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Manual

IDK® Bile Acids

Photometric test system for the in vitro determination of bile acids in serum

Valid from 2019-05-09

REF **K 7877CV**

Σ 40

+8°C +2°C

IVD

CE

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1. INTENDED USE

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

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 7877CV	STD	Standard 48 µmol/l, ready-to-use	1 x 250 µl
K 7877CV	CTRL1	Control, ready-to-use (see specification for range)	1 x 250 µl
K 7877CV	CTRL2	Control, ready-to-use (see specification for range)	1 x 250 µl
K 7877CV	SAMPLEBUF	Sample dilution buffer, ready-to-use	1 x 10 ml
K 7877CV	RGZ1	Reagent 1, ready-to-use	1 x 20 ml
K 7877CV	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 10–1000 µl single-use tips
- Disposable cuvettes (we recommend PS 1,5 ml semi-micro disposable cuvettes, 12,5 x 12,5 x 45 mm, Brand GmbH + Co KG, D-97861 Wertheim, Germany, Cat. No. 7590 15)
- Vortex
- Standard single-use laboratory glass or plastic vials, cups, etc.
- Cuvette photometer (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

- 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.**
- Reagents with a volume less than **100 µl** should be centrifuged before use to avoid loss of volume.

- **Sample dilution buffer** (SAMPLEBUF) is used as **blank**.
- All other 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 samples do not have to be diluted to be used in the assay.

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 (DOD) at 405 nm.

The bile acids concentration of the samples is determined directly from the accompanied standard.

Test procedure

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

We recommend to carry out the tests in duplicate.

1.	Pipet **450 µl reagent 1** (RGZ1) into the cuvette.
2.	Add each **30 µl standard/controls/blank/samples** into the respective cuvette and mix well.
3.	Incubate **5 min** at room temperature (15–30 °C).
4.	Add **150 µl reagent 2** (RGZ2) and mix well.
5.	Incubate **1 min** at room temperature (15–30 °C).

6.	Take the **first measurement** of absorption **directly after the 1 minute incubation**, cover the cuvette for **2 min** (please note the exact time interval) and then take a **second measurement**. The slope (**ΔOD)** 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

8. RESULTS

Calculation of sample concentration

$$\text{Concentration}_{\text{sample}} = \frac{\Delta OD_{\textbf{sample}} - \Delta OD_{\textbf{blank}}}{\Delta OD_{\textbf{standard}} - \Delta OD_{\textbf{blank}}} \times 48\ \mu\text{mol/l}$$

9. LIMITATIONS

Samples with a concentration of bile acids higher than the standard 48 µmol/l should be further diluted with sample dilution buffer and re-assayed. For the following analysis, the changed dilution factor has to be taken into consideration.

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

The reproducibility of three results in one measurement series was evaluated. Three samples were analysed 5 times by one person using the *IDK*® Bile acids.

Sample	Bile acids [µmol/l]	CV [%]
1	1.3	4.6
2	21.4	9.1
3	7.1	7.1

Analytical Sensitivity

The zero standard was measured 20 times. The detection limit was set as B_0 + 2 SD and estimated to be 0.19 µmol/l.

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 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 trademarks 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.

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Used symbols: