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Manual

IDK® Bile Acids

For the in vitro determination of bile acids in serum

Valid from 2022-11-07

REF **K 7877W**

Σ 96

+2 °C +8 °C -20 °C RGZ1 RGZ2

IVD CE

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1. INTENDED USE

This colourimetric assay is intended for the quantitative determination of bile acids in serum. For *in vitro* diagnostic.

2. INTRODUCTION

Bile acids are end products of the hepatic cholesterin metabolism and are secreted into the duodenum together with other bile components like cholesterin, bilirubin, phospholipids and proteins.

Bile acids perform important tasks like excretion of cholesterin via the intestine, absorption of fats and liposoluble vitamins in the small intestine and stimulation of intestinal motility.

Most of the daily secreted bile acids are reabsorbed in the terminal ileum, enter the liver via the portal vein and are again excreted as part of bile. The result of this enterohepatic circulation is a fecal excretion of only 3–5 % of bile acids per day.

Disturbances in the bile acid metabolism can occur at different points of the enterohepatic circulation:

- impaired bile synthesis in liver cells
- intrahepatic cholestase
- extrahepatic closure of the bile duct
- bile acid malabsorption in the intestine
- impaired bile acid reabsorption in the liver
- impaired intracellular metabolism (recycling) in liver cells

An hereditary or acquired dysfunction of the liver, e. g. caused by infections, metabolic diseases, drugs or toxins, causes detectable changes of bile acid concentrations in blood.

Bile acids in blood are a sensitive marker for liver diseases[1,2,3] and are suited for early indicator for e. g. toxic effects on the liver (drugs, alcohol, solvent exposure etc.).

Together with the rather unspecific symptom pruritus, elevated bile acid levels in blood define the clinical picture of intrahepatic cholestase during pregnancy (ICP).

Bile acids in blood are the most sensitive laboratory parameter for the diagnostics of ICP. In Europe, ICP occurs during 0,2 % of all pregnancies (highest prevalence in Sweden: 1–1,5 %)[4] and is mostly due to a genetic predisposition of the mother. In the second half of pregnancy, ICP is triggered by high hormone levels. The visible clinical symptom is pruritus, starting at palms and sole of the feet.

Untreated ICP bears the risk of premature birth (19–60 %), complications for the newborn (intrapartal emergency situation: 22–41 %) and intrauterine death (7.5–1.6 %)[4]. After an ICP occured, a cholestase is also possible on oral contraceptive, as the ICP is

caused by increased hormone levels. There is also a risk for recurrence of ICP during further pregnancies (45–70 %)[5].

3. MATERIAL SUPPLIED

Cat. No.	Label	Kit components	Quantity
K 7877W	PLATE	Microtiter plate	12 x 8 wells
K 7877W	STD	Standards, ready-to-use (96; 48; 24; 12; 6; 0 µmol/l)	6 x 500 µl
K 7877W	CTRL1	Control, ready-to-use (see specification for range)	1 x 500 µl
K 7877W	CTRL2	Control, ready-to-use (see specification for range)	1 x 500 µl
K 7877W	SAMPLEBUF	Sample dilution buffer, ready-to-use	1 x 10 ml
K 7877W	RGZ1	Reagent 1, ready-to-use	1 x 20 ml
K 7877W	RGZ2	Reagent 2, ready-to-use	1 x 6 ml

For reorders of single components, use the catalogue number followed by the label as product number.

4. MATERIAL REQUIRED BUT NOT SUPPLIED

- Ultrapure water*
- Calibrated precision pipettors and 10–1 000 µl single-use tips
- Foil to cover the microtiter plate
- Horizontal microtiter plate shaker
- Multi-channel pipets or repeater pipets
- Vortex
- Standard single-use laboratory glass or plastic vials, cups, etc.
- Microtiter plate reader (required filters see chapter 7)

* Immundiagnostik AG recommends the use of ultrapure water (water type 1; ISO 3696), which is free of undissolved and colloidal ions and organic molecules (free of particles > 0.2 µm) with an electrical conductivity of 0.055 µS/cm at 25 °C (≥ 18.2 MΩ cm).

5. STORAGE AND PREPARATION OF REAGENTS

Attention: Please unpack the kit components **RGZ1** (Reagent 1) and **RGZ2** (Reagent 2) from the transport packaging immediately upon receipt and follow the instructions for storage conditions printed on the product labels.

- **RGZ1** and **RGZ2** can be used until the expiry date stated on the label when stored at **-20 °C**.
- To run the assay more than once, ensure that reagents are stored at the conditions stated on the label. **Prepare only the appropriate amount necessary for each run.** The kit can be used up to 4 times within the expiry date stated on the label.
- Reagents with a volume less than **100 µl** should be centrifuged before use to avoid loss of volume.
- All test reagents are ready-to-use. Test reagents are stable until the expiry date (see label) when stored at **2–8 °C**.

6. STORAGE AND PREPARATION OF SAMPLES

Sample storage

Freshly collected serum can be stored at 2–8 °C for one week or for 3 months at -20 °C.

Sample preparation

Serum must be diluted **1:2 in sample dilution buffer (SAMPLEBUF)** before performing the assay. For example:

- **50 µl** sample + **50 µl** sample dilution buffer, mix well
  This results in a final dilution of 1:2.

For analysis, pipet **40 µl** of this **dilution** per well.

7. ASSAY PROCEDURE

Principle of the test

This assay is designed for the quantitative determination of bile acids in serum.

In the presence of excess thio-NAD, bile acids are converted to 3-keto steroids by the enzyme 3-a-hydroxysteroid dehydrogenase while thio-NADH is formed.

The rate of formation of thio-NADH can be determined by the change of absorbance (ΔOD) at 405 nm. A dose response curve ΔOD vs. concentration is generated, using the values obtained from measured standards. The bile acids concentration of the samples is determined directly from this curve.

Test procedure

Bring all **reagents and samples to room temperature** (15–30 °C) and mix well.

Mark the positions of standards/controls/samples on a protocol sheet.

Take as many microtiter strips as needed from the kit. Store unused strips covered at 2–8 ° C. Strips are stable until expiry date stated on the label.

For automated ELISA processors, the given protocol may need to be adjusted according to the specific features of the respective automated platform. For further details please contact your supplier or Immundiagnostik AG.

We recommend to carry out the tests in duplicate.

1.	Pipet **10 µl standard, control** (STD/CTRL) or **40 µl 1:2 diluted sample**, respectively, in each well.
2.	Add **150 µl reagent 1** (RGZ1) with a repeater pipet into each well. Take care not to contaminate the dispenser tip with standard/control/sample.
3.	Incubate **5 min** at room temperature (15–30 °C) on a horizontal plate shaker*.
4.	Add **50 µl reagent 2** (RGZ2) with a repeater pipet into each well. Take care not to contaminate the dispenser tip with the content of the well.
5.	Incubate **1 min** at room temperature (15–30 °C) on a horizontal plate shaker*.
6.	Determine **absorption immediately** with an ELISA reader at **405 nm** against 620 nm (or 690 nm) as a reference. If the photometer allows the measurement of reaction kinetics, record 10 measuring points in a time interval of 15 sec and determine the slope (Δ**OD)** by linear regression over all data points between 15 and 150 sec.. If only single measurement is possible, then determine absorption directly after the 1-minute-incubation, cover the plate for 2 min (please note the exact time interval) and then take a second measurement. The slope (Δ**OD)** corresponds to the difference of final OD and start OD divided by the time interval between the two measurements. ΔOD = (final OD - start OD)/time interval

* We recommend shaking the strips at 550 rpm with an orbit of 2 mm.

8. RESULTS

Point-to-point calculation

We recommend a linear ordinate for the change in optical density (Δ**OD)** and a linear abscissa for the concentration.

The plausibility of the duplicate values should be examined before the automatic evaluation of the results. If this option is not available with the programme used, the duplicate values should be evaluated manually.

Serum samples

The test results have to be divided by **2** to obtain the bile acids levels of the samples.

9. LIMITATIONS

Samples with concentrations above the measurement range (see definition below) can be further diluted and re-assayed. Please consider this higher dilution when calculating the results.

Samples with concentrations lower than the measurement range cannot be clearly quantified.

The upper limit of the measurement range can be calculated as:

highest concentration of the standard curve × *sample dilution factor to be used*

The lower limit of the measurement range can be calculated as:

LoB × *sample dilution factor to be used*

LoB see chapter "Performance characteristics".

10. QUALITY CONTROL

Immundiagnostik AG recommends the use of external controls for internal quality control, if possible.

Control samples should be analysed with each run. Results, generated from the analysis of control samples, should be evaluated for acceptability using appropriate statistical methods. The results for the patient samples may not be valid if within the same assay one or more values of the quality control sample are outside the acceptable limits.

Reference range

We recommend each laboratory to establish its own reference range.

11. PERFORMANCE CHARACTERISTICS

Precision and reproducibility

Intra-Assay (n = 30)

Sample	Bile acids [µmol/l]	CV [%]
1	8.7	9.4
2	25.5	7.2

Inter-Assay (n = 10)

Sample	Bile acids [µmol/l]	CV [%]
1	8.5	10.4
2	24.6	10.8

Spiking Recovery

Three samples were spiked with different concentrations of cholic acid and measured using this assay (n = 3).

Sample	Unspiked Sample [µmol/l]	Spike [µmol/l]	Bile acids expected [µmol/l]	Bile acids measured [µmol/l]
A	3.12	8.53	11.66	13.34
	3.12	6.37	9.5	8.75
	3.12	3.54	6.67	8.2
	3.12	2.13	5.26	5.21
B	2.85	10.92	13.76	17.33
	2.85	8.53	11.37	13.46
	2.85	6.36	9.2	10.67
	2.85	3.54	6.39	6.61
	2.85	2.13	4.98	5.57

Sample	Unspiked Sample [µmol/l]	Spike [µmol/l]	Bile acids expected [µmol/l]	Bile acids measured [µmol/l]
C	3.25	8.53	11.78	14.81
	3.25	6.36	9.61	11.14
	3.25	3.54	6.79	8.7
	3.25	2.13	5.38	5.73

Dilution recovery

Two samples were diluted and analysed. The results are shown below (n = 2).

Sample	Dilution	Bile acids expected [µmol/l]	Bile acids measured [µmol/l]
A	1:2	20.76	22.95
	1:4	10.38	9.09
	1:8	5.19	6.55
	1:16	2.6	3.45
	1:32	1.3	1.7
	1:64	0.65	0.84
	1:128	0.32	0.41
B	1:2	16.23	17.03
	1:4	8.11	9.4
	1:8	4.06	4.56
	1:16	2.03	2.27
	1:32	1.01	1.06
	1:64	0.51	0.59
	1:128	0.25	0.27

Analytical Sensitivity

The following values have been estimated based on the concentrations of the standard curve without considering possibly used sample dilution factors.

Limit of blank, LoB	0.195 µmol/l
Limit of detection, LoD	0.514 µmol/l
Limit of quantitation, LoQ	0.889 µmol/l

The evaluation was performed according to the CLSI guideline EP-17-A2. The specified accuracy goal for the LoQ was 20 % CV.

12. PRECAUTIONS

- All reagents in the kit package are for *in vitro* diagnostic use only.
- Kit reagents contain sodium azide or ProClin as bactericides. Sodium azide and ProClin are toxic. Avoid contact with skin or mucous membranes.

13. TECHNICAL HINTS

- Do not interchange different lot numbers of any kit component within the same assay.
- Control samples should be analysed with each run.
- Reagents should not be used beyond the expiration date stated on the kit label.
- Avoid foaming when mixing reagents.
- Do not mix plugs and caps from different reagents.
- The assay should always be performed according to the enclosed manual.

14. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- This assay was produced and distributed according to the IVD guidelines of 98/79/EC.
- The guidelines for medical laboratories should be followed.
- *IDK*® is a trademark of Immundiagnostik AG.
- Incubation time, incubation temperature and pipetting volumes of the components are defined by the producer. Any variation of the test procedure,

which is not coordinated with the producer, may influence the results of the test. Immundiagnostik AG can therefore not be held responsible for any damage resulting from incorrect use.

- Warranty claims and complaints regarding deficiencies must be logged within 14 days after receipt of the product. The product should be send to Immundiagnostik AG along with a written complaint.

15. REFERENCES

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Used symbols:

 Temperature limitation

Catalogue number

In Vitro Diagnostic Medical Device

To be used with

 Manufacturer

Contains sufficient for <n> tests

Lot number

 Use by

 Attention

 Consult instructions for use

Consult specification data sheet