German Society of Legal Medicine (DGRM), Society of Toxicological and Forensic Chemistry (GTFCh) and German Society for Traffic Medicine (DGVM)

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GUIDELINES FOR THE DETERMINATION OF BLOOD ALCOHOL CONCENTRATIONS (BAC) FOR FORENSIC PURPOSES
- BAC Guidelines -

Foreword

In 1966, the expert report commissioned by the German Health Agency compiled initial guidelines for the determination of blood alcohol concentrations in cases involving road traffic offences. These guidelines have been repeatedly adapted in line with the latest technological advances and judicial requirements. In October 1997, amendment recommendations were jointly issued by the German Society for Forensic Medicine (DGRM), the German Society for Traffic Medicine (DGVM) and the Society for Toxicological and Forensic Chemistry (GTFCh). Changes in organisation, reporting rules and new technological advances resulted in a further amendment in September 2007 (*Blutalkohol 44: 273-282*). Now, a further adaptation of these guidelines is necessary to comply with existing norms.

1. General Introduction

The purpose of determining blood alcohol concentrations is to provide blood alcohol concentrations with evidential value for forensic purposes. In the following, the term 'alcohol' refers to ethanol (ethyl alcohol, CAS 64-17-5).

In general, the alcohol concentration must be measured by two different methods, namely: (1) using the enzyme alcohol dehydrogenase (ADH), and (2) gas-chromatography (GC). Alternatively, two differing GC methods may be used.

Each analytical procedure must be performed in a separate laboratory workspace, with separate equipment, and by the technical staff allocated to each laboratory workspace. The analyses related to one analytical method must be carried out completely independently. Each analysis must be carried out from start to completion

of measurement by one person, i.e. division of workload is prohibited. It is vital that all results are combined only upon completion of the analysis. Calculations, interpretation and documentation may be carried out by a third party.

2. Clients and Requests

Clients are: courts of law, public authorities, institutes or private individuals. Blood samples may be drawn in accordance with the relevant regulations of a criminal, or civil, procedure, or given voluntarily after informed consent.

3. Sample Handling

The laboratory will analyse those blood samples received in accordance with paragraph **2**, above. The date of sample receipt must be recorded. Unpacking of samples must be performed and documented by two authorised persons. Poor condition of samples and transport packaging as well as deficiencies in labelling and accompanying documents must also be thoroughly documented. Sample labelling and accompanying documents must be immediately compared and checked for consistency. The client's sample code and label (if provided) must be used as intended. However, the laboratory's internal sample identification system may be used in addition.

4. Staff and Laboratory

4.1. Staff

The Head of Laboratory must hold a university degree in natural science or in medicine (Diploma, Master, State Examination or an equivalent qualification). For analysing samples for forensic purposes a further qualification in forensic science is mandatory, as well as appropriate continuous education.

For technical staff a qualified vocational education in laboratory analysis is required. Technical staff must provide evidence of continuing education in the specialist subject area of forensic laboratory analysis.

4.2 Laboratory

The determination of blood alcohol concentrations for forensic purposes must only be performed in designated laboratory spaces. Contamination of samples, standards, reagents and instruments with volatiles, especially alcohols (e. g. isopropanol), must

be strictly prevented. Both types of analytical methods must be performed in two separate, and independent workspaces.

5. Analysis

5.1. Preface

Results of duplicate measurements performed independently with each of the two methods must be quoted to three decimal points. The preparation of the sample aliquots must be carried out at room temperature. Thus, for each method, two equal volumes or masses (aliquots) of the sample are required. Analytical methods must be validated according to the GTFCh guidelines; however, proof of the suitability of an aqueous calibration may be omitted.

5.2. Gas chromatography (GC)

5.2.1. Preface

Determination of blood alcohol concentrations by gas chromatography is to be carried out by applying the laboratory's established method. Calibration and quality control must comply with paragraphs **6** and **7** of these guidelines.

For the analysis of serum/plasma samples, an internal standard must be added which does not typically occur in either serum or plasma. Interferences caused by typically occurring substances in the sample matrix must be excluded. Tertiary butanol has been proven to be an adequate internal standard. With mass-spectrometric detection, deuterated ethanol (hydrogen/deuterium exchange > 2, no deuterium bound to oxygen) has to be used.

5.2.2. Two different GC methods

Two GC methods are considered to be different when the following criteria are fulfilled:

- Two GC systems with comparable detectors (e.g. flame ionisation) but equipped with two columns of different polarity, which guarantee that the relative retention times of ethanol and the internal standard are different for both methods. Interferences by other potentially expected substances (especially congeners and endogenous volatile compounds such as ketones) must be excluded in the course of method validation.

- Two instrument systems with two different detectors (e.g. flame ionisation and mass spectrometric monitoring of at least two ions).

5.3. ADH method

The enzymatic determination of blood alcohol concentration must be carried out by the laboratory's established method. Calibration and quality control must comply with paragraphs **6** and **7** of these guidelines.

Should the analysis require a protein precipitation step (in the case of analysis of whole blood or haemolytic serum/plasma), then this must be done in two separately prepared replicates utilising the same protein precipitation procedure. Multiple analysis of a single deproteinised sample is prohibited. Care must be taken to exclude evaporation effects. Methods including a protein precipitation step must also be individually validated.

6. Calibration

Commercial water-based ethanol standard solutions must be used, the concentration of which is guaranteed by the manufacturer. At least five calibration concentrations must be used in single or multiple analyses. The calibrator concentrations of at least ≤ 0.20, 1.00, 2.00, and 3.00 g ethanol/L are mandatory. In contrast to ADH methods, the blank level in GC methods is not a calibration point. In ADH methods involving a deproteination step, the calibration must be performed with identically treated water-based ethanol standard solutions.

A calibration must be performed with every new analytical series; an exception being when all three quality control samples (low, middle and high - paragraph **7**) lie within the given ranges. In such a case, a previously measured calibration can be used.

Should authentic samples show concentrations greater than that of the highest calibrator, then a method involving a dilution step must be established.

A dilution step, involving e.g. isotonic saline, must be independently performed for both methods, if required.

7. Quality Control

7.1. General rules for the analysis of control samples

Internal laboratory quality control monitoring must be performed with suitable serum/plasma samples. These must be certified reference control samples the target

concentration and confidence range of which is guaranteed by the manufacturer. The manufacturers' indicated confidence ranges must not exceed the maximum permissible deviation (as set out in section **7.2**). Therefore, for forensic purposes, the manufacturers' confidence range may have to be restricted by the individual laboratories themselves.

Commercial control material should reflect the variation in matrix encountered in test samples and, therefore, it is recommended that control samples from different manufacturers are included. In-house prepared control samples are unsuitable for this purpose.

For each sample test series, at least one negative control sample and two differing positive control samples must be analysed in duplicate with the following levels: one positive control sample with a low concentration (≤ 0.50 g/L serum; ≤ 0.40 g/kg whole blood) and, at least alternating from series to series, one additional control sample with a middle concentration (0.50-1.60 g/L serum; 0.40-1.29 g/kg whole blood) or a high concentration (≥ 3.0 g/L serum; ≥ 2.43 g/kg whole blood).

In each analytical series a control sample must be analysed at least after every 10th test sample (20 measurements) and the analysis sequence must conclude with a further control sample.

All quality control results must be checked and documented. Charts for the documentation and control of the quality control (QC) results must be constructed into which two QC results per method must be filled in and checked. At least one duplicate result of each concentration for each method and analytical series has to be registered in the charts. It must be defined *a priori* which QC sample has to be registered.

7.2. Acceptance criteria

7.2.1. Control samples

The target value (middle line) and the maximum allowable deviation must be registered in the QC charts. The maximum allowable deviation from a target concentration ≤ 1.000 g/kg whole blood (1.236 g/L serum) is ± 0.05 g/kg (0.062 g/L) and from a target concentration > 1.000 g/kg whole blood (1.236 g/L serum) ± 5%.

If these deviations are exceeded, the cause must be ascertained and corrective measures implemented; if necessary, including repeated analyses of the entire test

series, including QC samples. In such a case, the report must contain only the results obtained after the repeated analysis.
Upon request, the measurement uncertainty can be determined according to the guidelines of the GTFCh.

7.2.2. Authentic samples

For each test sample, the spread between the individual results obtained with both methods must be checked as follows:
For average results ≤ 1.000 g/kg whole blood (1.236 g/L serum), the maximum allowable difference between the highest and lowest single value is 0.100 g/kg whole blood (0.124 g/L serum). For average results > 1.000 g/kg (1.236 g/L serum), a maximum spread of 10% is allowed.

8. External quality control

The laboratory must participate in at least four proficiency tests per year, which comply with all forensic aspects, including the forensically relevant measurement range of the analytical procedure. Proficiency test samples must be analysed in the same manner as the routine samples and in accordance with paragraph **5** and should therefore consist of a serum/plasma matrix. The laboratory must hold a valid proficiency certificate for measuring blood alcohol concentrations at any time.

9. Calculation and reporting

Alcohol concentrations must be determined in serum/plasma (where available). In the final report, however, the concentration must be given in whole blood. The concentration in whole blood is calculated based on the result in serum/plasma according to the distribution of water between serum/plasma and whole blood and thus using 1.20 as divisor. When calibrators with ethanol concentrations in mg/L or g/L are used, then the results must be converted to mg/g or g/kg using 1.03 as divisor (serum density). The combined divisor is therefore 1.236.
If neither serum nor plasma is available, whole blood must be used. In such a case, the density of whole blood must be taken into account. Therefore the final concentration, which must be reported in mg/g or g/kg, is calculated by applying the divisor 1.06. When using a diluted sample and a headspace method (at least fourfold dilution), no correction factor for the increased vapour pressure is required.

The calculated results for whole blood must always be given cut (not rounded) to two decimal points. The results of the four single determinations must be averaged and the result cut again to two decimal points.
All single results and their average must be reported as 'per mill' (g ethanol per kg blood).
Results falling below the level of the lowest calibrator (at which the concentration is ≥ the limit of quantification) must be reported accordingly.
In the final report, all variations/discrepancies must be stated and it must be affirmed, that the analysis was performed according to the valid guidelines and that the laboratory holds a valid certificate stating successful participation in a proficiency testing scheme at the time of the analysis.

10. Retention of records

All final reports, result protocols and quality control charts must be kept for six years.

11. Sample storage

The original sample container must be closed immediately after withdrawal of an aliquot for analysis. The addition of any substance or adulterant to the primary sample after arrival in the laboratory is prohibited. Blood samples must be immediately placed in a refrigerated store at ca. +4°C. For the purpose of re-quantification or analysis of congeners, serum/plasma should be separated from the remaining sample materials and stored at -15°C or below, subsequent to completion of the initial alcohol determination. This procedure, including sample labelling and documenting, must be performed in the presence of, and monitored by, a second person. The original and, where present, separated samples, must be stored for at least two years, as long as no judicial directive states otherwise.
The obligation to store samples is the responsibility of the head of laboratory.

12. Data protection

The client is the owner of the information. Relevant data protection measures must guarantee that no proprietary information is given to unauthorised third parties. This information not only includes the blood alcohol results but also the fact, that samples from a particular person have been received. All personnel must receive written instructions concerning data protection.

13. Quality management

The head of laboratory must guarantee that the analyses are performed according to these guidelines. In this context, the implementation of a quality management system, defined according to DIN EN ISO 17025 for forensic purposes, is mandatory.

14. Coming into effect

These guidelines are effective as of the day of publication in the Journal 'Blutalkohol'. Consequently, the September 2007 guidelines are no longer valid. Those determining blood alcohol concentrations for forensic purposes according to the previous valid guidelines may continue to do so for a maximum transition period of one year.

Only the original German version of these guidelines is applicable for forensic purposes.

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