UMWELTPROBENBANK
DES BUNDES

Guideline for Sampling and Sample Processing

Analysis of Clinical Chemical Parameters

Dominik Lermen, Martina Bartel-Steinbach, Nicole Jost

Fraunhofer Institute for Biomedical Engineering IBMT
Josef-von-Fraunhofer-Weg 1, 66280 Sulzbach, Germany

Umwelt Bundesamt

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Guidelines for Sampling, Transport, Storage and Chemical Characterization of Environmental and Human Samples

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1 German Environmental Specimen Bank

The German Environmental Specimen Bank (ESB) is an instrument for environmental monitoring of the Federal Ministry for the Environment, Nature Conservation, Building and Nuclear Safety (BMUB) subject to specialist and administrative coordination by the Federal Environment Agency (UBA). The ESB collects ecologically representative environmental and human samples, stores them and investigates them for environmentally-relevant substances (BMUB 2008).

The long-term storage is carried out under conditions which, as far as possible, exclude a change in state or a loss of chemical characteristics over a period of several decades. The archive therefore provides samples for retrospective investigation of substances for which the potential risk for the environment or human health is not yet known.

Comprehensive information on the ESB is available at www.umweltprobenbank.de.

2 Objective of this Guideline

This guideline describes all necessary work steps for photometric determination of selected clinical chemical parameters in 24-h urine collection and blood plasma.
In 2014, a comprehensive quality management system according to DIN EN ISO/IEC 17025 (Lermen et al. 2014) was established for the division of the ESB operated by the Fraunhofer Institute for Biomedical Engineering (IBMT), which includes the collection, storage and initial characterization of human samples. The following guideline represents a non-controlled excerpt of this QM system in relation to the analysis of selected clinical chemical parameters. In this form, it is not an integrated component of this QM system.

3 Areas of Application

This guideline describes the process for photometric determination of the concentrations of cholesterol (CHO), total protein (TP) and triglycerides (TRIGLY) in blood plasma and of creatinine in blood plasma (CRE) and 24-h urine collection (CRE-U) at the ESB.
The defined areas of application and detection limits for the relevant analytes are shown in table 1.
Human sample materials, the infection status of which is not further characterized, are generally seen as potentially infectious. When handling these, the requirements of the regulation on safety and health protection when working with biological substances (BioStoffV) must be strictly followed. The work must therefore be carried out in a laboratory of biological safety level 2 (BSL2) and the appropriate protective clothing must be worn.

4 Description of the Method

The determination of clinical chemical parameters is carried out in a photometric manner using the Cobas c111 analyzer from Roche.
The Cobas c111 system determines the absorption value in the sample liquid using absorption photometry. The concentration of the analytes of interest in the solution is calculated on the basis of the absorption. The determination is based on the following chemical physical principles.

Cholesterol (CHO)
CHOD-PAP method – the cholesteryl esters are split into free cholesterol and fatty acid under exposure to cholesterol esterase. The cholesterol oxidase catalyzes the oxidation of cholesterol to cholest-4-en-3-one and hydrogen peroxide. The hydrogen peroxide that is created forms a red dye with amino antipyrine and phenol under the catalytic effect of the peroxidase.
The intensity of the dye formed is directly proportional to the cholesterol concentration.

Total protein (TP)
Biuret method – divalent copper reacts in an alkali solution with the peptide bonds of proteins to form the characteristic purple-colored Biuret complex. Potassium sodium tartrate is added to the reaction to prevent the precipitation of copper hydroxide. The addition of potassium iodide prevents the autoreduction of copper.
The intensity of the Biuret dye reaction is directly proportional to the total protein content.

Creatinine (CRE, CRE-U)
Jaffe method – in alkali solution, creatinine forms a yellow-orange colored complex with picric acid. The rate of formation of the dye is proportional to the creatinine concentration.

Triglycerides (TRIGLY)
GPO-PAP method – lipoprotein lipase hydrolyzes triglycerides to glycerin. Subsequent oxidation leads to the formation of dihydroxyacetone phosphate and hydrogen peroxide. The created hydrogen peroxide forms a red dye under the catalytic effect of the peroxidase with 4-aminophenazone and 4-chlorophenol in a Trinder endpoint reaction. The intensity of the red dye formed is directly proportional to the triglyceride concentration.

5 Devices, Reagents and Materials

5.1 Analysis Device

Cobas analysis system, model: c111
Manufacturer: Roche Diagnostics GmbH

5.2 Reagents and Materials

Cobas c 111 specific materials
Cobas c 111 microcuvette segment
RD standard false bottom tube
Screw cap for false bottom tube

Test-specific reagents
The test-specific reagents are ready-made solutions from Roche. The following reagents are used for the determination of the analytes mentioned above:

- Cholesterol (ref. no.: 04718917190)
- Total protein (ref. no.: 04657586190)
- Creatinine (ref. no.: 05401755190)
- Triglycerides (ref. no.: 04657594190)

Zero calibrator
Ultrapure water (> 18.2 MΩ*cm) is used as the zero calibrator.

Calibrator
Calibrator f.a.s., order no. 10759350

Quality control
Precinorm U, order no. 10171735
Precipath U, order no. 10171760

Table 1: Areas of Application

Analyte	Matrix	Measuring range	Lower detection limit	Normal range
Triglycerides	Blood plasma	8.85–885 mg/dl	8.85 mg/dl	< 150 mg/dl
Total protein	Blood plasma	2.0–120 g/l	2.0 g/l	66–87 g/l
Cholesterol	Blood plasma	9.7–800 mg/dl	9.7 mg/dl	< 200 mg/dl
Creatinine	Blood plasma	0.2–12.4 mg/dl	0.2 mg/dl	0.50–0.90 mg/dl (f) 0.70–1.20 mg/dl (m)
	24-h Urine Collection	0.31–368 mg/dl	0.31 mg/dl	740–1570 mg/24 h (f) 1040–2350 mg/24 h (m)

6 Execution

The Cobas c111 analyzer must be commissioned and set up according to the manufacturer's instructions.

6.1 Measuring Analytes

In order to measure an analyte in a matrix, approx. 1 ml of the matrix must be pipetted into a prepared Cobas sample tube (RD standard false bottom tube). The sample tube must be placed in the Cobas c111 analyzer, the desired test must be selected in the user menu and the measurement must be started. The measurement is carried out automatically.

6.2 Calibration and Internal Quality Control

In order to ensure the measurements are accurate, the device must be calibrated every working day before each measurement series.
In addition to this, in order to check and guarantee that the measurement results are correct, before and after each measurement series internal quality control measurements of all measurement parameters must be carried out.
If the measurements for the quality controls after the end of a measurement series are outside of the tolerance range or if any other irregularities occur (e.g. frequent error messages on the Cobas c111 analyzer), then the analyses of the samples from this measurement series must be repeated and the head of the laboratory must be informed.

6.3 Method Validation

The validation of the method is based on the requirements from the standard DIN EN ISO/IEC 17025:2005 and DAkkS [German Accreditation Body] guideline 71 SD 4 019, which is based on this (revision: 1.1, January 14, 2015). For this purpose, the procedural parameters to be determined must be specified in a validation plan. The following procedural parameters must be identified for the determination of clinical chemical parameters:
Precision
- Repeat precision
- Laboratory precision
- Comparative precision

Correctness
Robustness
Specificity/selectivity
The procedural parameters determined must be presented and assessed in a validation report. The method must be approved by the head of the laboratory following assessment for the area of application.

6.4 External Quality Control

Participation in suitability tests is indispensable for checking and external quality assurance for the determined measurements for clinical-chemical parameters. Participation in an external quality control is based on the requirements of RiLiBäk [Guidelines of the German Medical Council]. For this purpose, once per year before the start of sampling (in October) the participation in an organized external quality assessment (e.g. Instand e.V.) is required. Furthermore, comparative tests are organized on an annual basis. In this case, after the end of the sampling approx. 20% of the samples that have not yet been frozen (25 samples per matrix) are cooled (2–8°C) and sent to an accredited laboratory for comparative testing.

7 Documentation

The results of the calibrations and quality control measurements must be documented. In this case the date and time of the calibration/measurement, any discrepancies, additional comments and the person carrying out the work must be recorded. All records must be archived.
The analysis results saved in the Cobas c111 must be read out from the device after each measurement series, checked for plausibility and redundantly stored. The results must be printed and archived.

As the analysis of clinical-chemical parameters of human samples of the ESB during sampling on location is carried out at the location of sampling and immediately after receipt of the samples at the mobile laboratory, deviations from the valid guideline must be documented in the report for the relevant sampling. The reports must be archived.

8 Literature

BMUB (German Ministry for the Environment, Nature Conservation, Building and Nuclear Safety, Ed.) (2008): German Environmental Specimen Bank – conceptual design (Status: October 2008); www.umweltprobenbank.de

DAkkS rule 71 SD 4 019 (2015): Validation and verification of test procedures as per the requirements of DIN EN ISO/IEC 17025 for test laboratories in the area of chemical and chemical-physical analysis in the area of department 4 (consumer health protection | agricultural sector | chemistry | environment).

DIN EN ISO/IEC 17025:2005 (2005): General requirements for the competence of testing and calibration laboratories.

Lermen D, Schmitt D, Bartel-Steinbach M, Schröter-Kermani C, Kolossa-Gehring M, von Briesen H, Zimmermann H (2014). A New Approach to Standardize Multicenter Studies: Mobile Lab Technology for the German Environmental Specimen Bank. PloS one, 9(8), e105401.