

## Publishable Summary for 16RPT02 ALCOREF Certified forensic alcohol reference materials

### Overview

The European status report on road safety of the WHO Regional Office for Europe demands better enforcement of drink-driving legislation in several European countries. This project addressed that need by building up long term capacities for the production and certification of ethanol in water reference materials suitable for calibration of evidential breath alcohol analysers as defined by recommendation R 126 of the International Organisation of Legal Metrology (OIML). Certification included characterisation of the materials, assessment of homogeneity, stability, and uncertainty. Ethanol concentrations meet regional legal limits for alcohol control. Interlaboratory comparisons have been conducted within the EURAMET Technical Committee Metrology in Chemistry (TC-MC) to test the materials and capabilities developed.

### Need

The European Commission has estimated that about one quarter of road traffic deaths are due to alcohol. However, there are big differences regarding the number of victims with the Nordic countries having far lower death rates than the Baltic countries or Southern Europe. The EU has set itself a target of halving the number of people killed by road traffic accidents by 2020. The European status report on road safety therefore stated that, among other measures, better legislation and enforcement of alcohol control is needed in several countries. In particular, the report demands that unrestricted access to alcohol breath testing, using breath analysers of equivalent and agreed standard, should be implemented throughout Europe.

The OIML recommendation R 126 sets high standards for type approval and calibration of such breath analysers. It requires a test gas similar to human breath, with a defined concentration of alcohol. Such gases are created by bubbling air through an aqueous ethanol solution (wet bath simulator). Large volumes of ethanol in water solutions are necessary and the ethanol content should ideally be certified, i.e. traceable to the SI, accompanied by uncertainty, stability, and homogeneity data. There were only two European NMIs that produced such materials when the project started. Due to the different national approaches and the large volumes needed, it was necessary that more European countries were able to produce their own traceable certified ethanol in water reference materials (CRMs) in large quantities tailored for their regional needs. However, the metrological quality (homogeneity, stability, uncertainty, traceability) of the materials developed at different NMIs should be equivalent in so they meet the demands of the OIML.

### Objectives

The overall objective of the project was to develop/establish regional research and metrological capacity for the development of certified forensic alcohol reference materials for the law enforcement of drink-driving regulations.

The specific objectives of the project were:

1. To develop traceable measurement and production capabilities for certified ethanol in water reference materials at NMIs/DIs. Materials should be eligible for the calibration of breath analysers as defined by OIML R 126. Target values and measurement ranges to be covered should address legal limits of regional drink-driving legislation.
2. To enable NMIs/DIs to produce forensic alcohol reference materials following an appropriate quality system according to ISO Guide 30 to 35. This includes assessment of homogeneity, short- and long-term stability, and uncertainty, as well as appropriate documentation, drafting of certificates and certification reports. Certified values should be traceable to the SI. Measurement capabilities and reference materials developed should be at a metrological level high enough for entries into the BIPM CMC database.

3. To conduct EURAMET TC-MC intercomparisons to test the reference materials and measurement capabilities developed within the project.
4. For each emerging NMI/DI (BRML, CEM, GUM, IMBiH, DMDM, TUBITAK, FTMC, IAPR), to develop a country specific strategy for the long-term development of their measurement and production capabilities by specifying the collaborations with regional stakeholders such as calibration authorities, reference materials producers, standardisation and accreditation bodies, and manufacturers of breath analysers. NMIs/DIs should develop plans to offer services and products from the newly established capacities. Individual strategies should be discussed within the consortium and with other EURAMET NMIs/DIs, to ensure that a coordinated and optimised approach is developed.

### Progress beyond the state of the art

The project aimed to develop the capacity to regularly produce certified forensic alcohol in water reference materials at NMIs/DIs for breath analyser type approval and calibration (objective 1). State of the art analytical methods for purity assessment of ethanol (objective 1) and for the accurate quantification of ethanol in aqueous media (objective 2) have been implemented at institutes that had no access to these methods before. The project partners now maintain newly established or improved quality assurance systems according to ISO 17034 and ISO Guides 30 to 35 which allow the reproducible production of the certified materials. Standard operating procedures (SOPs), draft certificates and certification reports are further important outcomes of the project. Certified values are traceable to the SI and are produced via validated methods and reported with an uncertainty budget to fulfil the requirements of the OIML. Target certified values and volumes address end-users' needs regarding legal limits of regional drink-driving legislation and the instrumentation for breath analyser calibration available in different countries.

Intercomparisons organised at the end of the project demonstrated the metrological equivalence of the materials developed and the analytical methods established (objective 3). Results of the intercomparisons are suitable to submit calibration and measurement capability (CMC) claims or underpin existing CMC claims in the BIPM key comparison database KCDB. This creates a larger and more established network of ethanol reference materials and measurement capabilities.

Within the project, the emerging NMIs/DIs agreed individual strategies for the long-term exploitation of the newly established capabilities (objective 4). This leads to an improvement in the European infrastructure beyond the lifetime of the project. The reference materials developed are available to breath analyser producers, calibration laboratories and the police for breath analyser calibration, verification and type approval after conclusion of the project and, consequently, the law enforcement of drink-driving regulations benefits from traceably calibrated/verified instruments.

### Results

#### Development of traceable measurement and production capabilities for certified ethanol in water reference materials at NMIs/DIs

All partners had defined target concentrations, volumes, maximum uncertainties and other parameters for the preparation of their ethanol in water CRMs in cooperation with potential end-users in their countries. Training sessions on the gravimetric preparation of ethanol in water CRMs and on purity assessment of ethanol were conducted in conjunction with the kick-off meeting in October 2017 in Berlin.

Ethanol in water CRMs were prepared by spiking a defined mass of high purity ethanol into water. All partners developed and implemented metrologically sound weighing and bottling procedures suitable to produce the CRMs as required by end-users. Methods are documented as Standard Operation Procedures (SOPs) and incorporated into each partner's Quality System to ensure consistent performance over the long term. Furthermore, methods for accurate purity assessment of ethanol were developed and implemented since knowledge of the purity of the ethanol used for the preparation of the CRMs is essential to determine the certified value and its uncertainty and to establish traceability to the SI. Most partners apply a mass balance approach for purity assessment. Water as the main impurity is determined by Karl-Fischer titration and organic impurities are determined by chromatographic methods. The sum of all impurities is subtracted from 100 %. GUM employs a density method, and IAPR and TUBITAK developed quantitative nuclear magnetic resonance (qNMR) methods to directly assess the ethanol purity.

With these methods, altogether 43 ethanol in water CRMs were developed within the project. CRMs are now fit for their intended purpose. They cover a mass fraction range of 0.1 mg/g to 7 mg/g which is relevant for the implementation of OIML R126. An uncertainty budget according to the principles of the *Guide to the expression*

of uncertainty in measurement was established for all CRMs. Relative expanded uncertainties between 0.2 % and 4.3 % were achieved. The majority of values meet the target uncertainties of OIML recommendation R 126 (between 0.56 % and 4.4 % dependent on concentration). The CRMs have been assessed for homogeneity, short- and long-term stability.

Calibrated balances are a further prerequisite for traceability. Partner institutes which had no regular and documented calibration regime for their balances have established such a system in cooperation with their national calibration authorities.

Objective 1 of the project was achieved successfully.

#### Techniques to certify forensic alcohol reference materials

All project partners developed and implemented at least one analytical method for an accurate quantification of ethanol in water. Most institutes applied gas chromatography with flame ionisation (GC-FID) or mass spectrometry (GC-MS) detection. Different injection modes such as headspace, cool on-column or split/splitless injection were used. N-propanol and deuterated ethanol were employed as internal standards for calibration of GC-FID and GC-MS measurements. GUM developed two different approaches: oscillation-type density measurements with reference to the International Alcoholometric Tables OIML R22:1975 and a wet gas simulator ("bubble train") coupled to an evidential breath alcohol analyser.

Methods were validated and documented as Standard Operation Procedures (SOPs) in the Quality System of each institute to ensure reliable performance in the long term.

Partners used the newly established methods for the verification of the gravimetric value of the CRMs prepared under objective 1 and for homogeneity and stability assessment. The results demonstrated sufficient homogeneity for all candidate CRMs. Stability under transport conditions even at elevated temperatures (40 °C to 60 °C) could be confirmed for almost all candidate CRMs, and all candidate CRMs showed sufficient long-term stability when stored at room or refrigerator temperature. Shelf life periods between 6 and 12 months were derived from the study.

Certified values are traceable through balances calibrated with traceable to the SI mass standards and purity assessment by a primary method such as coulometric Karl-Fischer titration or any other method traceable to the SI via national standards (qNMR, density measurements).

Draft certificates and certification reports which summarise the homogeneity and stability data were prepared. With these documents the candidate reference materials are certified and ready for dissemination to customers and stakeholders.

Objective 2 of the project was achieved successfully.

#### EURAMET TC-MC intercomparison to test reference materials and measurement capabilities

Three interlaboratory comparisons were conducted to compare the reference materials and measurement capabilities developed in the project with each other and with capabilities of NMIs/DIs outside of the consortium: EURAMET.QM-S13 "Comparison of value assigned forensic alcohol in water reference materials", EURAMET.QM-S14 "Measurement capabilities for the quantification of ethanol in water" and a project internal intercomparison of methods applied for purity assessment of ethanol.

In total 23 CRMs from 14 NMIs/DIs were included in EURAMET.QM-S13. Certified values were in the range 0.1 mg/g to 8 mg/g. Eighteen materials were provided from project partners, the remaining materials were from NMIs of Argentina, the USA, South Africa and Australia. All CRMs were analysed at LNE by isotope dilution GC-MS under repeatable conditions. Measurement results were analysed by two statistical approaches: the frequentist approach at LNE and the Bayesian approach at NIST. These approaches deliver different reference functions between assigned and measured values. Results demonstrated that most of the materials are consistent with the Key Comparison Reference Function.

For EURAMET.QM-S14, samples were prepared by the coordinating laboratory (BAM) at two concentration levels that are relevant for the calibration of evidential breath analysers. Sixteen institutes from 15 countries participated in the intercomparison, besides the project partners NMIs from Kenya, Argentina, Uruguay, South Africa and Saudi Arabia.

Participants mostly applied GC-FID or GC-MS, NMISA South Africa used titrimetry and GUM employed a test bench for breath analyser calibration ("bubble train") and a density method. Participants did either in-house purity assessment of their commercial high-purity ethanol calibrants by Karl-Fischer titration, chromatographic methods, qNMR and/or density measurements; or they used ethanol/water CRMs from other NMIs/DIs for calibration. Results and options for the Key Comparison Reference Value (KCRV) were discussed at

EURAMET TC-MC and CCQM Organic Analysis Working Group. It was decided to use a Gaussian Random effects model (REM) with Hierarchical Bayesian (HB) solution consensus value as KCRV. This approach was considered appropriate since it accounts for the dark uncertainty (excess variance) amongst datasets, as well as the participants' reported uncertainties.

Using a Monte Carlo technique, absolute Degrees of Equivalence (DoEs) and their uncertainties at the 95 % level of confidence were determined along with the KCRV with the NIST Consensus Builder. DoEs confirm that the methods developed in the project are equivalent with each other and with those of other NMIs/DIs participating in the intercomparison. Project partners have demonstrated the capability to successfully assign ethanol mass fraction values in aqueous media from 0.1 mg/g to 8 mg/g with a 95 % level of confidence relative uncertainty of  $\pm 2$  % or less.

The EURAMET intercomparison reports will be published in Metrologia Technical Supplement.

Samples for the project internal purity intercomparison consisted of a batch of commercial high purity ( $\geq 99.8$  %) ethanol in 1 L plastic bottles. The batch was characterized and assessed for homogeneity and stability before distribution to study participants. Participants were requested to determine the mass fraction of ethanol in the sample. Identification and quantification of impurities was optional.

Most participants applied a mass balance approach. That means water content was quantified by Karl-Fischer titration, and the content of structurally similar impurities was quantified by GC-FID or GC-MS. The impurity mass fractions were then subtracted from 100 %. Results showed that water is by far the main impurity, organic impurities were negligible. TUBITAK and GUM determined the purity directly by qNMR and density measurements, respectively. The participants' results were in good agreement, although different approaches have been used. Overall, the methods are suitable to determine the purity of ethanol used for the preparation of certified ethanol in water reference materials for breath alcohol control. However, the associated uncertainties were quite different from one institute to the other and should be reconsidered.

Objective 3 of the project was achieved successfully.

#### *Development of a country specific strategy for the long-term development of their measurement and production capabilities*

The new ethanol in water CRMs and measurement capabilities were incorporated into the range of services of partner institutes. Country specific strategies for the long-term exploitation were developed and discussed at the final project meeting. These strategies include cooperation with regional stakeholders, CMC claims, papers and conference presentations, accreditation according to ISO 17034:2017 - *General requirements for the competence of reference material producers*, seminars and workshops for stakeholders and new national reference materials projects.

Objective 4 of the project was achieved successfully.

## **Impact**

A web article entitled "Blowing for more safety" was published as part of the Analytical Sciences section of the BAM Website to raise the awareness of the project.

<https://www.bam.de/Content/DE/Standardartikel/Themen/Analytical-Sciences/artikel-alcoref-pusten-fuer-mehr-sicherheit.html>. Furthermore, the project was presented to the scientific community by 12 oral conference presentation and 8 posters at 5 international and 9 national conferences. Among them were highly reputed conferences such as BERM International Symposium on Biological and Environmental Reference Materials or CIM International Metrology Congress. Two peer-reviewed articles were published in the Proceedings of the 19th International Congress of Metrology 2019. Furthermore, all consortium partners have introduced the project to stakeholder groups in their countries, as explained in the following section.

#### *Impact on industrial and other user communities*

The main stakeholder groups which directly benefit from the outcome of the project are the end-user of the CRMs developed - manufacturers and service providers of breath analysers, the national calibration/verification authorities, calibration laboratories, and the national police. The Output and Impact report lists 25 stakeholders from the industry, 10 from other public bodies like the national police or national calibration authorities and 4 NMIs/DIs outside the consortium. DMDM and GUM already produced and delivered ethanol in water CRMs to end-users. Among them were verification laboratories, industry enterprises,



the Bureau of Metrology of Montenegro and the Bureau of Metrology of North Macedonia. A metrological laboratory from Lithuania is a new customer of BAM CRMs since 2020.

Ethanol in water CRMs from BRML are regularly used for metrological verifications and legal control of breath analyzers at the INM Physico-chemical Group in Romania. CRMs from CEM are used for breath analysers verification at the CEM ethylometers laboratory since October 2020.

Furthermore, laboratories from Spain, the UK and Bosnia-Herzegovina expressed interest in CRMs developed by project partners.

#### *Impact on the metrology and scientific communities*

NMIs/DIs in the consortium directly benefit from the project by their newly established production and measurement capabilities since they enable them to provide new services. These new services are displayed in 10 new or improved CMC claims drafted during the runtime of the project. Three CMC claims are already published in the BIPM database, 7 claims are ready for submission after approval of the project's intercomparison reports by BIPM CCQM.

Expertise from the ALCOREF project facilitates new national CRM projects at CEM and GUM, and the participation of BRML in a new EMPIR project on bioethanol.

Ten training courses for consortium NMI/DIs on the gravimetric preparation of ethanol in water CRMs, on purity assessment of ethanol and on methods for the quantification of ethanol in water were organised. Researchers from the Bureau of Metrology BOM, North Macedonia, attended a training course conducted at the DMDM laboratory. Furthermore, the project provided advice to the Slovenian NMI and to an Argentine governmental metrology laboratory by exchange of data, papers and presentations.

Seven NMIs/DIs outside of the consortium participated in the project's intercomparisons, namely NIST (USA), NMIA (Australia), NMISA (South Africa), INTI (Argentina), LATU (Uruguay), KEBS (Kenya) and SASO-NMCC (Saudi Arabia). The intercomparisons allowed them to benchmark their capabilities against the ones developed in the project and vice versa.

#### *Impact on relevant standards*

The project actively supported knowledge exchange with key international and European metrology and legal committees. Project results were presented to BIPM CCQM Organic Analysis Working Group, to EURAMET TC-MC Committee for Bio- and Organic Analysis, to OIML TC17 Instruments for physico-chemical measurements, SC7 Breath testers, to ISS KS Z076 Committee for medical devices (Serbia), to BAS TC25 Health protection technology (Bosnia and Herzegovina) and to CLEN Customs Laboratories European Network. GUM, LNE and CEM provided input to the revision of OIML R126 Evidential breath analysers, planned for publication in 2021. LNE holds the secretariat of the committee for revision of OIML R126.

#### *Longer-term economic, social and environmental impacts*

The main impact of this project has been achieved through new certified forensic ethanol in water reference materials in countries where they were not available before. These materials are now ready for the type approval and regular calibration/verification of evidential breath alcohol analysers according to OIML regulations and national requirements. Law enforcement of drink driving regulations is supported by the new CRMs because breath alcohol determination is faster and less expensive than blood alcohol determination. It can be performed directly on the road without medical or laboratory facilities.

Furthermore, the project helped to reduce the gap between experienced and developing European NMI/DIs. It enabled new services for customers in the long term. The CRMs together with a wet gas simulator ("bubble train") operated at the NMI/DI represent the national measurement standard for breath alcohol analysis. Therefore, the project helps to maintain comparable and equivalent national standards in the field Europe-wide.

#### **List of publications**

1. M. Buzoianu, M. Radu, G. V. Ionescu: Recent progress in chemical measuring capabilities in INM as a result of EMRP/EMPIR Programme. Proceedings of the 19<sup>th</sup> International Congress of Metrology, 20004 (2019). <https://doi.org/10.1051/metrology/201920004>

2. B. Lalere, F. Gantois, R. Philipp, S. Vaslin-Reimann: Certified reference materials for breath alcohol control – the ALCOREF project. Proceedings of the 19<sup>th</sup> International Congress of Metrology, 15002 (2019). <https://doi.org/10.1051/metrology/201915002>

This list is also available here: <https://www.euramet.org/repository/research-publications-repository-link/>

Project start date and duration:		01 September 2017, 42 months
Coordinator: Rosemarie Philipp, BAM      Tel: +49 30 81045893      E-mail: rosemarie.philipp@bam.de		
Project website address: <a href="https://www.bam.de/Content/EN/Projects/Alcoref/alcoref.html">https://www.bam.de/Content/EN/Projects/Alcoref/alcoref.html</a>		
Internal Funded Partners:	External Funded Partners:	Unfunded Partners:
1. BAM, Germany	9. FTMC, Lithuania	
2. BRML, Romania	10. IAPR, Greece	
3. CEM, Spain	11. UW, Poland	
4. GUM, Poland		
5. IMBiH, Bosnia and Herzegovina		
6. LNE, France		
7. DMDM, Serbia		
8. TUBITAK, Turkey		
RMG02: IMBiH, Bosnia and Herzegovina (Employing organisation); BAM, Germany (Guestworking organisation)		