear Kinetics Substrate depletion: Absorbance limit	1.0000
Linearity: Maximum deviation [%]	
Fixed Time Kinetics Substrate depletion: Absorbance limit	
Endpoint Stability: Largest remaining slope	
Prozone Limit [%]	

Reagents	
Decimals	
Units	

Sample	
Diluent	Stool buffer
Hemolysis:	
Agent [µL]	0 (no hemolysis)
Cleaner	
Sample [µL]	0
Technical limits	
Concentration technical limits-Lower	3.5000
Concentration technical limits-Upper	130.0000
SERUM	
Normal volume [µL]	5.0
Normal dilution (factor)	1
Below normal volume [µL]	
Below normal dilution (factor)	
Above normal volume [µL]	
Above normal dilution (factor)	
URINE	
Normal volume [µL]	5.0
Normal dilution (factor)	1
Below normal volume [µL]	
Below normal dilution (factor)	
Above normal volume [µL]	
Above normal dilution (factor)	
PLASMA	
Normal volume [µL]	5.0
Normal dilution (factor)	1
Below normal volume [µL]	
Below normal dilution (factor)	
Above normal volume [µL]	
Above normal dilution (factor)	
CSF	
Normal volume [µL]	5.0
Normal dilution (factor)	1
Below normal volume[µL]	
Below normal dilution (factor)	
Above normal volume [µL]	
Above normal dilution (factor)	
Whole blood	
Normal volume [µL]	5.0
Normal dilution (factor)	1
Below normal volume[µL]	
Below normal dilution (factor)	
Above normal volume [µL]	
Above normal dilution (factor)	

Results	
Decimals	2
Units	µmol/L
Correlation factor-Offset	0.0000
Correlation factor-Slope	1.0000

Range	
Gender	Male
Age	
SERUM	>=4.30 <=83.80
URINE	
PLASMA	
CSF	
Whole blood	
Gender	Female
Age	
SERUM	>=4.50 <=70.30
URINE	
PLASMA	
CSF	
Whole blood	

Contaminants
Please refer to r910 Carryover Pair Table

Calibrators details	
Calibrator list	**Concentration**
Cal. 1/Blank	0
Cal. 2	*
Cal. 3	
Cal. 4	
Cal. 5	
Cal. 6	
	Max delta abs.
Cal. 1	0.1
Cal. 2	0.1
Cal. 3	
Cal. 4	
Cal. 5	
Cal. 6	
Drift limit [%]	0.80

Calculations	
Model	X
Degree	1

* Enter calibrator value