FINAL PUBLISHABLE REPORT

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Project short name	ALCOREF
Project full title	Certified forensic alcohol reference materials

Project start date and duration:		01 September 2017, 42 months
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Internal Funded Partners: 1. BAM, Germany 2. BRML, Romania 3. CEM, Spain 4. GUM, Poland 5. IMBiH, Bosnia and Herzegovina 6. LNE, France 7. DMDM, Serbia 8. TUBITAK, Turkey	External Funded Partners: 9. FTMC, Lithuania 10. IAPR, Greece 11. UW, Poland	Unfunded Partners:
RMG02: IMBiH, Bosnia and Herzegovina (Employing organisation); BAM, Germany (Guestworking organisation)		

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1 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.

2 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.

3 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.

4 Results

4.1. Objective 1: Development of traceable production capabilities for ethanol in water certified reference materials

Certified ethanol in water CRMs for calibration/verification of evidential breath alcohol analysers should meet requirements of OIML recommendation R126 as well as national requirements regarding legal limits and instrumentation in the different countries. The OIML defines the uncertainty of the ethanol mass concentration in wet calibration gas to be equal to or smaller than one third of the maximum permissible error of the breath analyser's measurement result, i.e. between 40 % at 0.05 mg/L ethanol and 5 % at 1.95 mg/L ethanol in gas. A target expanded relative uncertainty between 4.4 % at 0.13 g/kg and 0.56 % at 5.0 g/kg ethanol in water can be derived from OIML recommendation.

All consortium partners contacted potential end-users for their ethanol in water CRMs in order to identify their needs. Among these end-users were breath analysers producers, calibration laboratories, national police departments and departments of NMIs/DI which regularly verify breath alcohol analysers. Based on this survey and the OIML recommendation, target certified values, uncertainties and volumes for the CRMs were agreed.

Ethanol in water CRMs are 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 CRMs is essential to determine the certified value and it's 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 then subtracted from 100 %. GUM employs a density method, and IAPR and TUBITAK developed qNMR methods to directly assess the ethanol purity. Comparability of these methods was demonstrated in a project internal intercomparison (see section 4.3.4). LNE obtained a new CMC claim for purity assessment of high purity ethanol in Cycle XIX (Figure 3 in section 4.2) which is already published in the BIPM database.

An uncertainty budget according to the principles of the *Guide to the expression of uncertainty in measurement* (http://www.bipm.org/utils/common/documents/jcgm/JCGM_100_2008_E.pdf) for the preparation process was established at all partner institutes.

Altogether, 43 ethanol in water CRMs were developed in the project (Table 1). Figure 1 shows the CRMs of GUM and DMDM as examples.

Institute	CRM identifier	Certified value	Expanded uncertainty (95 % CI)	Volume [L]
BRML	INM MRC-ETH01	1025 mg/kg	13 mg/kg	0.25 - 1
CEM	CEM-1	0.385 9 g/L	0.001 0 g/L	2
	CEM-2	0.514 6 g/L	0.001 3 g/L	
	CEM-3	0.643 2 g/L	0.001 6 g/L	
	CEM-4	1.029 2 g/L	0.002 6 g/L	
	CEM-5	1.543 8 g/L	0.003 9 g/L	
	CEM-6	1.801 1 g/L	0.004 5 g/L	
	CEM-7	2.444 3 g/L	0.006 1 g/L	
	CEM-8	3.859 4 g/L	0.009 6 g/L	

DMDM	DMDM-E01	0 g/L	0.0001 g/L	1
	DMDM-E02	0.2573 g/L	0.0007 g/L	
	DMDM-E03	0.6432 g/L	0.0014 g/L	
	DMDM-E04	1.0292 g/L	0.0025 g/L	
	DMDM-E05	1.8011 g/L	0.0043 g/L	
	DMDM-E06	2.4443 g/L	0.0059 g/L	
	DMDM-E07	3.8594 g/L	0.0092 g/L	
	DMDM-E08	5.0172 g/L	0.012 g/L	
	DMDM-E09	1.2252 g/L	0.0030 g/L	
	DMDM-E10	0.6126 g/L	0.0015 g/L	
FTMC	FTMC-1	0.390 mg/g	0.004 mg/g	1
	FTMC-2	0.640 mg/g	0.006 mg/g	
	FTMC-3	1.030 mg/g	0.010 mg/g	
	FTMC-4	1.800 mg/g	0.020 mg/g	
	FTMC-5	3.860 mg/g	0.040 mg/g	
GUM	GUM 12.1	0.1294 g/kg	0.00052 g/kg	1
	GUM 12.2	0.2588 g/kg	0.00052 g/kg	
	GUM 12.3	0.6470 g/kg	0.0013 g/kg	
	GUM 12.4	1.0352 g/kg	0.0021 g/kg	
	GUM 12.5	1.8119 g/kg	0.0036 g/kg	
	GUM 12.6	2.4593 g/kg	0.0049 g/kg	
	GUM 12.7	3.8842 g/kg	0.0078 g/kg	
	GUM 12.8	5.051 g/kg	0.010 g/kg	
IAPR	HRM 03 01 E001	0.2096 g/kg	0.0089 g/kg	1
	HRM 03 01 F001	0.4219 g/kg	0.0101 g/kg	
	HRM 03 01 A001	0.5167 g/kg	0.0141 g/kg	
	HRM 03 01 C001	1.0317 g/kg	0.0159 g/kg	
	HRM 03 01 D001	2.0623 g/kg	0.0198 g/kg	
	HRM 03 01 B001	3.8164 g/kg	0.0302 g/kg	
IMBiH	IMBiH02	0.6072 mg/g	0.003 mg/g	1
	IMBiH03	1.0329 mg/g	0.004 mg/g	
	IMBiH04	1.7964 mg/g	0.007 mg/g	
TUBITAK	UME CRM 1320 Level-1	0.207 mg/g	0.002 mg/g	0.5
	UME CRM 1320 Level-2	7.225 mg/g	0.090 mg/g	

Table 1: Ethanol in water CRMs developed by project partners

Figure 1: Ethanol in water CRMs of GUM (left) and DMDM (right)

CRMs are 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. Basically, partners are able to prepare any other mass concentration/mass fraction within the institute's range of CRMs in the table. The majority of CRMs in Table 1 also meets the uncertainty requirements of OIML R126. 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 measurement). CEM, LNE, GUM, DMDM, FTMC and BRML have prepared CMC claims for their CRMs that are ready for submission to the BIPM database in the upcoming Cycle XXIII (Figure 3 in section 4.2).

CRMs were assessed for homogeneity, short- and long-term stability as described in section 4.2. They were furthermore tested in an intercomparison EURAMET.QM-S13 *"Comparison of value assigned forensic alcohol in water reference materials"* as described in section 4.3.2.

Objective 1 of the project was achieved.

4.2. Objective 2: Development of measurement capabilities for ethanol in water CRMs

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/splittless 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. Methods were then used for the characterisation of a batch of candidate ethanol in water CRMs and for the assessment of homogeneity and short- and long-term stability of the materials. Homogeneity and stability data of each institute were statistically analysed in-house, and again all datasets were analysed together by DMDM using PROlab software for validation. 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.

Furthermore, a researcher from IMBiH validated a GC-FID method for ethanol quantification during a two months Research Mobility Grant at BAM. She also performed homogeneity, short- and long-term stability assessment of IMBiH candidate reference materials at BAM. The general conclusion was that produced reference material can be safely dispatched under conditions where the temperature does not exceed 50 °C and can be stored at room temperature up to five months.

All partners have drafted certificates and certification reports that contain homogeneity and stability data for their ethanol in water CRMs. Certificates of CEM and IAPR (EXHM) are displayed in Figure 2 as examples.

MINISTERIO DE INDUSTRIA, COMERCIO Y TURISMO

CERTIFICADO Nº 202474001

CEM CENTRO ESPAÑOL DE METROLOGÍA

CERTIFICADO DE CALIBRACIÓN
Certificate of Calibration

Expedido a: *Issued to* — Ministerio de Industria, Comercio y Turismo; Centro Español de Metrología; C/ del Alfar Nº2; 28760 Tres Cantos - Madrid

Instrumento: *Instrument* — MRC de Etanol en agua

Especificaciones: *Features* — Botella de 2 L

Fabricante: *Manufacturer* — Laboratorio de Química

Marca/Modelo: *Trademark/Type* — MRCs/CEM-01 (0,150 mg/L)

Nº Serie/Código CEM: *Serial number/CEM code* — 01.23112020.001

Fecha fin de calibración: *End of calibration date* — 23/11/2020

Este Certificado no refleja el grado otras características que las indicadas por los datos aquí contenidos. Los resultados se refieren al momento y condiciones en que se efectuaron las mediciones. No garantiza la trazabilidad al SI. (Véase https://www.cem.es/servicios/certificados-cem).
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ISO 14001

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El documento consta de un total de 3 folios. Folio 1 de 3 - Código Seguro de Verificación: 74266 07467282

EXHM

CHEMICAL METROLOGY LABORATORY (EXHM/GCSL-EIM)
GENERAL CHEMICAL STATE LABORATORY - HELLENIC METROLOGY INSTITUTE
CHEMICAL METROLOGY SERVICE, 16 TSOCHA STREET, 115 21 ATHENS, GREECE
tel: +30 210 6479136-8 fax:+30 210 6479114 email: metrology@gcsl.gr

EIM

CERTIFICATE OF ANALYSIS

HRM® - 03 01 A001

ethanol in aqueous matrix		
Measurand	Certified value [1]	Uncertainty [2]
	g in kg	
ethanol	0.5167	± 0.0103

1) The material is gravimetrically certified. Ethanol mass fraction in the reference material has been confirmed by GC-IT/MS. The certified value is traceable to the SI.

2) Estimated expanded uncertainty *U* with a coverage factor of $k = 2$, corresponding to a level of confidence of about 95%, as defined in the Guide to the expression of uncertainty in measurement JCGM 100: 2008. Uncertainty contributions arising from characterisation as well as from homogeneity and stability testing have been taken into account.

The certified properties will be valid for 6 months beginning from the dispatch of the material from EXHM, this validity may be extended as further evidence of stability becomes available.
The minimum sample intake is 10 mL.

NOTE
Hellenic Reference Material HRM®-03 01 A001 was produced and certified under the responsibility of Chemical Metrology Service (IAPR/EXHM/GCSL-EIM) according to the principles laid down in the technical guidelines outlined in the Quality Manual, in accordance with the requirements of ISO 17034.

It is accepted as an HRM®, Athens, 2019-10-15

Athens, 2019-10-15
GCSL
Chemical Metrology Service

(Head of Service)

HRM GENERAL STATE CHEMICAL LABORATORY
CHEMICAL METROLOGY SERVICE - FORM TITLE: HRM Certificate

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FORM ENT 27 01 7.14 02(E1)(22)/30.06.2016

Figure 2: Certificates (cover pages) of ethanol in water CRMs of CEM and EXHM

Furthermore, the newly developed methods were tested in an intercomparison EURAMET.QM-S14 *"Measurement capabilities for the quantification of ethanol in water"* organised by the project (see section 4.3.3). Based on the intercomparisons, altogether 10 new or improved CMC claims were prepared by project partners during the lifetime of the project (Figure 3). Claims of TUBITAK UME, EXHM/GCSL-EIM and LNE already passed the RMO and inter-regional review process and are published in the BIPM database. All other claims are ready for submission in the upcoming cycle XXIII in December 2021. CMC claims reflect the new CRMs and measurement capabilities developed in the project and the new services available in the institutes.

Country	NMI or Designated Service Provider	NMI Service Identifier	Matrix	Measurand		Dissemination Range of Measurement Capability			Range of Expanded Uncertainties as Disseminated						Range of Certified Values in Reference Materials			Range of Expanded Uncertainties for Certified Value						Mechanism(s) for Measurement Service Delivery
				Analyte or Component	Quantity	From	To	Unit	From	To	Unit	Cov. factor	Lev. of confid.	Is the expanded uncertainty a relative one?	From	To	Unit	From	To	Unit	Cov. factor	Lev. of confid.	Is the expanded uncertainty a relative one?	
TR	UME	G3OK-7300	water	ethanol	mass fraction	0,5	5	mg/g	1,0	2,0	%	2	95 %	relative										calibration
GR	EXHM/GCSL-EIM	EXHM 3.4 01	water	ethanol	mass fraction	0,5	5	mg/g	2,4	3	%	2	95%	relative										Provision of reference value for client materials, PT samples and reference materials, Calibration
ES	CEM	MRCe	water	ethanol	mass fraction	0.1	8	mg/g	0.25	0.25	%	2	95%	relative	0.1	8	mg/g	0.25	0.25	%	2	95%	relative	CRMs, MRCe
FR	LNE	CMI-37-300-20	water	ethanol	mass fraction	0,1	8	g/kg	2	3	%	2	95 %	relative	0,13	7,7	g/kg	0,0037	0,12	g/kg	2	95%	absolute	calibration and provision of CRM
PL	GUM	12.1 to 12.8	aqueous solution	ethanol	mass fraction	0,12	5,01	g/kg	0,5	0,5	%	2	95%	relative	0,12	5,01	g/kg	0,4	0,2	%	2	95%	relative	Calibration Service; provision of CRMs
RS	DMDM		aqueous solution	ethanol	mass concentration	0,2	8	mg/g	0,3	0,5	%	2	95%	relative	0,25	5,02	g/L	0,0007	0,012	g/L	2	95%	absolute	
LT	FTMC	CM-M7.2-05.1 to CM-M7.2-05.5	aqueous solution	ethanol	mass fraction	0,20	4,0	mg/g	0,5	1	%	2	95%	relative	0,35	3,90	mg/g	0,5	1	%	2	95%	relative	provision of CRMs
BA	NMI - IMBIH		water	ethanol	mass fraction	0,1	8	mg/g	1	2,5	%	2	95%	relative										reference measurement service
RO	BRML-INM	ETH01	single component organic solution (ethanol)	ethanol	mass fraction	0,6	4	mg/g	1.2	1.3	%	2	95%	relative	1,028	1,029	g/kg	0,007	0,007	g/kg	2	95%	absolute	CRM, calibration service
FR	LNE	CMI-37-300-30	high purity ethanol	ethanol	mass fraction	980.0	999.98	mg/g	0.01	2.0	mg/g	2	95%	absolute										calibration

Figure 3: CMC claims for measurement capabilities and CRMs developed in the project.

Objective 2 of the project was achieved.

4.3. Objective 3: Intercomparisons to test the CRMs and measurement capabilities

Three interlaboratory comparisons were conducted to test the reference materials and measurement capabilities developed in the project: 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 high purity ethanol. Results of these intercomparisons are presented below.

4.3.1 EURAMET.QM-S13 *"Comparison of value assigned forensic alcohol in water reference materials"*

The aim of this intercomparison was to directly compare the CRMs developed in the project with each other and with similar CRMs of NMIs/DIs outside of the consortium. Participants submitted their CRMs together with a certificate to the coordinating laboratory LNE, where they were analysed under repeatable conditions.

In total 23 CRMs from 14 NMIs/DIs were included in the intercomparison (Table 2). Certified values were in the range 0.1 mg/g to 8 mg/g. Eighteen materials were provided from project partners.

NMI	**Code**	**Material**	**Assigned value and uncertainty**		**Certification Method**
			V_i	$U_{95}(V_i)$	
BAM	E	BAM-K003	0.611 2 g/kg	0.000 6 g/kg	Gravimetric Preparation
	K	BAM-K001	1.03 13 g/kg	0.001 g/kg	Gravimetric Preparation
CEM	L	CEM-04-120919-004	1.031 4 mg/g	0.001 5 mg/g	Gravimetric Preparation
DMDM	M	DMDM-E04	1.031 3 mg/g	0.002 5 mg/g	Gravimetric Preparation
	T	DMDM-E07	3.869 3 mg/g	0.009 2 mg/g	Gravimetric Preparation
EXHM	D	HRM 0301A002	0.517 g/kg	0.014 1 g/kg	Gravimetric Preparation

	Q	HRM 0301B003	3.816 g/kg	0.030 2 g/kg	Gravimetric Preparation
FTMC	B	LTF-104-190627	0.388 mg/g	0.025 mg/g	Gravimetric Preparation
	R	LTF-504-190627	3.85 mg/g	0.25 mg/g	Gravimetric Preparation
GUM	F	GUM 12.3	0.6470 g/kg	0.001 3 g/kg	Gravimetric Preparation + Density measurement
	N	GUM 12.4	1.0352 g/kg	0.002 1 g/kg	
IMBiH	H	IMBiH03	1.0329 mg/g	0.004 1 mg/g	Gravimetric Preparation
	P	IMBiH04	1.7964 mg/g	0.007 2 mg/g	
INM	I	INM RM-ETOH1	102.9 mg/100g	0.70 mg/100g	Gravimetric Preparation + GC/FID
INTI	V	INTI-MRC003-E003	0.503 %	0.005 %	Gravimetric Preparation + chromatography
LNE	J	LNE-050919-1	1.0292 g/L	0.011 g/L	Volumetric Preparation
	S	LNE-050919-2	3.8594 g/L	0.059 g/L	
NMIA	O	ETOH 18-02	1.23 g/kg	0.020 g/kg	GC/MS
NMISA	C	ORG-001 Batch 0495/19	50.12 mg/100g	0.65 mg/100 g	Gravimetric Preparation + Titrimetry
	U	ORG-001 Batch 0496/19	502.1 mg/100g	5.0 mg/100 g	
NIST	G	SRM 2893a	0.07663 %	0.000 97 %	GC/FID + GC/MS
TUBITAK UME	A	UME-1	0.207 mg/g	0.002mg/g	Gravimetric Preparation
	W	UME-2	7.225 mg/g	0.090mg/g	

Table 2. Materials with assigned values and auxiliary information

All CRMs were analysed at LNE by isotope dilution GC-MS according to a design that was applied for a previous CCQM intercomparison CCQM-K79 *"Comparison of value-assigned CRMs and PT materials: Ethanol in aqueous matrix"* (Duewer, D. L., Gasca-Aragon, H., Lippa, K. A. et Toman, B. Experimental design and data evaluation considerations for comparisons of reference materials. Accred Qual Assur 2012; 17:567–588. DOI 10.1007/s00769-012-0920-4). The measurement design is displayed in Figure 4.

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. As an example, Figures 5 and 6 show the Key Comparison Reference Function (KCRF) obtained with the frequentist approach.

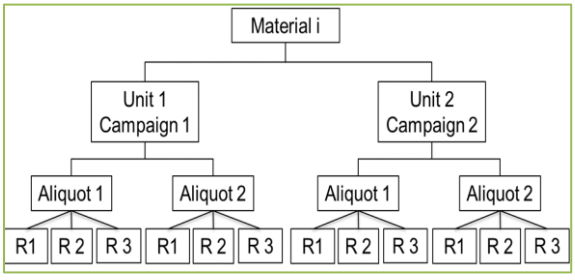

Figure 4. Repeatability measurement design for EURAMET.QM-S13, N_c = 2 number of measurement campaigns, N_a = 2 number of aliquots per unit, N_r = 3 number of replicates per aliquot.

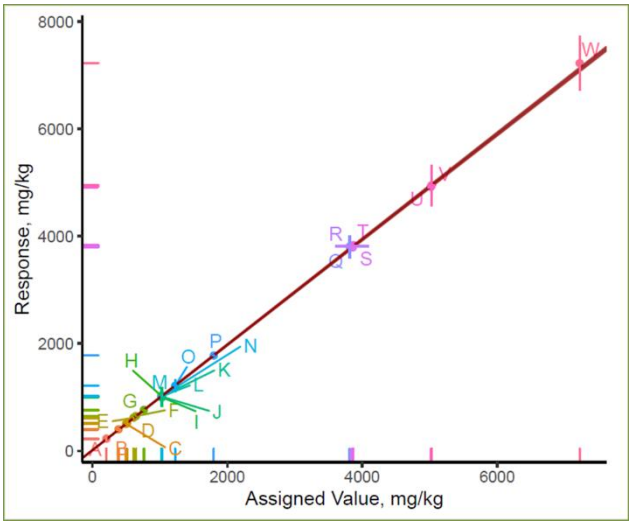

Figure 5: Overview of the relationship between assigned and measured values**.** See Table 2 for the association between the code and the material**.** The red lines represent the KCRF and its 95% confidence region**.**

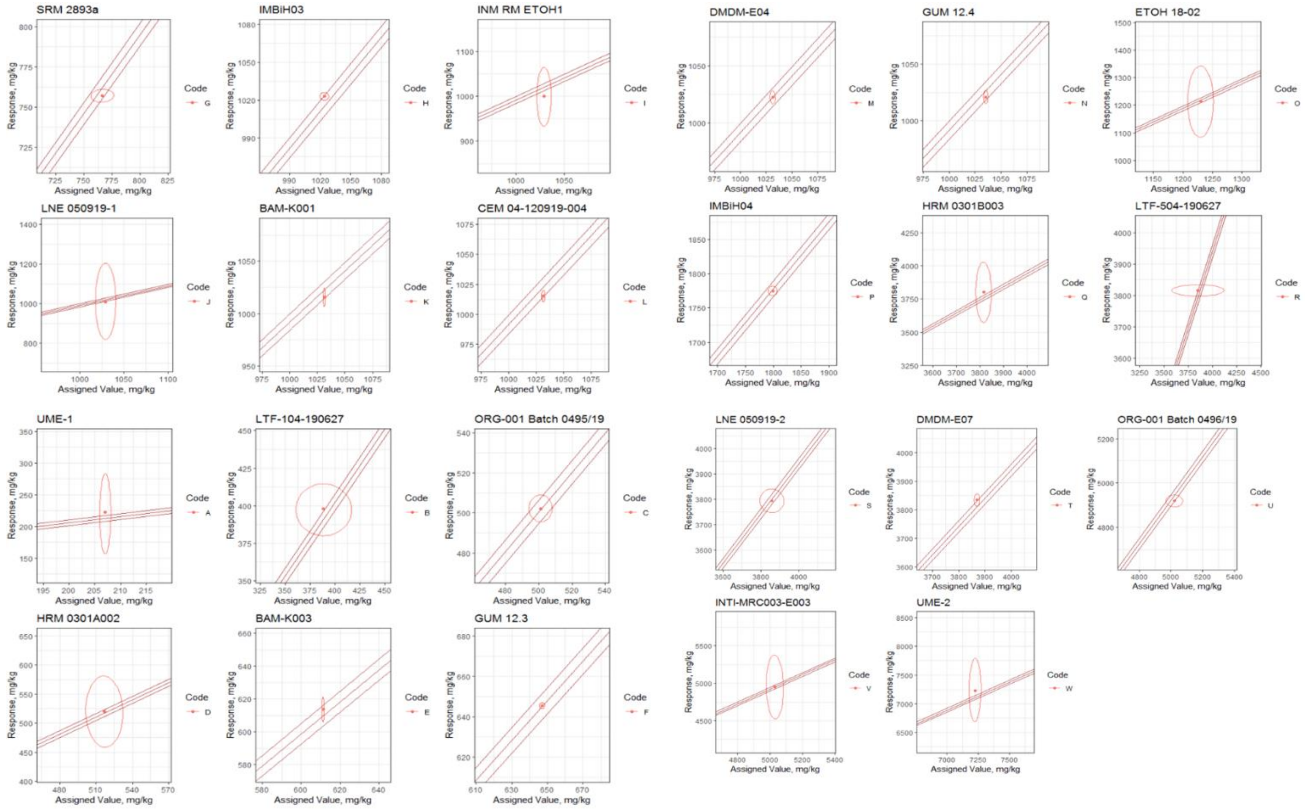

Figure 6. High-resolution display of the KCRF and its 95 % confidence region (red lines).

Figure 6 demonstrates that the 23 materials are consistent with the KCRF, all the ellipses enter the KCRF ± U_{95}(KCRF) interval but some do not overlap the KCRF line.

Furthermore, the influence on the KCRF by materials having small uncertainties was investigated by a Leave-one-out cross validation. Degrees of Equivalence for the materials and for the institutes were determined with both the frequentist and the Bayesian approach. These data show that the majority of the NMIs 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 ± 2 % or less, regardless of the method of value assignment.

The intercomparison report will be published in Metrologia Technical Supplement.

4.3.2 EURAMET.QM-S14 *"Measurement capabilities for the quantification of ethanol in water"*

The aim of this intercomparison was to directly compare the measurement capabilities developed in the project with each other and with capabilities of NMIs/DIs outside of the consortium.

Samples were prepared by the coordinating laboratory (BAM) at two concentration levels that are relevant for the calibration of evidential breath analysers: Level 1: (0.6112 ± 0.0010) g/kg; Level 2: (1.8239 ± 0.0029) g/kg. Samples were assessed for homogeneity, short- and long-term stability before distribution.

Sixteen institutes from 15 countries participated in the intercomparison. Participants mostly applied GC-FID or GC-MS, NMISA 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 NMIs/DIs for calibration (Table 3). GUM and NMISA submitted two sets

of data obtained with different methods. They declared one of their data sets as key comparison result, the other one was treated as a pilot study result. Results of the University of Warsaw (UW) were also considered as pilot study data since UW is not an NMI or DI. Figure 7 shows results reported for Level 1.

Institute	Analytical method	Calibrants
BAM, Germany	GC-FID, on-column injection	Commercial ethanol, in-house purity assessed by mass balance
CEM, Spain	GC-FID	Commercial ethanol, in-house purity assessed by mass balance
DMDM, Republic of Serbia	Headspace GC-FID	CRMs from NIST, LGC, BAM
EXHM/GCSL-EIM, Greece	GC-MS, GC-FID	CRM from NIST, commercial ethanol in-house purity assessed by mass balance and qNMR
FTMC, Lithuania	GC-FID	CRMs from BAM
GUM, Poland	„bubble train“ + breath analyser, density	Commercial ethanol, in-house purity assessed by density measurement
IMBiH, Bosnia-Herzegovina	GC-FID, on column injection	CRM from BAM
INM, Romania	GC-FID	CRM from LGC
INTI, Argentina	GC-FID	Commercial ethanol, in-house purity assessed by mass balance
KEBS, Kenya	GC-FID	CRM from NIST
LATU, Uruguay	GC-FID, on column injection	CRM from CENAM
LNE, France	Isotope dilution GC-MS	Commercial ethanol, in-house purity assessed by mass balance
NMISA, South Africa	Headspace GC-FID	CRM from NMISA for GC, CRM from NIST and NMIJ for titrimetry
SASO-NMCC, Kingdom of Saudi Arabia	Headspace GC-FID	CRM from NIST
TUBITAK UME, Turkey	Headspace GC-FID	Commercial ethanol, in-house purity assessed by qNMR
UW, Poland	GC-MS	CRM from GUM

Table 3: Participants in EURAMET.QM-S14, analytical methods and calibrants

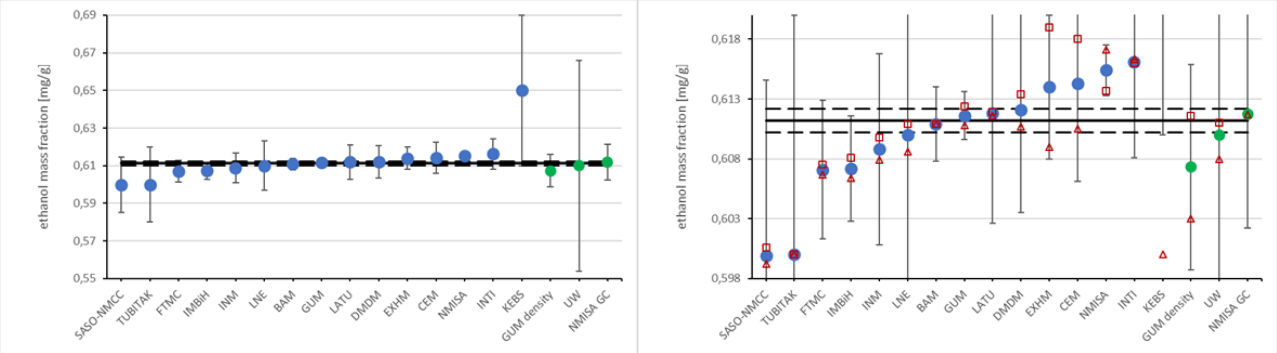

Figure 7: Results for Level 1; ● Key comparison, ● Pilot study, △□ individual results for bottle 1 and bottle 2; error bars: expanded uncertainties (95 % CI); solid and dashed lines: gravimetric value ± expanded uncertainty (95 % CI)

Results agree very well with the gravimetric value. They do not display a bias of one or the other method.

Results and options for the Key Comparison Reference Value (KCRV) were discussed at EURAMET TC-MC SCBOA and CCQM OAWG. 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. Results of KEBS were identified as technical outliers and therefore not included in the calculation of the KCRV.

The absolute Degrees of Equivalence (DoEs) for the participants were estimated as difference between the measured value and the HB consensus KCRV. Using a Monte Carlo technique, the DoEs and their uncertainties at the 95 % level of confidence were determined along with the KCRV with the NIST Consensus

Builder version 1.2 (https://consensus.nist.gov/app/nicob). Figure 8 displays the DoEs and their 95 % confidence interval.

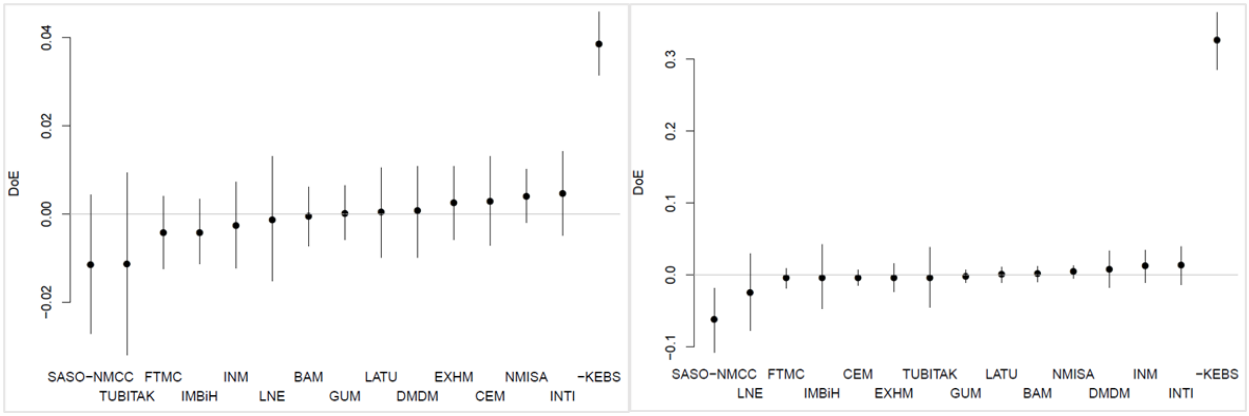

Figure 8: DoEs (in mg/g) and their expanded uncertainties at 95 % confidence for Level 1 (left), and Level 2 (right). Values of KEBS excluded from KCRV estimation.

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. All project partners have successfully quantified the ethanol in both samples.

The intercomparison report will be published in Metrologia Technical Supplement.

4.3.3 Project internal intercomparison for purity assessment of ethanol

Ethanol in water CRMs are prepared by spiking a defined mass of commercial high purity ethanol into water. Knowledge about the purity of the ethanol is essential to determine the certified value and its uncertainty and to establish traceability of the certified value to the SI. Therefore, a project internal intercomparison of methods for purity assessment was conducted to test and compare the capabilities of project partners in this field.

Intercomparison samples consisted of a batch of commercial high purity (≥ 99.8 %) ethanol in 1 L plastic bottles. The batch was characterized and assessed for homogeneity and stability by LNE 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 show that water is by far the main impurity, organic impurities are negligible. TUBITAK and GUM determined the purity directly by qNMR and density measurements, respectively. EXHM also reported a direct qNMR result additionally to a mass balance result. Figures 9 and 10 display the ethanol mass fraction and the water content reported.

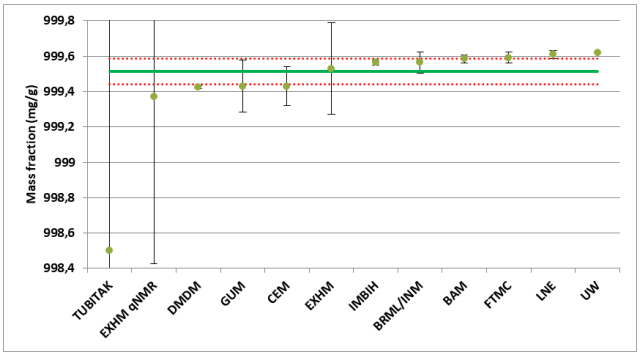

Figure 9: Participants' results for the ethanol mass fraction. green solid line: robust mean A15, red dotted lines 95 % confidence interval of the robust mean. Error bars: expanded uncertainties 95 % confidence interval.

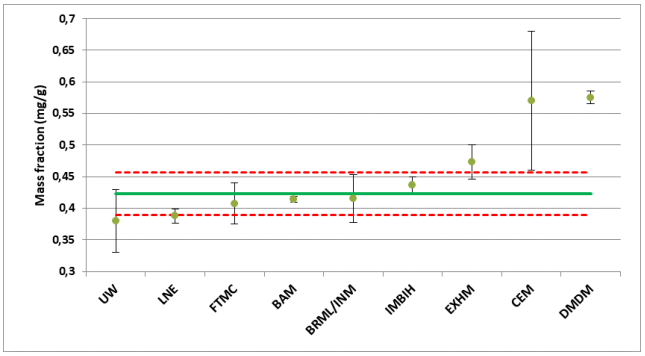

Figure 10: Participants' results for the water content in pure ethanol. green solid line: robust mean A15, red dashed lines 95 % confidence interval of the robust mean. Error bars: expanded uncertainties 95 % confidence interval.

A robust statistical evaluation of results was performed by EXHM. The robust mean A15 was selected as the best estimator for the Key Comparison Reference Value (KCRV). Fig. 11 shows the Degrees of Equivalence (DoEs) and their 95 % confidence interval calculated with this KCRV for the ethanol mass fraction.

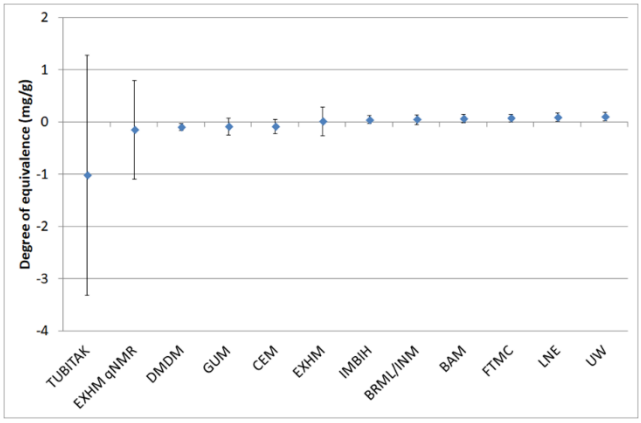

Figure 11: DoEs for ethanol purity determination. Error bars represent expanded uncertainties (95 %confidence interval)

The participants' results are in good agreement, although different approaches (e.g. Karl-Fischer titration, qNMR, densimetry measurements) 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 are quite different from one institute to the other and should be reconsidered.

Altogether, the outcome of the three intercomparisons demonstrates that the methods and materials developed in the project are comparable. Methods are suitable for the production of ethanol in water certified reference materials. Objective 3 of the project was achieved.

4.4 Objective 4: Individual strategies for the long-term operation and development of measurement and production capacities

The main achievements of the project are new ethanol in water CRMs for the calibration/verification of evidential breath alcohol analysers and new or improved measurement capabilities for the quantification of ethanol in water. Furthermore, project partners have gathered expertise and know-how about the general process of certification of reference materials according to ISO Guides 30 to 35 and ISO 17034:2017. With Objective 4 emerging NMIs/DIs in the project should establish plans for the long-term exploitation of these new capacities.

As a first step towards an individual strategy for exploitation, BRML, CEM, GUM, IMBiH, DMDM, TUBITAK, FTMC and IAPR have contacted breath analysers producers, national maintenance and calibration laboratories, national police departments and other potential stakeholders and end-users to discuss their needs. The Output and Impact report currently lists 41 stakeholders from the industry, public bodies and other NMIs/DIs outside the ALCOREF consortium. In some cases (BRML, CEM), the main end-user of the CRMs is a department of the institute itself which provides metrological verification of evidential breath analysers for customers. Based on the survey, key parameters for the CRMs such as target certified values, uncertainties and volumes were defined. CRMs are now ready for use. CMC claims prepared by all institutes will help to promote the CRMs and measurement capabilities. Some more institute specific planned activities for exploitation are given below.

BRML

- Certification of at least one more CRM of nominal mass fraction 4 g/kg by December 2021
- Further development of an isotope dilution GC method for ethanol in water quantification with improved uncertainties; submission of a publication of results by December 2022
- Expansion of the capabilities developed in the project to bioethanol; Development of CRMs and GC-MS and GC-FID measurement capabilities for bioethanol in the EMPIR project “New metrological methods for biofuel materials” BIOFMET (2020-2023)
- Intensified cooperation with other suppliers of calibration services for breath analysers operating in Romania (after approval of CMC)
- Cooperation with accredited laboratories for forensic testing in Romania (after approval of CMC)

CEM

- Continuous production of ethanol in water CRMs for the verification of evidential breath analysers for customers in the CEM ethylometers laboratory
- With the experience of the ALCOREF project, a new national CRM project called SACAREF is being developed. SACAREF has two objectives: 1. Development of reference materials for sucrose in water necessary for the metrological control of instruments designed to measure the sugar content of grape must, concentrated must and rectified concentrated must; 2. Development of certified sucrose in water reference materials for the calibration of refractometers
- Practical training courses for students in chemical metrology
- 2 articles for publication in the scientific literature: 1. Desarrollo de Materiales de Referencia Certificados para el control metrológico de los etilómetros evidenciales (Development of Certified Reference Materials for metrological control of evidential analyzers); 2. Assessment of the purity of ethanol used in certified reference materials
- Presentation at the 7th Spanish Metrology Congress
- Disclosure talk at CEM about the ALCOREF project

GUM

- Ethanol/water CRMs are offered as a new permanent service at GUM website and GUM catalogue of CRMs. https://www.gum.gov.pl/pl/uslugi/katalog-crm/3611,Katalog-CRM.html
- Preparation of ethanol/water CRMs for proficiency testing schemes if requested by customers
- Equipment (dry box, bottling stand) and software developed in the project will continue to be routinely used for the preparation of CRMs and calibration standards for breath analyzers.
- Paper in an international peer-reviewed journal (e.g. AQUAL), to inform the international community about the new GUM capabilities of CRMs production
- Presentation at an upcoming GUM seminar; stakeholders will be invited.
- Training courses on breath analyser calibration for calibration laboratories
- Further input to revision of OIML R 126 „Evidential Breath Analysers“; further participation in OIML TC 17/SC 7: Breath testers
- Expertise from the ALCOREF project will facilitate new CRM projects at GUM e.g. for elemental multi-component standard solutions and multi-parameter liquid certified reference materials of physicochemical properties

IMBiH

- Implementation of ISO 17034:2017 - General requirements for the competence of reference material producers

DMDM

- Full implementation and presentation of ISO 17034 quality system at next EURAMET TC-Q meeting

- Stakeholders' meeting/workshop for current and potential users of CRMs
- Presentation to Z076 Committee for Medical Devices at the ISS (Institute for Standardization of Serbia)
- Continuous optimization of the production process (e.g. replacement of glass by plastic bottles, as often requested by customers)
- Paper on CRM stability testing at DMDM
- Presentation of project results at conferences and workshops in Serbia and in the region
- Development of new CRMs based on stakeholders' needs

TUBITAK

- Contact with the national regulatory actors in the field to introduce the concept to the national legislation and promote the use of breath wet gas simulators instead of dry gas applications
- Set up a laboratory infrastructure for measurement of alcohol content by wet gas simulators to calibration/verification or type approval of alcoholmeters within TUBITAK premises
- Preparation and application for ISO 17034 accreditation on this CRM production
- Trainings, workshops or meetings with stakeholders for dissemination and sustainability of project results

FTMC

- Implementation of ISO 17034:2017
- Calibration and verification of evidential breath alcohol analysers at FTMC

IAPR

- IAPR performed a **SWOT** analysis to identify **s**trengths, **w**eaknesses, **o**pportunities and **t**hreats in the field of metrology for organic chemistry. As a result, a 5 years roadmap was developed that will be evaluated every 2 years. Provision of CRMs like the ethanol/water CRMs and provision of measurement services is part of this roadmap.

Strategies for exploitation of project results were presented and discussed at the final ALCOREF meeting. Consortium partners agreed to cooperate further beyond the end of the project by e.g. bilateral comparisons or exchange of (candidate) CRMs. UW will continue to support GUM with organic chemical analysis. Partners will also continue to work together in metrological and standards bodies such as OIML TC 17/SC 7: Breath testers, EURAMET-TC-MC or BIPM OAWG.

Objective 4 of the project was achieved.

5 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.

6 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 19th 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 19th 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/

7 Contact details

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