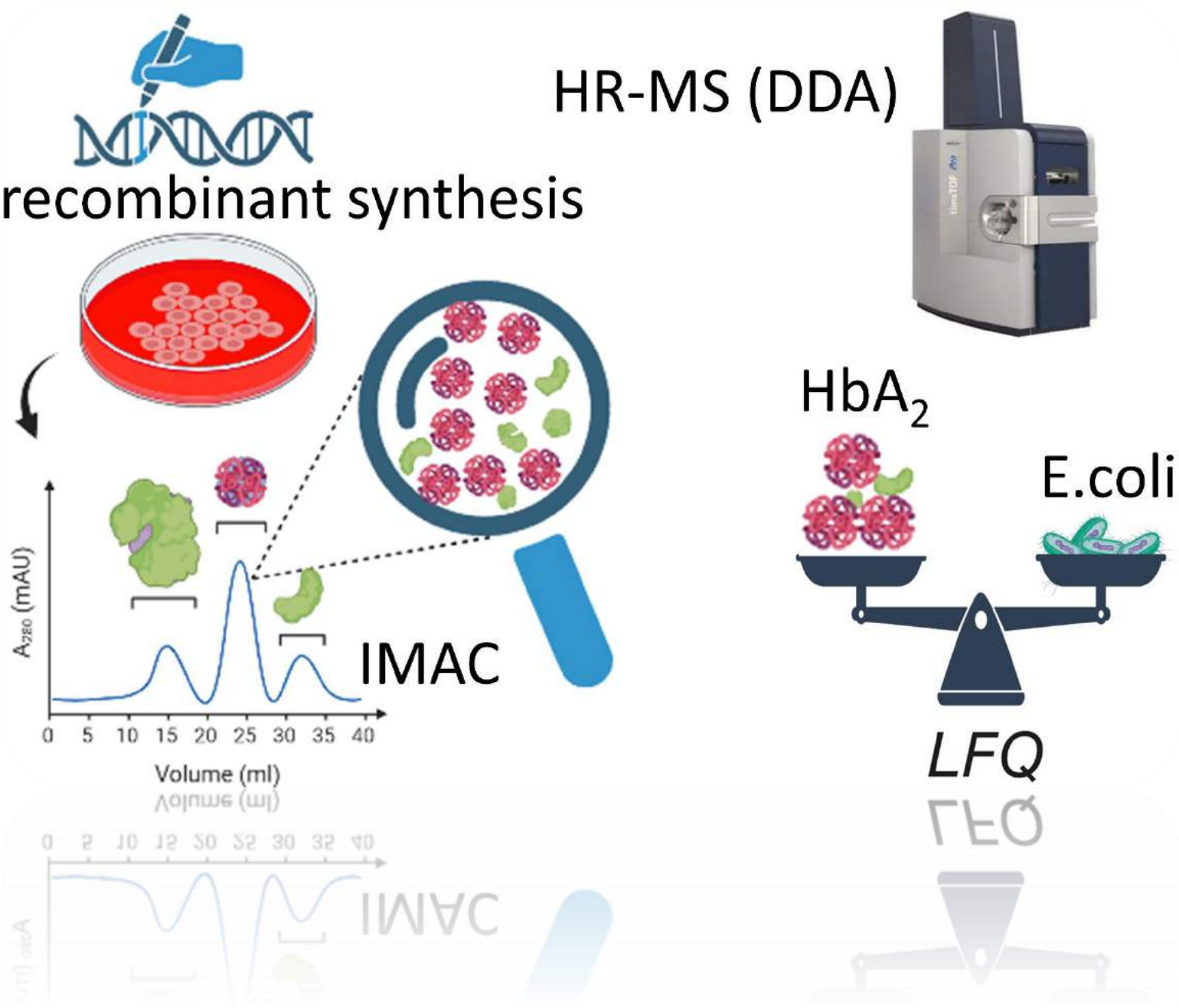
CITAC
Cooperation on International Traceability in Analytical Chemistry

CITAC NEWS

2024



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Chair: Prof. Zoltan Mester
National Research Council (NRC), Canada
E-mail: Zoltan.mester@nrc.ca

CITAC News Editor: Dr Tang Lin Teo
Health Sciences Authority, Singapore
E-mail: Teo_Tang_Lin@hsa.gov.sg

The cover page picture is provided courtesy of authors of CITAC best paper titled "Label Free Quantification of Host Cell Protein Impurity in Recombinant Hemoglobin Materials". Special thanks to André Henrion, Cristian Gabriel Arsene, Maik Liebl and Gavin O'Connor.

FOREWORD BY THE CHAIR

CITAC ACTIVITY IN 2023

Zoltan Mester // National Research Council (NRC), Canada

Dear friends,

CITAC is an international organization with members from all continents aiming to improve the worldwide comparability of chemical measurements through collaboration. We are doing this by observing the principles of mutual respect, peace, basic human rights, dignity and worth of every person as the foundation of our work.

In 2023, our Chair, Prof Bernd Güttler from the Physikalisch-Technische Bundesanstalt (PTB) has completed his three years term, serving through the exceptionally difficult times of COVID and the breakout of war in Ukraine. Bernd has obtained his university and PhD degree from Leibnitz University Hannover, followed by research appointments at the University of Cambridge. In 1990 he joined PTB as senior researcher in solid-state chemistry. In 2002, he become the first head of the Department of Metrology in Chemistry then the head of the Division of Chemical Physics and Explosion Protection in 2015. For twenty some years he represented PTB at the Consultative Committee for Amount of Substance: Metrology in Chemistry and Biology (CCQM) were he led the Working Group on the Mole providing significant contribution to the redefinition efforts and drafting the Mise en pratique for the unit mole in the SI. He served the wider metrology community as the Chair of the Technical Committee for Metrology in Chemistry TC-MC of EURAMET between 2007-2011 and as the Chair of CITAC ending his term in May 2023.

On behalf of CITAC I would like to thank Bernd's exceptional leadership and dedication to our organization and in general to the advancement in metrology in chemistry. I am very much looking forward continuing working with you, Bern for years to come!

In 2023, we are also fortunate to have Dr. Tang Lin Teo from the Health Sciences Authority (HSA) in Singapore, appointed to the CITAC Executive Committee for the first time. She is a recipient of a fellowship from Singapore's Agency for Science, Technology and Research for her postgraduate studies, and earned her Ph.D. in Chemistry from the National University of Singapore in 2007. Dr. Teo currently serves as the Division Director of the Chemical Metrology Division at HSA. Notably, she has been elected as the next Chair of the Technical Committee for Amount of Substance under the Asia Pacific Metrology Programme (APMP), set to commence her 3-year term after the 40th APMP General Assembly at the end of 2024.

I had the pleasure for working with Tang Lin for many years on various metrology related projects and I am delighted to welcome her to the committee!

Digital transformation has remained the key theme of the year in metrology. In early 2022,

standardization organizations banded together and issued a Joint Statement of intent on the digital transformation of the international scientific and quality infrastructure. The statement provides a platform for the signatories for the development, implementation, and promotion of the International System of Units (SI) Digital Framework as part of a wider digital transformation of the international scientific and quality infrastructure.

(https://www.bipm.org/en/liaison/digital-transformation)

In March 2023, the International Committee for Weights and Measures (CIPM) decided to establish a Forum on Metrology and Digitalization to develop the SI Digital Framework. This new Forum was intended to provide a platform for discussion between National Metrology institutes, BIPM/CIPM Consultative Committees and stakeholder organizations in the international science and quality infrastructure. The kick of meeting of the Forum was held in October 2023. (bipm-ws/forum-dig/2023-11-23 - BIPM)

In association with the SciDataCon 2023, BIPM held a Webinar on 'Digital References for Metrology' discussing among other issues digital identifiers for chemicals. The recordings of the meeting could be accessed from: bipm-ws/bipm-ws-dig-ref/2023-10-12 - BIPM.

Check out InChi at IUPAC (https://iupac.org/who-we-are/divisions/division-details/inchi/)

Another significant digital transformation effort relevant to the chemical measurement science community is the IUPAC Gold Book project which aims to bring all currently analog, paper based, IUPAC recommendations and definitions into the digital era, following FAIR data principles of being findable, accessible, interoperable, reusable. Learn more about the IUPAC Gold Book and the current renewal process @ Defining Chemical Concepts - The IUPAC Gold Book - History, Updates and Semantics - YouTube (https://www.youtube.com/watch?v=uLkONO3MLOU)

One key goal of CITAC is the building of chemical measurement science as distinct field of scientific enquiry. We do this by celebrating outstanding contributions to our field. The CITAC Best Paper in Metrology Award in 2023 was given to :

Abneesh Srivastava and Joseph T. Hodges, Primary Measurement of Gaseous Elemental Mercury Concentration with a Dynamic Range of Six Decades, Analytical Chemistry 94 (2022) 15818-15826
https://pubs.acs.org/doi/abs/10.1021/acs.analchem.2c03622

Mengyun Pan et al. Absolute Quantification of Total Hemoglobin in Whole Blood by High-Performance Liquid Chromatography Isotope Dilution Inductively Coupled Plasma-Mass Spectrometry, Analytical Chemistry 94 (2022) 11753-11759
https://pubs.acs.org/doi/abs/10.1021/acs.analchem.2c01324

A. Briones et al., An SI-traceable reference material for virus-like particles, iScience 25 (2022) 104294
https://doi.org/10.1016/j.isci.2022.104294

Award Ceremony and Lectures of the CITAC Best Paper held on June 27, 2023. https://youtu.be/eXKu1UXB7nI?si=hquhf3GXSmew8FUE

Congratulations to the winners of 2023 and I would like to thank all the CITAC members for supporting the nomination and selections process.

I hope to see all of you, in person, in 2025!

Zoltan Mester
CITAC Chair

ADDRESS OF THE VICE-CHAIR

HOW DO GOOD METROLOGY PRACTICES GET TO YOUR PLATE?

Ricardo Bettencourt da Silva // University of Lisbon, Portugal

Metrology institutes, together with Metrology Institutes Networks and following BIPM references and guidelines, contribute to the development of measurement capabilities and manage the use of the International System of units in specific countries. For some measurement capabilities, these institutes even contribute directly to routine measurements by calibrating balances, thermometers, flowmeters, etc. Routine measurements are, for instance, the ones regularly performed before delivering products to their end-user and to check environmental and individual health.

However, many other relevant measurements are performed using some measurement capabilities from the International System of Units, such as the determination of the mass of the tested portion, but depending on major effects far beyond the technical competence of metrology institutes. Some examples of these effects on measurement in chemistry are the impact of tested item matrix in the determinations. In some of these measurement fields, laboratories have developed reference materials and proficiency tests mimicking the practices of metrology institutes to manage the measurements fitness of their use. In measurement fields with more relevant socio-economic impact, measurement methods are standardised, where this harmonisation can be extended to how measurement procedures are validated, routine analysis controlled, results reported and decisions on tested items taken. The guidelines are set, balancing the reliability of determinations and the cost of analysis, by experts of the analytical field with rare contributions from the end-users of the analytical result. Analytical protocols are typically developed from lively negotiations between experts more concerned with the risk of bad measurements and those more focused on analysis cost. In some fields, this equilibrium for sure would be more balanced towards results reliability if participated by the end users of the analysis results directly affected by bad measurements.

Accreditation bodies have developed procedures for assessing if the technical state of the art is being correctly applied in routine measurements in a structure where additional quality assurance requirements must be fulfilled. The ISO/IEC 17025 accreditation standard defines the internationally accepted Quality Assurance

Requirements that guarantee that the technical competence is applied consistently, impartially and results adequately reported, considering client needs.

However, the management of the technicalities of the analysis is beyond the direct competence of accreditation bodies that rely on expert's and laboratory's interpretation of how analytical service should be delivered. The divergent opinions of the stakeholders should be communicated and discussed by independent experts following procedures set and supervised by the accreditation bodies.

In addition to each sector's technical requirements, accreditation bodies define general technical requirements such as the need to train laboratory staff properly, calibrate relevant equipment, validate measurement procedures and evaluate the measurement uncertainty. These requirements must be fulfilled in all accredited measurement capabilities considering the scientific state of the art. Some general requirements are set specifically to ensure good metrological practices in measurement areas beyond the competent of metrology institutes.

In fields where there are no standardised measurement procedures or methodologies to manage the validity of test results, the assessment of technical competence is more difficult, requiring more energy in discussing divergent positions about the fulfilment of the general accreditation requirements.

Accreditation requirements are the ones distilled directly from the accreditation standard and their interpretation from the International Laboratory Accreditation Cooperation (ILAC), regional cooperation bodies and accreditation bodies. In cases where no higher-level documents indicate how to manage a specific competence requirement, national accreditation requirements are set. One of those examples is the need to report the measurement uncertainty from all accredited tests set by some accreditation bodies and not followed by others.

The way relevant routine measurements are managed is difficult to improve because it must rely on both technical and economic constraints. However, for sure some analytical fields would benefit from informed inputs from the end-users of test reports to improve the quality and objectivity of results reporting and interpretation. The same applies to the implementation of some accreditation requirements made explicit in the latest edition of the ISO/IEC 17025 standard such as the need to report measurement results with uncertainty and of quantifying the risk of false conformity decision.

Let's hope the analytical community reminds that we are all direct customers of accredited tests and good metrological practices are not a luxury.

Ricardo Bettencourt da Silva
CITAC Vice-chair

MESSAGE FROM THE CITAC SECRETARY

METROLOGY AND EXAMINOLOGY APPLIED IN PHARMACEUTICAL INDUSTRY

Felipe Rebello Lourenço // University of São Paulo, Brazil

Metrology and Examinology play fundamental roles in ensuring the quality and reliability of results obtained in chemical laboratories, especially in pharmaceutical industrial contexts. The trueness and precision of measurements are crucial to ensure the quality, efficacy, and safety of pharmaceutical products, as well as to comply with the rigorous regulatory standards established by competent authorities.

Metrology, the science that studies measurements, is essential for establishing reference standards, performing calibrations, and ensuring the traceability of measurements carried out in laboratories. In a pharmaceutical environment, where small variations in substance quantities can have significant impacts on the efficacy and safety of medications, the reliability of measurements is imperative. Metrology provides the methods and instruments necessary to perform reliable measurements of mass, volume, concentration, purity, and other chemical properties essential for the formulation and production of medications.

Examinology, on the other hand, is the science dedicated to the study of qualitative analyses, playing an essential role in the identification and characterization of substances in chemical samples. Using a variety of techniques and analytical methods, such as chromatography, spectroscopy, and specific chemical tests, Examinology allows for the determination of the properties and composition of substances present in a sample, providing valuable information about their nature and quality. Examinology is crucial in a wide range of fields, including forensic chemistry, the pharmaceutical industry, and quality control of chemical products, contributing to ensuring the safety and efficacy of medicines.

The importance of the reliability of quantitative and qualitative results in pharmaceutical industrial applications cannot be underestimated.

Errors in measurements can lead to failures in pharmaceutical manufacturing and quality control, resulting in ineffective or unsafe medicines for patients. Furthermore, non-compliance with regulatory standards can result in severe legal sanctions, damaging the reputation and credibility of pharmaceutical companies.

To ensure the reliability of results, pharmaceutical laboratories must adopt good practices in metrology and examinology, including regular calibration of measuring equipment, validation of analytical methods, and participation in external quality control programs. The implementation of quality management systems, such as ISO 9001 and ISO/IEC 17025 standards, also plays a fundamental role in standardizing processes and continuously improving quality.

Additionally, the qualification and training of professionals working in laboratories are essential to ensure technical competence and result integrity. Analysts must be familiar with the analytical techniques used, as well as with the regulatory requirements applicable to the pharmaceutical sector. Continuing education and training in good laboratory practices are indispensable for maintaining the highest standards of quality, efficacy, and safety.

Another important aspect to consider is the implementation of robust and secure data management systems, ensuring the integrity and traceability of analytical results. In a regulated environment such as the pharmaceutical industry, it is essential to maintain detailed records of all activities carried out in laboratories, from sample reception to the generation of analysis reports.

Therefore, metrology and examinology play critical roles in ensuring the quality, efficacy, and safety of pharmaceutical products. The reliability of quantitative and qualitative results is essential to meet regulatory requirements, protect patient health, and maintain the integrity of companies in the sector. Investing in good practices in metrology and examinology is fundamental to the success and sustainability of pharmaceutical operations.

Felipe Rebello Lourenço
CITAC Secretary

EDITORIAL

WHY METROLOGICAL TRACEABILITY IS BECOMING EVEN MORE IMPORTANT IN THE AGE OF BIG DATA

Tang Lin Teo // Health Sciences Authority, Singapore

Despite the advancements in data science, effectively translating big data into actionable outcomes requires careful consideration. This is primarily due to the significant challenges stemming from the inherent uncertainties in big data, including inconsistencies, incompleteness, accuracy, and reliability. These uncertainties are commonly acknowledged as issues of 'data quality' or the fourth dimension of big data, known as 'veracity,' which encompasses inconsistencies, biases, noise, and ambiguities within the data.

The substantial generation of data by clinical laboratories daily contributes to big data in healthcare, offering the potential to uncover patterns and translate them into actionable knowledge for precision medicine and decision-makers. Metrological traceability is crucial for ensuring the veracity and reliability of big data in healthcare. Whenever possible, traceability to internationally recognized standards and reference materials is essential for maintaining the quality and reliability of healthcare data, particularly in the overwhelming context of big data. Traceable measurements facilitate interoperability between different medical devices.

The Directive 98/79/EC of the European Parliament and of the Council of 27 October 1998 on in vitro diagnostic medical devices mandated manufacturers to provide traceability information, demonstrating that assays were anchored to internationally accepted reference materials and/or reference methods. Clinical laboratories rely on calibrators supplied by in-vitro diagnostic manufacturers to calibrate medical devices. Despite performing calibration, devices can still experience drift over time due to

wear and tear. Additionally, studies have indicated that errors primarily arise from pre- and post-analytical measurements.

External quality assessment (EQA) programmes offer a means of evaluating the performance of a laboratory, albeit as a snapshot in time. It reflects the quality of pre-analytical, analytical and post-analytical performances. When utilising EQA data to derive deeper insights, the performance of a clinical laboratory should be taken into account.

For example, the HSA Chemical Metrology Laboratory had leveraged data for lipid clinical markers from clinical laboratories participating in its regular EQA programmes across 5 years (1). LDL-C values were calculated using the conventional Friedewald equation and alternative Martin/Hopkins and Sampson equations, then compared against reference values determined by CML's beta-quantitation-isotope dilution mass spectrometric (BQ-IDMS) method with traceability to the International System of Units (SI). The finding led to the conclusion that the alternative Martin/Hopkins equation is more reliable at higher triglyceride levels, also supports the increasing use of non-fasting lipid tests, facilitating early detection and medical intervention. The conclusion cannot be drawn had there been no metrologically traceable assigned values to ensure confidence on the 5-years' data from the clinical laboratories.

In conclusion, metrological traceability plays a critical role in ensuring the veracity of big data in healthcare, ultimately contributing to improved patient outcomes and healthcare delivery.

(1) Tan HT, Yong S, Liu H, Liu Q, Teo TL, Sethi SK. Evaluation of low-density lipoprotein cholesterol equations by cross-platform assessment of accuracy-based EQA data against SI-traceable reference value. Clin Chem Lab Med. 2023 Apr 4;61(10):1808-1819. doi: 10.1515/cclm-2022-1301. PMID: 37013650.

Tang Lin Teo
Editor

JOINT PUBLICATIONS FROM CITAC

IUPAC/CITAC GUIDE: EVALUATION OF RISKS OF FALSE DECISIONS IN CONFORMITY ASSESSMENT OF A SUBSTANCE OR MATERIAL WITH A MASS BALANCE CONSTRAINT (IUPAC TECHNICAL REPORT)

Francesca R. Pennecchi // Istituto Nazionale di Ricerca Metrologica (INRiM), Italy; Ilya Kuselman // Independent Consultant on Metrology, Israel; and D. Brynn Hibbert // University of New South Wales, Australia

Francesca R. Pennecchi

Ilya Kuselman

D. Brynn Hibbert

A methodology for evaluation of risks of false decisions in conformity assessment of a substance or material with a mass balance constraint was developed as part of the IUPAC project, https://iupac.org/project/2019-012-1-500/, by the task group consisted of Angelique Botha, NMISA, South Africa; Aglaia di Rocco, UniTO, Italy; Francesca R. Pennecchi, INRiM, Italy; and D. Brynn Hibbert, UNSW, Sydney, Australia; chaired by Ilya Kuselman, Independent Consultant on Metrology, Israel. The work was supported by CITAC. The corresponding IUPAC/CITAC Guide has been published by Francesca R. Pennecchi, Ilya Kuselman and D. Brynn Hibbert in the official IUPAC journal Pure and Applied Chemistry, vol. 95, 2023, https://doi.org/10.1515/pac-2022-0801; and at the CITAC website, https://www.citac.cc/guides/.

Risks expressed as probabilities of false decisions on conformity of chemical composition of a multicomponent material or object due to measurement uncertainty were defined earlier using a Bayesian approach in the previous IUPAC/CITAC Guide on the topic: I. Kuselman, F. R. Pennecchi, R. J. N. B. da Silva, D. B. Hibbert. Pure and Applied Chemistry, vol. 93, 2021, https://doi.org/10.1515/pac-2019-0906.

According to Bayes' theorem, a prior knowledge of the measurand values (actual contents of components in the items) and new information acquired during the measurement (modelled by a likelihood function) are combined in a posterior probability density function (pdf). Such posterior pdf, containing all the available information, is then used for the evaluation of risks. The probability of accepting the item after comparing a measured value with the acceptance limit of a content of an item component, when it should have been rejected, is called the "consumer's risk," whereas the probability of falsely rejecting the item is the "producer's risk". This Guide, in order to take into account the multivariate nature of the

materials or objects under conformity assessment, provides models and methods for calculation of total global risks of a false decision on conformity of their chemical composition (as a multivariate integral of the corresponding joint pdf of the actual/true and measured property values) and total specific risks (as a multivariate integral of the posterior density function of property values on the multivariate specification domain of the item compositions). Such approach, however, is not able to cope with cases when component contents of a substance or material are subject to a mass balance constraint (sum of the actual contents – mass fractions or amount fractions – is 1 or 100 %). Measured values of these kinds of component contents are called "compositional data". These data are intrinsically correlated because of the constraint, and the relevant correlation was named by Karl Pearson in 1897 as "spurious".

There is a strong message in the literature stressing how traditional statistical techniques may produce inadequate results if applied to raw compositional data without suitable transformation. Compositional Data Analysis (CoDA) based on an isometric logratio transformation of the original measured values was developed in the 1980s by John Aitchison. Mathematical aspects of CoDA and its applications in environmental analysis, geology and other fields have been intensively developed during the past 40 years. However, CoDA applies the constraint directly to the measured values, not considering their associated measurement uncertainties. The present Guide is intended for study of the risks caused by measurement uncertainties in conformity assessment of a multicomponent substance or material with a mass balance constraint. The document is intended for quality control, measurement and testing chemical analytical laboratories, metrologists and analytical chemists, specialists involved in the laboratory accreditation activity, laboratory customers, quality managers, and regulators.

When *n* actual component contents c_i of a substance or material are subject to a mass balance constraint ($\sum_{i=1}^{n} c_i = 1$ or 100 %), measured values c_{im} of the component contents are "compositional data", that may be depicted in a multidimensional simplex, as in Fig. 1, in which, in general, Euclidean geometry cannot be blindly applied. For the model $c_1 + c_2 = 100\,\%$, the correlation between the two components is exactly equal to -1. For the model $c_1 + c_2 + c_3 = 100\,\%$, the first component can be derived from the measurement of the other two, i.e., $c_1 = 100\,\% - c_2 - c_3$, and the corresponding analytical correlation coefficients are $r_{1,2} = -s_2^2/(s_2^2 + s_3^2)$ and $r_{1,3} = -s_3^2/(s_2^2 + s_3^2)$, respectively, where s_i is the standard deviation of the *i*th component. Note that a mass balance constraint is applicable to actual component contents c_i, while the sum of measured values c_{im} cannot in general be exactly equal to 1 (or 100 %) or another constant because of measurement uncertainties u_{im} associated with the measured values.

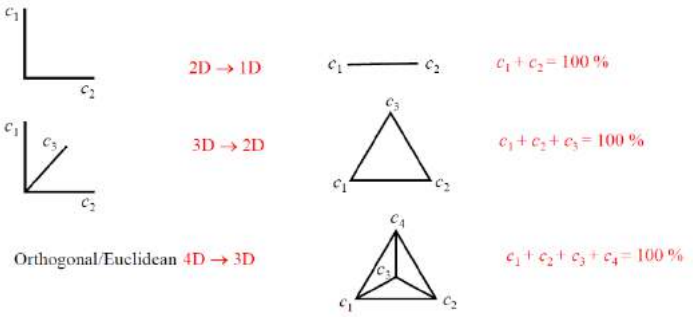

Fig.1: Orthogonal coordinates of Euclidean space and simplices. The variables c_i (%) are contents of a material components *i* = 1, 2, 3, and 4; each simplex vertex corresponds to c_i = 100 %; D is dimension. In each case, mass balance constraint reduces the number of dimensions by one.

The vector of actual ("true") component contents $\boldsymbol{c} = [c_1, c_2, \ldots, c_n]$ describes the *n*-component (*n*-part) composition of a material or substance and lies within the compositional space, i.e., the simplex

$$S^n = \left\{ \boldsymbol{c} = [c_1, c_2, \ldots, c_n] \mid c_i > 0, i = 1, 2, \ldots, n, \sum_{i=1}^{n} c_i = k \right\}$$

where *k* is usually equal to 1 (100 %). As c_i are positive quantity ratios, a vector of content values $\boldsymbol{c}$ multiplied by any positive constant contains the same information as the original one, i.e., represents the same composition and can be considered as an equivalence class. This property is termed "scale invariance." In other words, if $\boldsymbol{c}$ is scaled by a constant, e.g., content values c_i change from parts-per-unit to percentages, the information which $\boldsymbol{c}$ conveys is completely equivalent. Therefore, it is natural to select a representative of the equivalence class to facilitate data analysis and interpretation of

corresponding results. This selection is formalized by the closure operation:

$$clo(\boldsymbol{c}) = \left[\frac{kc_1}{\sum_{i=1}^{n} c_i}, \ldots, \frac{kc_n}{\sum_{i=1}^{n} c_i}\right]$$

As vector $\boldsymbol{c}$ represents the actual n-component composition of a substance or material, component contents c_i, are the measurands in conformity assessment, and $\boldsymbol{c}$ is the vector of the measurands. Using a multivariate Bayesian approach, knowledge about a composition $\boldsymbol{c}$ can be modelled by a random multivariate posterior variable and expressed in terms of its probability density function (pdf). Such a pdf combines prior knowledge about the measurands and new information acquired during the measurements:

$$g(\boldsymbol{c}|\boldsymbol{c}_m) = C\ g_0(\boldsymbol{c})\ h(\boldsymbol{c}_m|\boldsymbol{c})$$

where $g(\boldsymbol{c}|\boldsymbol{c}_m)$ is the multivariate posterior pdf; C is a normalizing constant; $g_0(\boldsymbol{c})$ is the multivariate prior pdf; and $h(\boldsymbol{c}_m|\boldsymbol{c})$ is the multivariate likelihood function taking into account the measurement uncertainties and possible covariance terms.

The total global risks of a false decision on the material conformity is evaluated according to the following formulae, for the consumer and the producer, respectively:

$$R_{\mathrm{c}} = \int_{T^{\mathrm{c}}} \int_{A} g_0(\boldsymbol{c})\ h(\boldsymbol{c}_m|\boldsymbol{c})\, d\boldsymbol{c}_m d\boldsymbol{c}$$

$$R_{\mathrm{p}} = \int_{T} \int_{A^{\mathrm{c}}} g_0(\boldsymbol{c})\ h(\boldsymbol{c}_m|\boldsymbol{c})\, d\boldsymbol{c}_m d\boldsymbol{c}$$

where, T is the multivariate tolerance/specification domain $T_1 \times T_2 \times \ldots \times T_n$; A is the multivariate acceptance domain $A_1 \times A_2 \times \ldots \times A_n$; the integral symbols indicate multiple integrals; superscript "c" of T in the formula for R_c means "complementary" for at least one T_i, whereas the integration with respect to all c_{im} is performed within A; the superscript "c" of A in the formula for R_p means "complementary" for at least one A_i, whereas the integration with respect to all c_i is performed within T.

The total specific risks for the consumer and the producer are calculated, respectively, by:

$$R_{\mathrm{c}}^{*} = 1 - \int_{T} g(\boldsymbol{c}|\boldsymbol{c}_m)\ d\boldsymbol{c}$$

when $\boldsymbol{c}_m$ is in A

$$R_{\mathrm{p}}^{*} = \int_{T_1} \ldots \int_{T_v} \int_{0}^{100} \ldots \int_{0}^{100} g(\boldsymbol{c}|\boldsymbol{c}_m)\ d\boldsymbol{c}$$

when c_{im} are outside A, for $1 \le i \le v$

where R_{c}^{*} is the probability that at least one of corresponding actual content values c_i is actually outside its tolerance interval, when all the measured content values c_{im} are within their acceptance intervals (false conforming); symbol v in the equation for R_{p}^{*} indicates the number of those components whose measured content values c_{im} are outside their acceptance intervals.

PRIOR PDF

A large enough dataset of results of tested items of the same material produced at the same factory, as well as results of monitoring the same environmental compartment, can be used for approximation of the prior pdf. The assumption is that the actual concentration values are approximated by the test/measurement results adequately, since measurement uncertainty is negligible in comparison with item-to-item (batch-to-batch) variations caused by changes of conditions of the material production, environmental conditions, etc. According to the principle of maximum entropy, a multivariate normal distribution is usually considered as the prior pdf for the vector of actual component contents $\boldsymbol{c}$ when the vector of mean values $\boldsymbol{\mu}$ and covariance matrix $\boldsymbol{V}$ constitute the only available information about the vector quantity. In a case of a mass balance constraint, the data properties need to be taken into account when assigning a corresponding pdf. Since the vector $\boldsymbol{c}$ is non-negative, and the first two moments of the distribution (the expected value and variance) are known, the maximum entropy distribution is a truncated multivariate normal distribution TMN($\boldsymbol{\mu}$,$\boldsymbol{V}$) on the nD region $[0, k]^n$.

Three scenarios of modeling the prior distribution are considered in the Guide (more details are available in the document):

1) Modeling all the actual values of the components' contents by applying the closure operation $clo(\boldsymbol{c})$ to $\boldsymbol{c}$ which follow a TMN($\boldsymbol{\mu}$,$\boldsymbol{V}$);
2) Modeling actual values of n-1 components' contents by a TMN($\boldsymbol{\mu}$,$\boldsymbol{V}$) on a (n-1)D region and then deriving the nth as 100 % minus the sum of the other components' values;

3) Sequentially modelling one component at a time by a (univariate) truncated normal distribution, whose domain of definition depends on the previously modelled component c_i value.

LIKELIHOOD FUNCTION

The likelihood is a function describing the plausibility of the actual values of a component concentration at a given measurement result. In practice, a distribution of measured values at a given actual concentration $\boldsymbol{c}$ of a multicomponent material or object, caused by measurement uncertainty, is available from the analytical method validation data. The modeling of the likelihood function for measured content values $\boldsymbol{c}_m$ is based on the idea that an appropriate pdf with zero expectation is chosen for an error vector $\boldsymbol{e}_m$ and then translated to the vector of actual ("true") content values $\boldsymbol{c}$ generated for the prior. Therefore, vector $\boldsymbol{c}_m$ is recovered as $\boldsymbol{c}_m = \boldsymbol{c} + \boldsymbol{e}_m$. The covariance matrix $\boldsymbol{U}$ associated with $\boldsymbol{c}_m$ contains the squared measurement uncertainties u_i^2 and the covariance terms u_{ij} whose corresponding correlation coefficients are the same as for $\boldsymbol{V}$. The modeling of the likelihood for a multicomponent composition follows that of the prior (see the bullet list above).

POSTERIOR PDF

The posterior distribution is the normalized product of the prior and the likelihood, according to expression $g(\boldsymbol{c}|\boldsymbol{c}_m) = C\, g_0(\boldsymbol{c})\, h(\boldsymbol{c}_m|\boldsymbol{c})$. Since the posterior pdf predicts further actual content values (after accumulation of the dataset used for modeling the prior pdf) taking into account what may happen during the measurement process *via* the likelihood function, the closure operation is not appropriate for the posterior data. In other words, the sum of the calculated (predicted) actual component content values may differ from 100 % because any predicted value has its associated prediction uncertainty.

COMPUTATIONAL DETAILS

To evaluate the total global risks in the case of variables related to a mass balance, prior pdf, and likelihood function are modeled according to the relevant sections, then, a large number of MC simulations of values drawn from the joint pdf $g_0(\boldsymbol{c})\, h(\boldsymbol{c}_m|\boldsymbol{c})$ are performed. The three models for the prior and likelihood give three ways to obtain the joint pdf. The R function "rtmvnorm" is applied in the first and the second model, and the function "rtnorm" in the third model. The total global producer's risk is evaluated considering the fraction of simulated vectors in which all the actual values are within the corresponding tolerance region T, while at least one of the measured values is out of its acceptance interval A_i. The total global consumer's risk is estimated as the fraction of vectors in which all the measured values are within the acceptance region A, while at least one of the actual values is out of its tolerance interval T_i.

As for the total specific risks, for each specified vector of measured values, the integrals of the posterior pdf involve ratio of multiple integrals of the joint pdf with respect to variables c_i over appropriate domains. The numerical evaluation of such integrals is performed again by simulation of M random vectors $[c_1, c_2, \ldots, c_n, c_{1\mathrm{m}}, c_{2\mathrm{m}}, \ldots, c_{n\mathrm{m}}]$, generated according to the prior modeling for c_i values and the likelihood modeling for $c_{i\mathrm{m}}$ values.

LIMITATIONS

There are limitations of the Bayesian approach: the use of any model as a simplified reflection of reality; the assumption of negligible definitional uncertainty of actual component content c_i (in particular, inhomogeneity and/or instability of an item of the multicomponent substance or material leading to an increase of the standard deviation of the prior pdf and its skewness); adequacy of treatment of a dataset of item-to-item (batch-to-batch) test/measurement results for modeling a prior pdf; goodness-of-fit of experimental and theoretical distributions, etc.

EXAMPLES

Examples of evaluation of the risks are provided for conformity assessment of a PtRh alloy, pure potassium trioxidoiodate, a sausage, and synthetic air.

LIAISON REPORTS 2023 OF THE SISTER INTERNATIONAL ORGANIZATIONS

AFRIMETS REPORT

Angelique Botha // National Metrology Institute of South Africa (NMISA), South Africa

APMP REPORT

Hongmei Li // National Institute of Metrology (NIM), China

EURAMET REPORT

Michela Sega // INRiM, Italy

EURACHEM REPORT

Stephen Ellison // LGC, UK

IUPAC REPORT

Stephen Ellison // LGC, UK

IMEKO REPORT

Michela Sega // INRiM, Italy, IMEKO TC8 Chair

REPORT FROM ISO/TC 334 REFERENCE MATERIALS

Angelique Botha // NMISA, South Africa, ISO/TC 334 Chair

LIAISON REPORTS

AFRIMETS ACTIVITIES

Angelique Botha // NMISA, South Africa

SUMMARY OF GENERAL ISSUES

AFRIMETS (the African regional metrology organisation) held its 16th General Assembly and related meetings from 16 to 20 July 2023 hosted by the National Institute of Standards (NIS) in Egypt. Most of the technical committees including the Technical Committee for Quality Systems (TC-QS) met before the General Assembly.

In terms of the election of executive officers, no new members were elected during 2023. Dr Henry Rotich from the Kenya Bureau of Standards (KEBS) is currently the Chairperson of AFRIMETS until 2025. Mr John Paul Musimami from the Uganda National Bureau of Standards (UNBS) is the Vice-chair responsible for Legal Metrology and Mr Matthew Ranganai from SIRDC-NMI in Zimbabwe is the Vice-chair for Scientific Metrology.

Some good progress has been made with the development of the AFRIMETS Services Database. The first concept draft of the database was developed by the contractor. The database has been tested and data entry has commenced. It mimics the Key Comparison Database of the BIPM (KCDB) but is designed to capture the quality confirmed (3rd party accredited or peer reviewed) calibration and measurement capabilities (CMCs) of the national metrology institutes (NMIs) in Africa (and later the Legal Metrology Bodies). The AFRIMETS Capabilities and Services Database (ACSD) will support the mutual recognition of metrology capabilities for the African Continental Free Trade Agreement (AfCFTA). The African States that are party to the Metre Convention and the Associates of the CGPM will continue to publish their CMCs in the KCDB, for international recognition of their services.

The review of the AFRIMETS quality systems by the AFRIMETS TC-QS confirmed that the calibration and measurement capabilities published in the international key comparison database (KCDB) are supported by total quality managements systems implemented in accordance with ISO/IEC 17025 and ISO 17034 as required. The review or re-review of the quality systems of EMI in Ethiopia, GSA in Ghana, SIRDC-NMI in Zimbabwe, INRAP in Tunisia, NSI in Namibia, TBS in Tanzania and ZMA in Zambia was completed in 2023. The review of the quality system of BOBS in Botswana has not been completed yet.

The General Assembly decided that a minimal membership fee will be implemented per country from 2025 onwards. AFRIMETS will be registered as a non-profit company with a bank account.

CURRENT TC AND WORKING GROUP CHAIRS AND CONTACT DETAILS

The AFRIMETS structure includes working groups to mirror the international consultative committee working groups (CC-WGs) and are identified as TC-(parameter).

The contact details of the TC-Chairs important to Chemistry are listed below:

Function	Name	Details
TC-QM Vice-Chair (Bio analysis)	Dr Angelique Botha Ms Désirée Prevoo-Franszen	National Metrology Institute of South Africa (NMISA), Private Bag X34, Lynnwood Ridge, 0040, RSA Tel: +27 12 947 2705 e-mail: abotha@nmisa.org Tel: +27 12 947 2738 e-mail: dprevoo@nmisa.org
TC-Mass and Related Quantities	Mr Thomas Mautjana	National Metrology Institute of South Africa, Private Bag X34, Lynnwood Ridge, 0040, RSA Tel: +27 12 947 2880 e-mail: tmautjana@nmisa.org
TC-QS Vice-Chair (CMCs)	Dr Noha Emad Khaled	National Institute for Standards (NIS), Tersa Street, El Haram, Giza, 12211 Egypt Tel: +(202)33862322 e-mail: nemadnis@yahoo.co.uk or nemadnis@netscape.net

AFRIMETS CMCs

As of 1 April 2024, there were a total of 768 AFRIMETS CMCs accepted in Appendix C of the KCDB.

The CMCs originate from:

South Africa (NMISA) = 542
(123 CMCs in Chemistry and Biology)

Egypt (NIS) = 108
(2 CMCs in Chemistry)

Zimbabwe (SIRDC-NMI) = 19

Kenya (KEBS) = 31
(2 CMCs in Chemistry)

Tunisia (DEFNAT) = 25

Zambia (ZMA) = 11

Namibia (NSI) = 7

Morocco (LPEE) = 20

Botswana (BOBS) = 3

DEVELOPMENT WORK IN CHEMISTRY AND BIOLOGY

LNMc-INRAP, who is the designated institute for metrology in chemistry in Tunisia, is currently in the process of implementing a national strategy in Metrology in Chemistry to achieve comparability of measurement results to meet the national needs in terms of production of certified reference materials (CRMs) and organisation of proficiency testing (PT) schemes. INRAP has developed a roadmap for the implementation of the national strategy in metrology in chemistry with the support of BIPM and AFRIMETS experts.

With the support of the AFRIMETS TC-QM and PTB, INRAP has implemented a gravimetric unit for CRM production in accordance with ISO 17034 and has produced three certified calibration solutions so far: aflatoxin B1 in acetonitrile; patulin in acetonitrile and an elemental copper (Cu) calibration solution. INRAP is currently commissioning an air-conditioned laboratory for the gravimetric unit.

Furthermore, the laboratory participated in a capacity building comparison of the BIPM (CCQM-K148.d) for patulin in acetonitrile in October 2022 and participated in a bilateral comparison with the BIPM for aflatoxin B1 in acetonitrile and obtained excellent results. During 2023, LNMc-INRAP participated in several key comparison studies: CCQM-K154.b1: Subsequent bilateral key comparison study - organic solvent calibration solution - gravimetric preparation and value assignment of aflatoxin B1 (AfB1) in acetonitrile (ACN); CCQM-K180: Mass fraction of metronidazole in pig muscle. In the beginning of 2024, LNMc-INRAP has organised a bilateral comparison with PTB in Germany: LNMc-BC-01: Copper Standard Solution in Nitric Acid: SRM-Cu Bilateral Comparison.

Pending the approval of the AFRIMETS TC-QM, the LNMc-INRAP is planning to pilot an AFRIMETS key comparison with the same copper calibration solution produced by LNMc-INRAP. As a capacity building and knowledge transfer (CBKT) participant, LNMc-INRAP will be participating in the key comparison study CCQM-K154.e: Organic solvent calibration solution - gravimetric preparation and value assignment of Ochratoxin A (OTA) in acetonitrile (ACN).

LNMc-INRAP has registered to participate in the supplementary comparison, AFRIMETS.QM-S1 - Pesticides in yellow plum. This comparison will allow INRAP to support the planned CMC claims for analysing pesticides in vegetable and fruit matrices. also, in the framework of this activity INRAP is currently planning a training activity with the NMISA in South Africa to continue building on core competencies and knowledge transfer.

NIS in Egypt is planning new CMC claims in the field of Chemistry and Biology that includes their measurement capabilities for pesticides in olive oil and metal alloy analysis.

The schedule for the AFRIMETS supplementary comparison for pesticides in fruit (AFRIMETS.QM-S1) has been finalised and the registration is still open for interested NMIs/DIs. The comparison will enable AFRIMETS NMIs, such as LNMc-INRAP in Tunisia, who has an advanced capability in this area, to claim new CMCs. Much interest has also been shown by developing NMIs from the SIM region, such as Columbia, Bolivia, and Argentina as well as from other NMIs active in the Organic Analysis Working Group (OAWG) of the CCQM.

AFRIMETS is also supporting Phase 2 of the Pan African Quality Infrastructure (PAQI) project Harnessing quality infrastructure in the cassava value chain with the organisation of a proficiency testing (PT) scheme for cassava. The PT scheme was planned for four (4) PT rounds for the determination of the moisture and protein content in cassava; toxic and nutritional elements in cassava flour; pesticides in cassava and hydrocyanic acid (HCN) in cassava. All four PT rounds have been completed and an on-line workshop was held in February 2024 to discuss the results with the participants.

The participants were invited to present their methods including how they established metrological traceability, validated their methods, and performed quality assurance. Based on the outcomes of the PT scheme and the feedback from the participants, PTB in Germany has agreed to support two repeat rounds of the PT scheme that will be run from April to June 2024.

These two repeat PT rounds will be for the determination of toxic and nutritional elements in cassava and the determination of mycotoxins in cassava.

In response to the training needs identified during the Pan African Food Testing Capability Survey in 2022, AFRIMETS, with the support of PTB, hosted a range of summer schools for the African food testing laboratories at the NMISA in South Africa in 2023. The summer schools involved dedicated training in several measurement techniques, such as gas and liquid chromatography for organic analysis with a focus on the analysis of mycotoxins in food as well as the elemental analysis of food with the use of the measurement techniques of inductively coupled plasma optical emission spectrometry (ICP-OES) and inductively coupled plasma mass spectrometry (ICP-MS). The hands-on training shared experiences with the trainees in instrument operation, maintenance and troubleshooting followed by training on the practicalities of food analysis for a range of analytes, including pesticides, mycotoxins, heavy metals, and nutritional elements. The training comprised of two-week sessions for the trainees where they attended lectures on the theory of the techniques and the sample analysis approaches during the first week and then had practical sessions where they worked on the instruments, prepared samples, and performed sample analysis during the second week.

***Figure 1**: Students at summer school for toxic and nutritional elements in food*

FUTURE ACTIVITIES

The summer schools will be repeated in 2024 with the mycotoxins in food summer school scheduled for August 2024 and the toxic and nutritional elements in food summer school scheduled for July 2024.

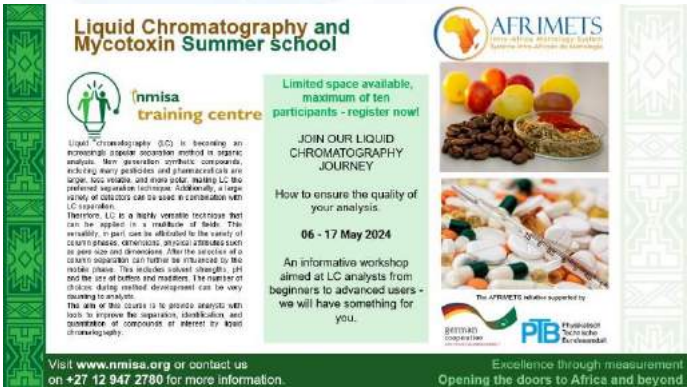

***Figure 2**: Brochure of the next summer school for liquid chromatography and mycotoxins in food to be held in August 2024*

For any further information on the activities in AFRIMETS or the activities of the TC-QM for Chemistry, please contact:

AFRIMETS Chair: Dr Henry Rotich (KEBS, Kenya): Rotich Henry: rotichh@kebs.org

TC-QM Chair; Dr Angelique Botha (NMISA, South Africa): abotha@nmisa.org

TC-QM Vice-Chair; Ms Désirée Prevoo-Franszen (NMISA, South Africa): dprevoo@nmisa.org

LIAISON REPORTS

APMP ACTIVITIES

Hongmei Li // NIM, China

ASIA PACIFIC METROLOGY PROGRAMME (APMP) ACTIVITIES

THE 39TH APMP GENERAL ASSEMBLY & RELATED MEETINGS

The 39th APMP General Assembly & Related Meetings was held from 27 November to 3 December 2023 in Shenzhen, China. Dr. Hyun-Min Park, the APMP Chairperson, declared the opening of the meeting and expressed special thanks to Mr. Xiang Fang and all organizers from NIM (China) who contributed to the organization of the APMP 2023 meetings. About 600 representatives from APMP member institutes, relevant international organizations, academia and industries attended the meeting.

TCQM

There were 28 full-member economies (47 institutes) and 13 associate-member economies (14 institutes) as of 1 June 2023.

As of 31 January 2023 (prior to Cycle XXIV), the calibration and measurement capabilities (CMCs) from APMP TCQM constituted to 42.2% of the total CMCs in the key comparison database (KCDB). The percentage of contributions from APMP in the categories of "High purity chemicals" and "Food" were 59.3% and 65.3% of total CMCs in KCDB, respectively. In 2023, 274 CMCs from APMP were reviewed and 220 CMCs were submitted to the Working Group on Key Comparisons and CMC Quality (KCWG). A total of 199 new CMCs were published in Cycle XXIV.

Since 1999, APMP TCQM had organised 16 key comparisons (KCs) – three on-going; 25 supplementary comparisons (SCs) – five on-going; and 38 pilot studies – six on-going.

FOOD SAFETY FOCUS GROUP (FSFG)

FSFG Annual Meeting: On 28 November 2023, the APMP Food Safety Focus Group (FSFG) annual meeting was held in Shenzhen, China. 20 participants from 11 economies attend the meeting. Representatives from BIPM, VNIIM Rusia, SNSU-BSN Indonesia, ITDI-DOST Philippines, GLHK Hong Kong, KIMIA Malysia, HSA Singapore, NIM China, NIMT Thailand, NMIA Australia, NMISA South Africa and KRISS Korea were invited to introduce food safety activities or related techniques in their economies.

THE APMP-APAC JOINT PROFICIENCY TESTING WORKING GROUP (PTWG)

The PTWG was formed in 2013 as a collaboration between APMP and the Asia Pacific Laboratory Accreditation Cooperation (APLAC). Following the establishment of the Asia Pacific Accreditation Cooperation (APAC) in 2019, the collaboration was renamed as the "APMP-APAC Joint Proficiency Testing Working Group." The PTWG is overseen by two Co-Convenors and permanent members representing both APMP and APAC.

The joint efforts of APMP and APAC aim to create real impact in various industries. Proficiency testing (PT) programmes are tailored for metrology institutes from developing economies, as well as testing/calibration laboratories nominated by APAC's member accreditation bodies. Additionally, PT needs surveys and capability building activities aimed at enriching measurement capabilities and fostering a deeper understanding of international standards are conducted. These initiatives are pivotal in driving progress and excellence in the field of metrology and accreditation across the Asia Pacific region.

BCEIA CHEMICAL METROLOGY AND REFERENCE MATERIAL SESSION

The 20th Beijing Conference and Exhibition on Instrumental Analysis (BCEIA 2023) was held on 7 & 8 September 2023 at China International Exhibition Center (Tianzhu New Hall), Beijing. The 'Chemical Metrology & Reference Materials sub-session' was organized by NIM, with Prof. Hongmei Li being the Chair, and over 100 participants attended the event. It invited a total of 26 presentations covering the following topics: State-of-the-art RM technology, green manufacturing, nutrition, and health.

THE 4TH TD-MSQS WORKSHOP

Between 5-7 May 2023, under the theme of "Measurements, Standards, Quality and Safety", the 4th Therapeutics and Diagnostics: Measurements, Standards, Quality and Safety (TD-MSQS) workshop was co-organized by NIM China and the BIPM in Chengdu, China. A total of 300 participants attended the meeting either in-person or online. Three sub-sessions were held, and these included:

A: Drug characterization and quality assurance

B: Research and quality control for in-vitro diagnostics

C: Reference standards, regulation and metrology

LIAISON REPORTS

EURAMET ACTIVITIES

Michela Sega // INRiM, Italy

EURAMET is the Regional Metrology Organization (RMO) of Europe. It coordinates the cooperation of National Metrology Institutes (NMI) in Europe in different fields of metrology, such as traceability of measurements to the SI units, international recognition of national measurement standards and related Calibration and Measurement Capabilities (CMC), research projects, etc. Through the knowledge transfer and cooperation among its members, EURAMET facilitates the development of the national metrology infrastructures.

EURAMET is currently chaired by Jörn Stenger (PTB, Germany); the two Vice-Chairpersons are Miruna Dobre (SMD, Belgium) for the General Assembly (GA) and Maguelonne Chambon (LNE, France) for the *European Metrology Programme for Innovation and Research* (*EMPIR*) and the European Partnership on Metrology related matters. In 2023, Dolores Del Campo (CEM, Spain) was elected as new chair: her mandate will start during the GA in 2025. The EURAMET 17th GA took place as a hybrid meeting from 30 May to 2 June 2023, in Tallin, Estonia, hosted by Metrosert, the Estonian NMI. An extraordinary GA was held on 17 January 2023, as an online meeting. The EURAMET 18th GA will take place from 30 May to 2 June 2023, in Teddington, UK, hosted by NPL, the British NMI.

European Partnerships are a key implementation tool of the European Commission's Horizon Europe; an ambitious research and innovation programme, running from 2021 to 2027. Among these, the European Partnership on Metrology (Metrology Partnership, EPM) aims at bringing together the measurement science community and stakeholders to deliver on global challenges including health and climate, support the European Green Deal, and underpin innovation in industry through collaborative research. The EPM is co-funded by the Member States and the European Union with an expected budget of around 690 million euro. The expected impact of the European Partnership on Metrology is manifold, as it will support a wide range of policies, commerce and advancement of key European challenges. It will comprise seven call cycles between 2021 to 2027, covering topics such as Green Deal, Health, Digital Transformation, Fundamental Metrology, Integrated European Metrology, Industry needs, Pre-normative and Knowledge transfer and capacity building measures. The Partnership builds on the progress achieved under the previous European Metrology Research Programmes, and aims to break new ground by contributing to the development of self-sustaining, coordinated metrology infrastructures, with the capacity to continue joint research and innovation after 2030.

In 2023 a call within the EPM framework was launched, via the usual two stage process, on the following major topics, addressing specific challenges: fundamental metrology, metrology

for industry, metrology for pre- and co-normative research, metrology support for research potential, capacity building coordination. Stage 1, opening on 11 January, aimed at offering stakeholders from any country the opportunity to influence the projects undertaken by the European Community by identifying potential research topics. The highest priority topics received at Stage 1 provided the basis for Stage 2 which opened on 23 June 2023. In November 2023 the review conference was held, after which the call ranked list was published, thus establishing the projects to be financed within the call. In 2024, another call within the EPM framework was launched on the following major topics: Green Deal, digital transformation, research potential, normative. Stage 1 opened on 10 January 2024 and closed on 19 February. The highest priority topics received at Stage 1 will provide the basis for Stage 2 which will open on 26 June 2024. Major information on the calls can be found at https://www.metpart.eu/.

One of the most important initiatives undertaken by EURAMET for the promotion of cooperation conceived in a broader scope towards better partnership, communication, and harmonization is the European Metrology Networks (EMNs). These are collaborative structures which go beyond joint research to increase the coordination of measurement science across Europe, addressing scientific and societal challenges, infrastructure and services in response to European and global metrology needs. Currently there are twelve EMNs: Advanced Manufacturing, Climate and Ocean Observation, Energy Gases, Mathematics and Statistics, Pollution Monitoring, Quantum Technologies, Radiation Protection, Safe and Sustainable Food, Smart Electricity Grids, Smart Specialisation in Northern Europe, Traceability in Laboratory Medicine and Clean Energy, the last been approved. Each EMN, by providing a single point of contact, aims at underpinning regulation and standardization by establishing a comprehensive and longer-term infrastructure, promoting best practice and disseminating knowledge in its respective fields. Further EMNs are in preparation or under consideration. More information on EMNs can be found at https://www.euramet.org/european-metrology-networks/.

Technical collaboration in EURAMET is organized within ten Technical Committees (TCs), focusing on specific areas which represent the forum for scientific and technical cooperation in the respective fields. In addition, two Committees deal with the overall topics Quality and Interdisciplinary Metrology https://www.euramet.org/technical-committees. The TCs are responsible for the execution of the activities required by EURAMET as RMO for the fulfilment of the Mutual Recognition Arrangement of the International Committee of Weights and Measures (CIPM-MRA). The types of technical cooperation carried out within the TCs are: cooperation in research, comparison of measurement standards, metrological traceability, and consultation on facilities.

One of the ten TCs is devoted to Metrology in Chemistry (Technical Committee for Metrology in Chemistry, TC-MC), which is concerned with primary methods and reference materials for chemical measurements and research in metrology to support different sectors in the amount of substance fields https://www.euramet.org/technical-committees/tc-mc.

NEWS FROM EURAMET TECHNICAL COMMITTEE IN METROLOGY IN CHEMISTRY (TC-MC)

TC-MC is chaired by Teemu Näykki (SYKE, FI). 31 EURAMET member countries are represented in TC-MC plus the European Commission. BIPM has the status of observers.

The technical activities are carried out within the four technical Sub-committees dealing with gas analysis (SC-GA), inorganic analysis (SC-IA), electrochemical analysis (SC-EA), bio and organic analysis (SC-BOA). The convenors of the subcommittees are: Janneke van Wjik (VSL, NL) for SC-GA, Rainer Stosch (PTB, DE) for SC-IA, Mine Bilsel (UME, TR) for SC-BOA and Matilda Roziková (CMI, CZ) for SC-EA. In addition, a strategy working group, chaired by the TC-Chair, is also active on the following tasks: advice to TC-Chair and subcommittee convenors, strategic planning of

comparisons, support actions, coordination, organization of workshops.

The TC-MC members are actively participating in the European Programmes on Metrology, being involved in all the targeted programmes; in addition, they cooperate within various EMN, among which Climate and Ocean Observation, Energy Gases, Mathematics and Statistics, Pollution Monitoring, Safe and Sustainable Food, Traceability in Laboratory Medicine, thus indicating the cross-disciplinary nature of the TC itself.

TC-MC MEETING IN 2023

The 2023 annual meeting of the TC-MC was held in Paris, France, hosted by LNE from 31 January to 2 February. It was the first meeting in presence, after three years of Covid-19 pandemic. The Strategy WG meeting had an online meeting on 27 January.

The four technical subcommittees reconvened, as usual, ahead of the annual TC-MC plenary meeting on 31 January 2023. A review of new claims as well as the obligatory re-review of a range of existing claims were carried out. Running and new projects and comparisons in the framework of EURAMET, EMPIR, EMP and also proposals for the upcoming EMP call were discussed in detail in all sub-committees.

The plenary meeting took place on 1 and 2 February 2023. Some highlights on EURAMET, BIPM/CIPM, CCQM strategy and activities within its main working groups were given. The conveners of the subcommittees gave an overview of the activities of each subcommittee and of the main outcomes of the meetings carried out in the previous day. A section of the meeting was devoted to EMNs dealing with topics related to the amount of substance field. An overview was given on the EMN for Climate and Ocean Observation (coordinated by NPL), EMN for Energy Gases (coordinated by VSL), EMN for Laboratory Medicine (coordinated by LNE), EMN for Environment Pollution (coordinated by LNE) and EMN for Safe and Sustainable Food (coordinated by INRiM). On 1 February a specific workshop in preparation for the 2023 EMP call was held, addressing all the different targeted programmes.

TC-MC MEETING IN 2024

The annual meeting of the TC-MC was held in Delft, the Netherlands, hosted by VSL from 6 to 8 February 2024. The Strategy WG meeting had an online meeting on 2 February.

The four technical subcommittees reconvened, as usual, ahead of the annual TC-MC plenary meeting on 6 February 2024. A review of new claims as well as the obligatory re-review of a range of existing claims were carried out. Running and new projects and comparisons in the framework of EURAMET, EMPIR, EMP and also proposals for the upcoming EMP call were discussed in detail in all sub-committees.

The plenary meeting took place on 7 and 8 February 2024. Some highlights on EURAMET, BIPM/CIPM, CCQM strategy and activities within its main working groups were given. Presentations on the activities carried out within liaison organisations like Eurachem and Eurolab were also given. The conveners of the subcommittees gave an overview of the activities of each subcommittee and of the main outcomes of the meetings carried out in the previous day. A section of the meeting was devoted to EMNs dealing with topics related to the amount of substance field. An overview was given on the EMN for Climate and Ocean Observation (coordinated by NPL), EMN for Energy Gases (coordinated by VSL), EMN for Laboratory Medicine (coordinated by LNE), EMN for Environment Pollution (coordinated by LNE) and EMN for Safe and Sustainable Food (coordinated by INRiM). On 7 February, a specific workshop in preparation for the 2024 EMP call was held, addressing all the different targeted programmes.

The next TC-MC meeting will be held at the beginning of February 2025, hosted by RICE, Sweden.

LIAISON REPORTS

EURACHEM ACTIVITIES

Stephen Ellison // LGC, UK

MEMBERSHIP

Eurachem currently has 36 full and associate members. The Chair is Isabelle Vercruysse (Belgium), supported by vice chairs Eugenia Eftimie Totu (Romania) (incoming) and Vicki Barwick (UK) (past chair). The Secretary is Philippe Maesen, Univ. Liege, supported by BeLab, Belgium.

EURACHEM GENERAL ASSEMBLY AND WORKSHOP

The 2023 Eurachem GA in May was held in person at METAS in Bern, Switzerland – the first in-person GA since 2019. The associated workshop on 'Ensuring reliable and accurate results of analytical processes' (see below), was also an in-person event, held in the same week.

The 2024 Eurachem GA will be hosted by Eurachem Lithuania, in Vilnius on 16 and 17 May. The week will also include a workshop on 'Quality Assurance in Chemical, Medical and Microbiological Laboratories' on 13 and 14 May, organised by the Eurachem Education and Training WG. Registration and programme details can be found on the Eurachem website.

EURACHEM AND EURACHEM / CITAC EVENTS 2023-4

May 2023: Ensuring reliable and accurate results of analytical processes. There were 80 participants from 4 continents and 24 countries. Presentations are available on the Eurachem website (www.eurachem.org). Available presentations are available (PDF format) on the Eurachem website. A number of refereed papers related to the event are available in an online collection for the journal Accreditation and Quality Assurance https://link.springer.com/collections/aagagafdcb.

Note: Not all papers in the collection are open access.

October 2023: 10th Eurachem Workshop on Proficiency Testing in Analytical Chemistry, Microbiology and Laboratory Medicine. This International workshop, held as an in-person event in Windsor in the UK, had been deferred because of Covid-19 travel restrictions. The in-person event attracted a total of 194 participants from 45 countries. Four well attended training sessions were held on the day before the workshop. Available presentations are available (PDF format) on the Eurachem website. A Special issue of Accreditation and Quality Assurance will include refereed papers from the event.

March 2024: Training event: Recent developments in Quality Assurance. This in person training event was hosted by Eurachem Cyprus with the support of the Pancyprian Union of Chemists and the Eurachem Education & Training Working Group. The event attracted 35 participants from Georgia, Greece, Italy and

Cyprus. Nine Eurachem speakers participated, including three online.

EURACHEM GUIDANCE UPDATES

NEW AND UPDATED GUIDES

Second edition of "Terminology in Analytical Measurement" (May 2023)

In this second edition the scope and structure, and the terms and concepts discussed, remain unchanged from the first edition. However, all sections have been reviewed and, where necessary, the text has been revised to improve clarity and ensure consistency with current guidance.

Third edition of "Accreditation for Microbiological Laboratories" (May 2023)

The principal changes include:

- Updates for recent trends in microbiology, e.g. PCR (polymerase chain reaction) techniques for the detection of microorganisms
- Addition of a section on risk-based thinking;
- References to the use of a decision rule;
- A new Annex C on reporting confidence intervals;
- A new Annex D on estimation of uncertainty from sampling.

NEW AND REVISED INFORMATION LEAFLETS

Information Leaflet on "Interlaboratory Comparisons other than Proficiency testing". (April 2024)

The new leaflet explains the three main purposes of interlaboratory comparisons (ILCs) and how the different objectives affect the design of the comparison.

Updated leaflets related to Proficiency testing

All of the existing leaflets related to PT have been updated to reflect the new ISO/IEC 17043 and related Standards.

TRANSLATIONS

- The guide 'Measurement uncertainty arising from sampling' which was published in 2019 has been translated into German
- The Eurachem/CITAC guide on "Assessment of performance and uncertainty in qualitative chemical analysis" is now additionally available in German and Farsi.
- The supplementary Guides on "Planning and Reporting Method Validation Studies" and "Blanks in method validation" are now available in Italian, in a single volume.

Many Information leaflets have also appeared in additional translations. The News section of the Eurachem website includes all of the updates.

WORKING GROUP UPDATES

NOTE: Eurachem WG updates for May 2024 are not yet available. Information below is based on mid-year reports supplemented by information available for the most recent executive meeting in February 2024.

PROFICIENCY TESTING

Further to the 10th PT workshop and the Information Leaflet updates described above, the Eurachem Proficiency Testing Working Group (PTWG) is working on:

- Organisation of the 11th International Workshop on Proficiency Testing in Analytical Chemistry, Microbiology and Laboratory Medicine. This is provisionally scheduled for 2026.
- Preparation and translation of a series of information leaflets regarding topics of interest to proficiency testing, to encourage good practice across Europe.
- Production of guidance on the evaluation of qualitative results in PT schemes.

EDUCATION & TRAINING

- The revised Eurachem reading list, coordinated by the ETWG, was published on the Eurachem website in June as a pdf and webpages.
- The terminology leaflet – 'You talk, we understand – The way out of the tower of Babel' has undergone a minor revision to bring it into line with the 2nd edition of the terminology guide.
- The ETWG is now focusing its efforts on the revision of the Guide to Quality in Analytical Chemistry. Task groups have been established to progress the work and a draft is under consideration within the WG.
- The ETWG is developing a new leaflet covering the revision of ISO 15189 (similar to that prepared for the revision of ISO/IEC 17025).
- The WG has agreed the slides and script for a short video promoting the importance of terminology and highlighting the revised terminology guide. Vicki Barwick will record the voiceover.
- The Pancyprian Union of Chemists (PUC) and ETWG ran a two-day workshop on quality assurance in Nicosia in early March 2024 (see above).

MEASUREMENT UNCERTAINTY AND TRACE-ABILITY (JOINT WG WITH CITAC)

The MU and Traceability working group is a joint working group with CITAC. The group currently has 34 members. The WG has met four times (excluding drafting task group meetings) since the May 2023 CITAC Annual meeting. The next scheduled meeting is in person in May in Lithuania.

The measurement uncertainty guide (QUAM:2012) is due for revision. Revision has been deferred pending completion of work on asymmetry and completion of the 'Uncertainty from in-house validation data guidance' (see below).

The current work programme includes:

- Preparation of draft guidance on the evaluation of uncertainty from in-house validation data. Drafts of examples were circulated to the WG for comment in January 2023.
- Work on handling asymmetry in the context of compliance assessment is ongoing. Simulation work to date has shown that, for two-sided compliance intervals, asymmetry does not greatly affect the overall producer and consumer risk, but the risks of incorrect conformity decisions at the upper and lower end of the interval can change considerably.
- The WG is investigating the effect on measurement uncertainty of non-linearity in measurement models, with a view to preparing brief guidance on the degree of nonlinearity that can be accepted before it becomes necessary to use advanced methods such as Monte Carlo analysis.
- The WG is continuing to collect examples of measurement uncertainty evaluation using 'bottom-up' models with a view to possible publication.

METHOD VALIDATION

- The MVWG have started the work on review/revision of the guide (3rd edition due around 2020-21).
- The MVWG is working on illustrative examples (validation protocols) on application of the principles recommended in the "Fitness for Purpose" guide.
- A Task Group on Validation of Non-Targeted Methods has been established. The group is planning to arrange a series of short webinars on the subject in cooperation with AOAC-E.
- A Joint Task Group (JTG), in cooperation with the Sampling Uncertainty WG and with inputs from Eurolab and NMKL, is ongoing to prepare Supplementary Guidance on the Validation of Measurement Procedures that Include Sampling.
- Lorens Sibbesen, Chair of the MVWG for many years, has stepped down and Helen Cantwell (IE), formerly Vice-Chair, has taken over as Chair.

QUALITATIVE ANALYSIS AND TESTING

- The QAWG is preparing leaflets on Likelihood ratios, Bayes' rule and (qualitative) medical laboratory analysis.
- The working group is also preparing an online event on production of RMs with qualitative properties, in collaboration with members of ISO TC334, the ISO committee responsible for Reference Materials.
- A collaboration with the Method Validation Working Group on the validation of qualitative analysis methods is foreseen.

REFERENCE MATERIALS

- Work continues on revision of the Guide 'The Selection and use of Reference Materials'. Task Groups have been established to work on separate topics.

SAMPLING UNCERTAINTY

- The WG is collaborating with the Method Validation working group in a Joint Task Group on Validation of Measurement Procedures that Include Sampling. The main focus of the JTG is the preparation of supplementary guidance (SG). A paper that underpins the main approach taken in the developing guidance, has been accepted for publication by Acqual (see Ramsey M.H., and Rostron P. (2024) Measurement uncertainty from sampling and its role in validation of measurement procedures. https://doi.org/10.1007/s00769-024-01575-0
- Recent effort by the UfS-WG has included input to Codex/CCMAS on the role of UfS in Acceptance Sampling for the revision of their Guidance on Sampling document (CXG 50). Following recent discussions (27 September 2022), the WG are awaiting their proposed rewording describing how CXG 50 allows for UfS.
- Further technical outputs include a discussion paper by two WG members on the role of sampling in the context of conformity assessment, a paper on Improved coverage factors for Expanded Measurement Uncertainty calculated from two estimated variance components https://doi.org/10.1007/s00769-024-01579-w
- Work ongoing includes preparation of a joint AMC-Eurachem Technical Brief on the role of UfS within Acceptance Sampling, and development of new software (RANOVA4) that enables users to calculate the improved coverage factors that are described in the paper above.

COMMUNICATIONS

- Eurachem operates two Twitter accounts (@EurachemEurope and @EurachemEvents), a YouTube channel (see https://eurachem.org/youtube) and a LinkedIn page (https://www.linkedin.com/company/eurachem/), which are used to further increase the promotion and dissemination of Eurachem guidance documents and events.
- Eurachem has a regular e-newsletter. Anyone wishing to receive periodic updates on Eurachem activities can subscribe at https://www.eurachem.org/subscribe

FUTURE WORKSHOPS

- Quality Assurance in Chemical, Medical and Microbiological Laboratories. 3-14 May 2024, Vilnius, Lithuania.
- An online event on production of reference materials with qualitative values, covering the principles of the forthcoming ISO 33406 on the same topic, is in preparation for September 2024.

For details see the Eurachem website.

LIAISON REPORTS

IUPAC ACTIVITIES

Stephen Ellison // LGC, UK

GENERAL IUPAC NEWS

GENERAL ASSEMBLY AND WORLD CHEMISTRY CONGRESS, 2023

The latest Biennium Council Meeting took place, face to face in The Hague. The Bureau met for the last time and is now being replaced by two new Boards – The Science Board and the Executive Board. The purpose of this restructure is to provide greater scientific steer to the activities of IUPAC by separating science from management and administrative activities. The new structure can be found at the following address https://iupac.org/who-we-are/organizational-chart/

Details of the Council meeting and associated decisions are available on the IUPAC website https://iupac.org/actions-taken-at-iupac-council-the-hague-2023/. The Council Elected the following officials for the 2024/25 Biennium

Ehud Keinan (Israel, **IUPAC President**, Chair)
Mary Garson (Australia, **Vice President**)
Javier García Martínez (Spain, **Past President**)
Zoltán Mester (Canada, **Secretary General**)

THE EXECUTIVE BOARD

Ehud Keinan; Hemda Garelick (UK); Richard Hartshorn (New Zealand); Bonnie Lawlor (USA); Christine Luscombe (Japan); Bipul Saha (India); Zhigang Shuai (China/Beijing); Mary Garson; Zoltán Mester (Canada); Wolfram Koch (Germany, Treasurer); Javier García Martínez (Spain, Past President); Greta Heydenrych (ED, ex officio)

THE SCIENCE BOARD

Mary Garson; Eva Åkesson (Sweden); Pierre Braunstein (France); Alejandra Palermo (UK); Peter Schreiner (Germany); Chi-Huey Wong (China/Taipei); Lidia Armelao (Italy); Derek Craston (UK); Igor Lacík (Slovakia); Michelle Rogers (USA); Frances Separovic (Australia); Ehud Keinan (Israel); Zoltán Mester (Canada); Greta Heydenrych (ED, ex officio)

KEY ACTIVITIES

The latest (GWB2024) IUPAC Global Women's Breakfast "Catalysing Diversity in Science"

The latest IUPAC Global Women's Breakfast was held on February 27, 2024. This included 420 events covering 77 countries and an estimated 40,000 participants worldwide. This made the largest ever GWB, and the event was featured in **DE GRUYTER CONVERSATIONS (27.02.2024)**

GWB2025 will be held on 11 February 2025 on the International Day of Women and Girls in Science.

TOP TEN TECHNOLOGIES

IUPAC has released its call for proposals to identify the top ten emerging technologies in chemistry with the results to be announced later in 2024.

This is an activity that IUPAC has been running annually since 2019, and many of the technologies identified have had strong links with measurement science such as nanosensors, rapid diagnostics for testing, chemiluminescence for biological use, single cell metabolomics and wearable sensors.

A full list of technologies captured by this initiative can be found at the following web address https://iupac.org/what-we-do/top-ten/

COMMISSION ON ISOTOPIC ABUNDANCES AND ATOMIC WEIGHTS

The Commission on Isotopic Abundances and Atomic Weights (**CIAAW**) is an international scientific committee of IUPAC under its Inorganic Chemistry Division. Since 1899, CIAAW has been entrusted with periodic critical evaluation of atomic weights of elements and other cognate data, such as the isotopic composition of elements. CIAAW has been a part of IUPAC since the first General Assembly of IUPAC in1920 and met again at the Biennium meeting in the Hague. Examples of on-going projects are:

- The assessment of absolute isotope ratios for the international isotope delta measurement standards
- Machine-Accessible Periodic Table

ANALYTICAL CHEMISTRY DIVISION OF IUPAC

Division Details - IUPAC | International Union of Pure and Applied Chemistry

DIVISION MEMBERSHIP

As results of the 2023 election of Analytical Chemistry Division membership the composition of the division for the 2024-2025 biennium is as follows:
Division President – Derek Craston
Division Past President – David Shaw
Division Secretary – Luisa Torsi
Titular Members – Aura Tintaru, Vasilisa B. Baranovskaia; Jiri Barek; Takae Takeuchi; Franziska Emmerling; Hongmei Li;
Associate Members – Erico Marlon de Moraes Flores; Ivo Leito;
National Representatives - D. Brynn Hibbert, Mariela Piston, Rufus H. Sha'Ato, Resat Apak; Ilya Kuselman; Susanne Wiedmer

ANALYTICAL CHEMISTRY AWARDS

In 2020, the Analytical Chemistry Division of IUPAC established two awards:

- The Emerging Innovator Award in Analytical Chemistry – an award to recognize outstanding work undertaken by an emerging analytical scientist that corresponds to the aims of the Analytical Chemistry Division.
- The IUPAC Analytical Chemistry Medal – an award to recognize significant lifetime contribution to the aims of the Analytical Chemistry Division.

In 2023, Janusz Pawliszyn received the IUPAC Analytical Chemistry Medal recognizing a lifetime of world leading, foundational research in analytical sample preparation and the invention of solid phase microextration. He is Canada Research Chair Professor, University of Waterloo, Ontario, Canada. His acceptance lecture, "Significance of Fundamentals in Development and Optimization of Sustainable Sampling and Sample Preparation Technologies" is available on **YOUTUBE**.

Xin Yan, Assistant Professor, Texas A&M University received the 2023 Emerging Innovator Award in Analytical Chemistry recognizing her ground-breaking work in electrochemical reactions in droplets using mass spectrometry.

Professor Yan's award lecture "Microdroplet Mass Spectrometry for Lipid Isomer "Analysis and Accelerated Discovery of Transition Metal Catalysis" is also available on **YOUTUBE**.

Nominations for the 2025 Awards will be launched in the Autumn of 2024.

Nominations for the 2025 Awards will be launched in the Autumn of 2024.

DIVISIONAL PROJECTS

A full list of projects currently running within the Division can be found at: https://iupac.org/body/500/#:~:text=The%20Analytical%20Chemistry%20Division%20is,of%20the%20chemical%20measurement%20process.

The following projects were initiated in the last 18 months.

2023-028-1-500
IUPAC BRIEF GUIDE TO METROLOGICAL TERMS IN CHEMISTRY

The aim of this project is to provide a clear description of selected terms used in metrology in the chemical sciences. An IUPAC Brief Guide will be published in Pure and Applied Chemistry (PAC), and possibly as a stand-alone brochure. The target audience comprises researchers, educators, chemistry students, and working chemists who make measurements but might not have had much training in metrology. The focus will be on measurements that chemists make, and the goal is to provide easy-to understand description of the major terms which, in turn, could be used to update the International Vocabulary of Metrology (VIM) and fill gaps in its coverage of contemporary measurements of chemical properties.

2023-016-1-500
IUPAC/CITAC Guide for interlaboratory comparison of nominal (qualitative) and ordinal (semiquantitative) characteristics of a substance or material

This project will produce a joint IUPAC/CITAC Guide for interlaboratory comparison of nominal (qualitative) and ordinal (semi-quantitative) characteristics of a substance or material. The guide will be helpful for application of relevant metrological and mathematical methods for statistical design of interlaboratory comparison of nominal and ordinal characteristics of a substance or material, and analysis of the obtained data.

2023-010-2-500
ELECTROANALYTICAL FLOW THROUGH SYSTEMS FOR MONITORING OF BIOLOGICALLY ACTIVE SPECIES

Over the last decades, low-cost electrochemical detection platforms and procedures have emerged as a simple and robust alternative to conventional tests allowing translation into field conditions. The aim of this project is to critically review related developments and to assess the benefits that these might bring to the chemical community in environmental protection, diagnostic and food quality control, as an aide memoire to potential platform developers and manufacturers and the wider analytical community.

2023-006-1-500
ASSESSING THE NEED FOR TERMINOLOGY, STANDARDS AND GUIDELINES FOR WEARABLE DEVICES THAT PROVIDE CHEMICAL / BIOCHEMICAL MEASUREMENT READOUTS

Wearable sensors have been developed over recent years based on technological advancements in smartphones and other mobile devices. Initially, most wearable sensors were used to track mobility or other physical parameters (heart rate), but more recently attention has been diverted towards biological/chemical measurements. There is, however, a lack of terminology and guidelines

on the validation and standardization of these wearable devices that could lead to confusion and inappropriate use of outputs. This project will review the state of the art and needs for standardization and terminology in this emerging area of science and make recommendations on appropriate IUPAC contributions.

2022-031-1-500
RARE EARTH METAL SULFATES IN WATER AND AQUEOUS SYSTEMS (SDS SERIES)

The project includes complete compilations and critical evaluations for the solubility of the rare earth metal sulfates in water and various aqueous systems. This volume continues the series of previous assessments dealing with rare earth metal halides (published in 2008, 2012 and 2014) and nitrates (in 1983). As previously, the plan is to publish in three parts: I - Sc, Y and La; II - Ce to Eu; III - Gd to Lu

2022-008-4-500
INTRODUCING THE IUPAC SEAL OF APPROVAL FOR A WIDER ADOPTION OF IUPAC RECOMMENDED SYMBOLS, TERMINOLOGY AND NOMENCLATURE: STAGE 1 – SYMBOLS

IUPAC makes great efforts to standardize chemical nomenclature, symbols, and terms, but it is evident that the authors of textbooks and scientific publications have no motivation for adopting these recommendations. The present project represents the first step to introduce a "IUPAC Seal" to be conferred by IUPAC on books, textbooks or other scientific publications that adopt IUPAC recommendations. The Seal will add value to the work that receives it. This initial project will focus on IUPAC expert review of human-readable depictions of IUPAC symbols in analytical and physical chemistry publications.

IUPAC ANALYTICAL CHEMISTRY PUBLICATIONS IN 2023

IUPAC Technical Report: Analytical chemistry of engineered nanomaterials: Part 2. analysis in complex samples. Jan Labuda, Jiři Barek, Zuzana Gajdosechova, Silvana Jacob, Linda Johnston, Petra Krystek, Zoltan Mester, Josino Moreira, Veronika Svitkova and Kevin J. Wilkinson 2023, Pure and Applied Chemistry, https://doi.org/10.1515/pac-2022-0401.

IUPAC Technical Report: Chemical data evaluation: general considerations and approaches for IUPAC projects and the chemistry community. David G. Shaw, Ian Bruno, Stuart Chalk, Glenn Hefter, David Brynn Hibbert, Robin A. Hutchinson, M. Clara F. Magalhaes, Joseph Magee, Leah R. McEwen, John Rumble, Gregory T. Russell, Earle Waghorne, Thomas Walczyk and Timothy J. Wallington 2023, Pure and Applied Chemistry. https://doi.org/10.1515/pac-2022-0802.

IUPAC Technical Report: IUPAC/CITAC Guide: Evaluation of risks of false decisions in conformity assessment of a substance or material with a mass balance constraint. Pennecchi, Francesca R., Kuselman, Ilya and Hibbert, D. Brynn Pure and Applied Chemistry, 2023. https://doi.org/10.1515/pac-2022-0801.

Risks of false decisions on conformity of a sausage with a mass balance constraint. Kuselman I, Pennecchi FR, Hibbert DB, Semenova AA. Journal of Physics: Conference Series. 2022 2022/03/01;2192(1):012021. https://dx.doi.org/10.1088/1742-6596/2192/1/012021.

IUPAC-NIST Solubility Data Series Volume 105: Solubility of Solid Alkanoic Acids, Alkenoic Acids, Alkanedioic Acids and Alkenedioic Acids Dissolved in Neat Organic Solvents, Organic Solvent Mixtures, and Aqueous-Organic Solvent Mixtures. III. Alkanedioic Acids and Alkenedioic Acids, E. Acree, Jr. and W. E. Waghorne, Journal of Physical Chemistry Reference Data, 52, 033102-1 (2023). https://doi.org/10.1063/5.0147933.

Compendium of Terminology in Analytical Chemistry: IUPAC Orange Book. prepared for publication by D Brynn Hibbert, The Royal Society of Chemistry, 2023 (ISBN 978-1-78262-947-4); DOI: https://doi.org/10.1039/9781788012881. The story of the 15-

year, 57-contrubutor effort to produce the Orange Book.

IUPAC Technical Report: Methods for the SI-traceable value assignment of the purity of organic compounds, Steven Westwood, Katrice Lippa, Yoshitaka Shimuzu, Béatrice Lalere, Takeshi Saito, David Duewer, Xinhua Dai, Stephen Davies, Marina Ricci, Annarita Baldan, Brian Lang, Stefan Sarge, Haifeng Wang, Ken Pratt, Ralf Josephs, Mikael Mariassy, Dietmar Pfeifer, John Warren, Wolfram Bremser, Stephen Ellison, Blaza Toman, Michael Nelson, Ting Huang, Ales Fajgelj, Ahmet Goren, Lindsey Mackay and Robert Wielgosz 2023 Pure and Applied Chemistry, https://doi.org/10.1515/pac-2020-0804.

LIAISON REPORTS

IMEKO ACTIVITIES

Michela Sega // INRi M, Italy, IMEKO TC8 Chair

IMEKO, the International Measurement Confederation, founded in 1958, is a non-governmental federation of 42 Member Organizations individually concerned with the advancement of measurement technology. It has a consultative status with UNESCO and UNIDO. Its fundamental objectives are the promotion of international interchange of scientific and technical information in the field of measurement and instrumentation and the enhancement of international co-operation among scientists and engineers from research and industry. Prof. Frank Härtig (Germany) is the current President of IMEKO, Prof. Paolo Carbone (Italy) is the President Elect and Chair of the Technical Board, Prof. Masatoshi Ishikawa (Japan), as past President, is the Advisory President and Chair of the Advisory Board.

The IMEKO Secretariat is located in Budapest (Hungary). More information about IMEKO and its structure can be found on the IMEKO website (www.imeko.org).

In 2023, due to the return to the normal operations after the pandemic emergency, many IMEKO events were organized in hybrid form or fully in presence. The IMEKO 2023 General Council Sessions took place on 8 and 9 September in Budapest (Hungary) hosted by SZTAKI, the Institute for Computer Science and Control, during which the 65th anniversary of IMEKO was celebrated. 32 countries' Member Organisations represented their Institutes at the General Council Sessions. The Technical Board meeting was also very well attended, with some Technical Committees sending more than one officer. 32 new members within IMEKO Technical Committees and 6 new TC officers were approved. As a part on the joint international effort on digitalization, IMEKO established the cross-IMEKO Working Group on Digitalization which will be coordinated by TC6 Vice Chair Dr Hugo Gasca. Some statistics on the continuously increasing success of the IMEKO Journals were presented. IMEKO Partners APMP, EURAMET, EUROLAB, GULFMET participated in the General Council Sessions and presented the latest developments and activities in their organizations and future plans.

Many TC events were organized, among which:

- 7th Conference on Pressure and Vacuum Measurement (together with the CCM - the Consultative Committee for Mass and Related Quantities of the BIPM), Washington DC, US, 15-19 May 2023
- IMEKO TC20: International Conference on Measurements of Energy, Braunschweig, Germany, 4-6 September 2023
- IMEKO TC4 International Symposium 2023 - Academia meets Industry, Pordenone, Italy, 20-21 September 2023
- 19th IMEKO TC10 Conference "MACRO meets NANO in Measurement for Diagnostics,

Optimization and Control", Delft, The Netherlands, 21-22 September 2023

- IMEKO TC8, TC11 and TC24 Joint Conference, Madeira, Portugal, 11-13 October 2023
- 7th International IMEKOFOODS Conference "Worldwide food trade and consumption: quality and risk assessment", Paris, France, 25-27 October 2023.

The TCs can select best contributions to IMEKO events to be published, as enhanced versions of the corresponding papers, in Measurement, the official journal of IMEKO, after the events. Additional contributions to IMEKO events can be also published in the IMEKO Online Journal ACTA IMEKO, which published its four issues in 2022 (https://acta.imeko.org/index.php/acta-imeko). In addition, three more specific open access journals are also available, in line with the rapid growth of studies on the relevant topics: Measurement: Sensors, Measurement: Food, and Measurement: Energy. Periodic newsletters are prepared by IMEKO Secretariat and can be accessed through IMEKO webpage.

The preparation for the XXIV IMEKO World Congress is ongoing. It will take place at the new Congress Center of Hamburg (Germany) on 26-29 August 2024, hosted by PTB, the German Member Organization of IMEKO. More information and updates can be found on the dedicated website (https://www.imeko2024.org/).

The following IMEKO World Congress will be organized by Professor Paolo Carbone, the IMEKO President-Elect, and will be held in Rimini, Italy, in 2027.

LIAISON REPORTS

ISO/TC 334 REFERENCE MATERIALS ACTIVITIES

Angelique Botha // NMISA, South Africa, ISO/TC 334 Chair

ANNUAL MEETING OF ISO/TC 334 IN JUNE 2023

The fourth meeting of ISO/TC 334 was held as an on-line meeting during the second week of June 2023. The number of participants who joined the meeting ranged between 60 to 70 with representatives from 15 member bodies (56% of the P-members) and 2 O-members also participated in the meeting. Representatives from 6 international organisations attended the meeting representing 46% of the external liaisons, and 3 of the ISO internal liaisons (other technical committees) representing 30% of the ISO/TC 334 membership attended the meeting. Currently, ISO/TC 334 has 10 internal (ISO) liaisons in force, 13 category A liaisons and 4 category C liaisons with external organisations. There are also three liaisons managed at ISO level (JCGM/WG1 responsible for the Guide to the Expression of Uncertainty in Measurement (GUM), JCGM/WG2 responsible for the International Vocabulary for Metrology (VIM) and JCTLM responsible for measurement traceability in laboratory medicine).

Some specific liaison activities of ISO/TC 334 during 2023 included working with ISO/TC 212 on the revision of ISO 15193 for the requirements for reference measurement procedures for in vitro diagnostic medical devices and ISO 15194 for the requirements of reference materials (RMs) for in vitro diagnostic medical devices. The 2nd committee draft of the fourth edition of the VIM (VIM4) was launched for balloting during the year and ISO/TC 334 commented extensively on the terminology related to RMs. ISO/TC 334 also wrote a letter to JCGM/WG2 about the developments related to terminology for reference materials in working group 10 (WG10) that is working on the revision of ISO 33400 – Reference materials - Vocabulary.

PROGRAM OF WORK OF ISO/TC 334

The program of work of ISO/TC 334 have now been updated based on the decisions taken at the fourth meeting and are listed below. The program includes the transformation of the existing ISO/REMCO Guides as well as the new work items of the committee that will also be transformed into international standards. ISO/TC 334 currently has eight (8) projects in hand in the development of the ISO 33400 series of standards. During the fourth meeting of the committee, it was noted that there was no immediate need to consider revision or transformation of the technical reports developed by the committee.

Figure 1 below depicts the relationships between the different documents previously developed by ISO/REMCO and currently being developed by ISO/TC 334.

- ISO/CD 33400 (previously ISO Guide 30) – Reference materials – Vocabulary
- ISO 33401 (previously ISO Guide 31) – Reference materials – Contents of certificates, labels and accompanying documentation
- ISO/CD TR 33402 (previously ISO Guide 80) – Good practice in reference materials preparation
- ISO 33403 (previously ISO Guide 33) – Reference materials – Requirements and recommendations for use
- ISO 33405 (previously ISO Guide 35) – Reference materials – Approaches for the characterization and assessment of homogeneity and stability
- ISO 33406 (previously WD/ISO Guide 85) – Approaches for the production of reference materials with qualitative properties
- ISO 33407 (previously WD/ISO Guide 86) – Guidance for the production of pure organic substance certified reference materials
- ISO/CD 33408 (previously AWI/ISO Guide 87) – Guidance for 'pure' reference materials for metals and metalloids

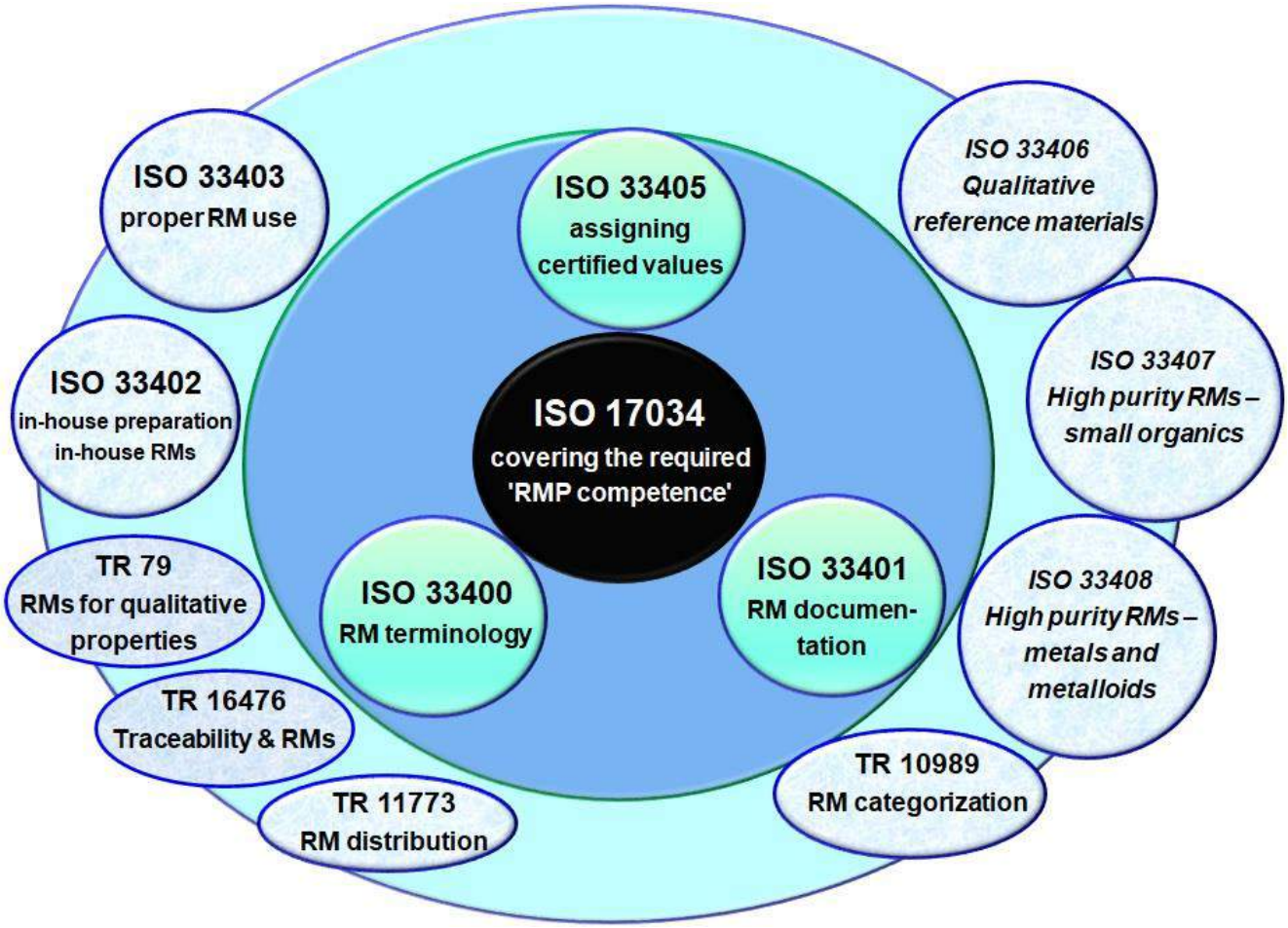

***Figure 1**: Schematic diagram of the planned ISO 33400 series standards for the transformation of the ISO/REMCO Guides 30 to 35 to international standards as well as the other projects of ISO/TC 334*

UPDATE ON THE PROGRESS WITH THE TRANSFORMATION OF THE ISO/REMCO GUIDES INTO THE ISO 33400 SERIES OF STANDARDS

The first two of the new standards of the ISO 33400 series was published in the beginning of 2024, these include ISO 33401 Reference materials – Contents of certificates, labels and accompanying documentation and ISO 33407 Guidance for the production of pure organic substance certified reference materials. Several of the rest of the new standards of the ISO 33400 series have progressed well. The final draft international standard (FDIS)-ballot of these standards have been submitted and the ballots are scheduled to close at the end of March 2024. These include ISO 33403 on the use of reference materials, ISO 33405 for the characterization of reference materials including the assessment of homogeneity and stability as well as the new standard ISO 33406 for the production of reference materials with qualitative properties.

ISO 33400 for the terms and definitions related to reference materials has progressed to the committee draft (CD) stage. Within the working group there has been some discussions about including new terms in the standard, such as operationally defined measurand. There is also strong support in the working group to refer to a certified reference material as a reference material with one or more certified property value and to specify the characteristics of a certified value, such as the requirement for a value with an associated uncertainty and established metrological traceability in the definition of certified value.

ISO 33408 for the production of high purity reference materials of metals and metalloids is at the final working draft (WD)-stage. The working draft still needs more information on purity determination by indirect, rather than direct methods. The convenor of the working group, Dr Zoltan Mester invited ISO/TC 334 members to nominate experts from commercial and industrial reference material producers to ensure wider representation of the community in the working group.

The next meeting of ISO/TC 334 will be a virtual meeting during the week of 3 to 6 June 2024.

CITAC BEST PAPER AWARD 2023

SUMMARY OF "LABEL FREE QUANTIFICATION OF HOST CELL PROTEIN IMPURITY IN RECOMBINANT HEMOGLOBIN MATERIALS"

André Henrion, Cristian-Gabriel Arsene, Maik Liebl and Gavin O'Connor

SUMMARY OF "CALIBRATION MODEL AVERAGING IN CHEMICAL ANALYSIS: A CASE STUDY FOR THE METHOD OF STANDARD ADDITIONS"

Enea Pagliano and Juris Meija

SUMMARY OF "CLINICALLY AND INDUSTRIALLY RELEVANT INCURRED REFERENCE MATERIALS TO IMPROVE ANALYSIS OF FOOD ALLERGENS, MILK, EGG, ALMOND, HAZELNUT AND WALNUT"

Gill Holcombe, Michael J. Walker, Malvinder Singh, Kirstin Gray, Simon Cowen, Stephen L.R. Ellison, Adrian Rogers, Anuradha Balasundaram, Malcolm Burns, E.N. Clare Mills

CITAC BEST PAPER AWARD 2023

SUMMARY OF "LABEL FREE QUANTIFICATION OF HOST CELL PROTEIN IMPURITY IN RECOMBINANT HEMOGLOBIN MATERIALS"

Analytical and Bioanalytical Chemistry. 2024, 416, 387−396

André Henrion[1], Cristian Gabriel Arsene[1], Maik Liebl[1] and Gavin O'Connor[1,2]

[1] Physikalisch-Technische Bundesanstalt (PTB), Bundesallee 100, 38116 Braunschweig, Germany

[2] Department of Biochemistry and Bioinformatics, Technische Universität Braunschweig, 38106 Braunschweig, Germany

André Henrion

Cristian Gabriel Arsene

Maik Liebl

Gavin O'Connor

INTRODUCTION

The source and traceability of primary calibrators is a contributing factor to the overall measurement uncertainty in most chemical analyses. For the provision of reference measurement procedures, where lower estimates of measurement uncertainty are often essential, the contribution of the primary calibrator can be excessive[1,2]. Therefore, researching methods for assessing the purity of primary reference materials has been a major activity for those developing reference measurement procedures. Methods and approaches for determining the quantity of impurities in small organic molecules intended for use as calibrators are well established[3]. However, applying the same approaches is not possible for large complex protein structures. Primary calibrators, used in targeted protein quantification, normally consist of peptides unique to the protein of interest. Alternatively, well characterised protein solution standards, where these are available in known concentrations, can be used[4-6]. Due to the amounts of material normally available the main strategies for assigning mass fractions to these calibrators rely on the amino acid analysis of hydrolysed peptides or proteins. Due to the synthesis methods, clean up approaches used and the relative simplicity of the peptides, a one-by-one identification and quantification of all amino acid-based impurities is possible[7-10]. However, protein-based calibrators are normally purified from their over-expression in cell cultures. During the purification the removal of all other cell derived proteins is desired, but the presence of many different host cell proteins (HCPs) is normal. The sheer number of these HCPs makes a one-by-

one identification and quantification prohibitive[11]. Therefore, a fit for purpose approach, whereby the mass fraction of HCPs could be quantified with measurement uncertainty estimations of less than 15% for proteins intended for use as calibrators is required.

Here, we systematically investigated the use of conventional proteomics based label free quantification (LFQ) approaches for the accurate quantification of the mass fraction of HCPs in a purified protein standard[12-13]. The basic hypothesis of the concept is that the average signal intensity for a peptide obtained on a mass spectrometer is directly related to the amount concentration of the peptide present in the sample. The quantification of proteins is normally achieved via enzymatic digestion of all proteins to peptides. Due to the frequency and natural distribution of lysine and arginine in proteins, trypsin is the protease of choice. For most proteins, the cleaved peptides have an average length of eight amino acids which results in peptides ideally suitable for mass spectrometric detection. Combing the instrumental response, the average size of the peptides and the fact that the number of peptides increase with the increasing molecular mass of the protein, results in the sum of all peptide signals being directly related to the relative mass fraction of the impurity proteins present in the sample. This is slightly different to the normal use of LFQ where normally the top three most intense ions from a protein are used to determine the amount concentration of a single protein. To assess and demonstrate the practical use of this, we investigated the addition of different mass fractions of a background proteome and a mixed standard of purified proteins for determining the unknown mass fraction of HCP in purified protein samples.

EXPERIMENTAL SECTION

Study Materials. The materials to be studied were three separate preparations of a recombinant HbA_2 protein[14,15]. A natural and an isotopically labelled form of the protein was expressed in E. Coli, purified and prepared in buffer at an approximate mass fraction of 0.5 mg/g. The protein of natural isotopic concentration was further purified using ion exchange chromatography. The purified material was prepared at the same mass fraction as the unpurified materials.

Calibrators. A commercially sourced HbA_2 material (99% protein purity as defined by the supplier) was used to prepare a stock standard protein solution in buffer at a mass fraction of 1 mg/g. The material was purity assessed using the LFQ approach and amino acid quantification was used to determine the mass fraction of the target protein in the solid material. An aliquot of this solution was used to provide the constant protein/peptide content present in each of the calibrators (red in Figure 1A)

A lyophilised E. coli protein isolate was commercially sourced. The material was dissolved in buffer and a mass fraction assigned using amino acid analysis. A series of mixed calibration standards were prepared with all solutions containing 0.4 mg of the HbA_2 calibration material per gram of solution and between 10 and 150 mg of total E. coli protein per gram of HbA_2. (red and green respectively in Fig. 1A). A similar series of calibrators were prepared from the commercially sourced total protein extracts from yeast (saccharomyces cerevisiae) and a human K563 cell line. A stock solution containing nine purified proteins was prepared. The purified proteins were assigned mass fractions using amino acid analysis. If the protein was available as a certified reference material the mass fractions provided by the supplier were used. A set of calibration standards, where the total protein mass per mass of HbA_2 matched those of the proteome standards above were prepared. A separate set of standards where the mass fraction of total protein to HbA_2 was between 0.2 and 1.5 mg/g were prepared from the nine-protein mixture, and this was used for the determining the mass fraction of HCP in the purified HbA_2 study materials.

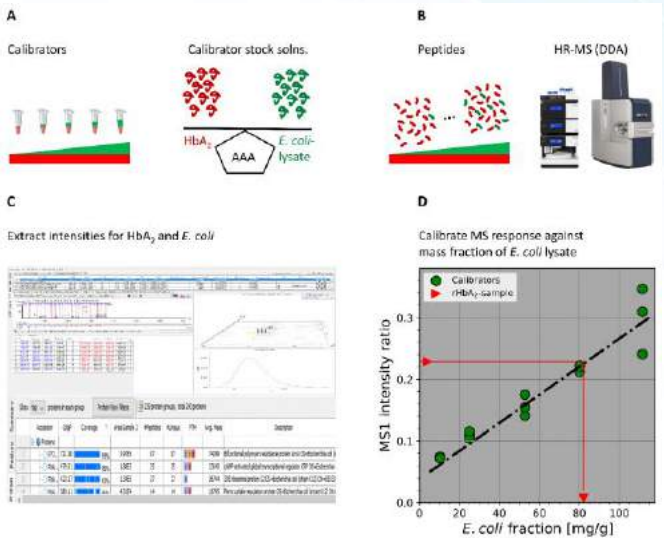

Figure 1. *LFQ-based measurement of HCPs (E. coli) mass fraction in recombinant HbA_2. A Calibrators were obtained by spiking aliquots of HbA_2 stock solution (red) with increasing amounts of E. coli lysate (green). Amounts per mass (mg) of both components (HbA_2 and E. coli) and mass fractions of E. coli were hence known for each calibrator through amino acid analysis. B Quantitative information was acquired by shotgun proteomics. C MS1 intensities were integrated over all features associated with peptides identified from either E. coli or HbA_2. D The sum of all MS1 peak intensities from peptides associated with E. coli per sum of all peak intensities associated with HbA_2 peptides for the calibrators were plotted vs. mass fractions. The fitting linear function was then used to calculate the E. coli fraction in the investigated materials from sample measurements (red line and arrows) (Copied without alteration from Henrion et al. Analytical and Bioanalytical Chemistry (2024) 416:387-396. http://creativecommons.org/licenses/by/4.0/.)*

Proteolysis and analysis. A 30 µL aliquot of sample or calibration standard was mixed with 70 µL of buffer. To each of these solutions 10 µL of a 1 mg/mL porcine trypsin solution was added. Further 10 µL aliquots of trypsin were added after 10, 70, 130, 190 and 250 min. Simultaneously, 40 µL aliquots of acetonitrile were added after 10, 30, 60, 90, 120 and 150 min. Digestion was allowed to proceed overnight. The samples were reduced with DTT and alkylated with iodoacetamide. The digestion reaction was quenched using 10 µL of a 10% aqueous solution of formic acid. The samples were cleaned and desalted using C18 solid phase extraction before being lyophilised. The residue was redissolved in 40 µL of 0.1% aqueous formic acid prior to LCMS analysis.

LCMS Analysis and data processing. The peptides were first trapped on-line using a C18 pre column before being separated using an acetonitrile gradient on a C18 nanoflow column. The nanoflow liquid chromatography system was coupled with a Bruker tims-TOF Pro mass spectrometer. The peptides were ionised using a captive spray, nano electrospray ionisation source. The mass spectrometer was run in a data dependant trapped ion mobility MS/MS mode. Spectra were acquired in a *m/z* range of 100 to 1700 with a ramped collision energy dependant on ion mobility.

The raw precursor and product ion data were processed using PEAKS Studio Xpro software. This was used for feature selection and assignment of protein-based precursor intensities. The peptide sequences for all E. coli and yeast proteins as well as the nine proteins used in the protein mixed standard were used as the protein database. The intensities of precursor ions of all peptides greater than seven amino acids in length which were identified with a false discovery rate of 1% were summed at the protein level. These were further processed using Python scripts to give the sum of all intensities relative to the sum of HbA_2 ion intensities.

RESULTS AND DISCUSSION

The mass fraction of the HCPs in the test materials was determined using a common bottom-up shotgun proteomics approach. The sum of all precursor ion intensities from all peptides derived from E. Coli, as identified via their product ion spectra and data base searching, were ratioed to the sum of all HbA_2 peptide precursor ion intensities. This ratio was plotted against the known mass fraction of the calibration standard as determined via amino acid analysis. The resulting calibration plot (Figure 2A) from 3 replicate injections at each concentration was used to determine the mass fraction of HCP in the samples.

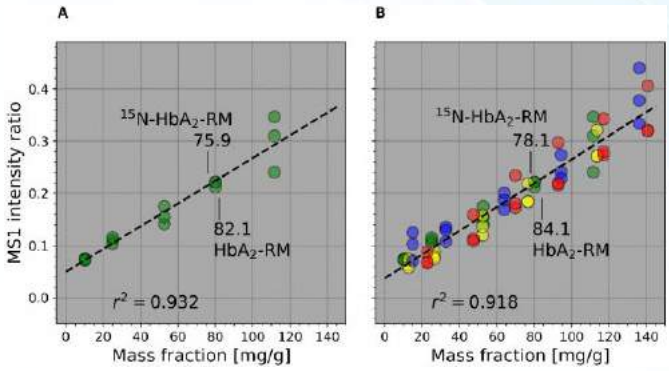

Figure 2. *Calibrating MS1 intensity ratios (proteome ÷ HbA$_2$) vs. mass fractions of HCPs impurity in HbA$_2$. A Calibration using E. coli lysate. B Joint calibration using a set of different proteomes in addition to E. coli: E. coli lysate (green), yeast (yellow), human K562 cell line (blue), and a mix of nine neat proteins (red). Dashed lines: linear regression fit. Annotations: results for the HbA$_2$ material and the U-15N-labeled HbA$_2$ material using these calibrations. (Copied without alteration from Henrion et al. Analytical and Bioanalytical Chemistry (2024) 416:387-396. http://creativecommons.org/licenses/by/4.0/.)*

As expected, the peptide and protein profiles from the whole cell lysate were markedly different from the HbA$_2$ samples. As is common practice, the materials had been purified using immobilized metal affinity cleanup. This resulted in a much smaller subset of co-purified proteins with just three protein impurities making up 60 – 70% of the HCPs in the HbA$_2$ samples. A principal components approach was used to highlight the differences between the background impurities in the calibrators and the sample materials. This demonstrated the inability to predict the background HCP mass fraction using individual proteins from another model. However, the model created from the sum of intensities from all peptides from all proteins ratioed to the sum of the HbA$_2$ peptide intensities did enable prediction. This supports the hypothesis that the peptides are representative of a common statistical population. If true, this would enable the prediction of other HCP mass fractions from different cell lines or even a set of model proteins[16-18]. This is shown in the calibration in Figure 2B where the intensities of the different cell lines and the standard nine-protein mixture are plotted on the same axis as those from the E. Coli peptides (Figure 2A).

The estimation of the overall uncertainty of the method, combined the uncertainty associated with the mass fraction of the calibrators, the uncertainty associated with the linear prediction model and the repeatability of the method[17,18]. This resulted in an overall assessment of the impurity mass fractions as 84 ± 30 mg HCP impurity/g HbA$_2$ and 78 ± 14 mg HCP impurity/g HbA$_2$ in the natural and isotopically labelled protein samples, respectively. In terms of protein purity, this results in protein purities of 923 and 928 mg HbA$_2$/g total protein with relative expanded uncertainties of 2.8% and 1.3%, respectively. In terms of protein calibrators, these uncertainties would be considered fit for purpose for most applications. However, to demonstrate the potential of the approach a smaller calibration range was prepared and the mass fraction of the HCP was determined in an ultra-purified HbA$_2$ material. The result predicted a mass fraction of 0.86 ± 0.22 mg HCP/g HbA$_2$ protein. This is equivalent to a predicted purity of 999.1 mg HbA$_2$/g total protein with an uncertainty of 0.02%.

The results suggest that in practice a set of peptides from known mass fractions of a proteome can be used to determine the mass fraction of the impurity proteins in a purified protein. However, the approach described here requires the peptide molar intensities to be derived from a statistical population that is shared between calibrator and sample. To determine the size and complexity of the peptide population required for accurate prediction and its impact on the estimation of uncertainty, a simulation was performed using the nine-protein mixed standard and the natural HbA$_2$ sample. The prediction model shown in Figure 3 suggests that as few as five representative proteins could provide peptides suitable for determining HCP mass fractions with uncertainties less than 15%, obviating the need for reference to complex cell-derived materials such as E.coli, yeast or K562 for calibration.

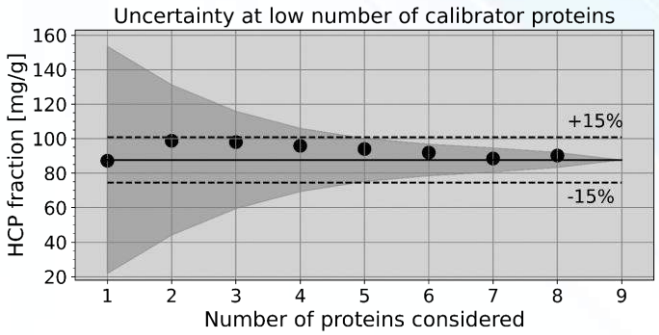

Figure 3 *Estimating the sampling error caused by the finiteness of the number of proteins/peptides used for calibration or present in the sample. The data shown are results for the HbA_2 material after stepwise reduction of the number of proteins included in calibration with the protein mix. Solid line: median obtained at n = 9 (87.7 mg/g), dashed: ± 15%. Scatterpoints: median results after recalculating the calibration function using random drafts of n = 1–8 out of the originally nine proteins. Dark grey area: corresponding standard deviations (shown here relative to the solid line, rather than to the medians) (Copied without alteration from Henrion et al. Analytical and Bioanalytical Chemistry (2024) 416:387-396. http://creativecommons.org/licenses/by/4.0/.)*

Finally, an assessment of an approach using just the top 5 E. coli proteins in the IMAC purified HbA_2 samples was performed. The results suggested that such an approach could result in an over-estimation of the purity by as much as 2.5%. This may still be deemed fit for purpose for many applications.

CONCLUSIONS

The LFQ approach outlined was suitable for the quantification of co-purified HCPs in bioengineered proteins. The approach of calibrating the sum of relative intensities of all HCPs peptides to the sum of the peptides from the purified protein enable the relative mass fraction of background protein to that of the purified protein to be easily determined. The hypothesis that the peptides act as a random statistical pool common between sample and standards, no matter the complexity of this pool was proven via the use of a simple nine-protein mixture to obtain equivalent results to those produced from the whole cell lysates. The method's expanded uncertainty for the IMAC purified proteins at approximately 920 mg HbA_2/g total protein was between 1- 3%. As is common in most purity assessment this uncertainty reduced to as low as 0.02% for an ultra-pure material (>99%).

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CITAC BEST PAPERS AWARD 2023

SUMMARY OF "CALIBRATION MODEL AVERAGING IN CHEMICAL ANALYSIS: A CASE STUDY FOR THE METHOD OF STANDARD ADDITIONS"

Metrologia. 2023, 60, 035003

Enea Pagliano and Juris Meija

National Research Council Canada, 1200 Montreal Road, Ottawa ON K1A 0R6, Canada

Enea Pagliano

Juris Meija

INTRODUCTION

Quantitation of chemical substances requires a reliable set of empirical observations along with a mathematical model that turns the experimental data into the measurement result. Since the advent of instrumental analytical methods, significant efforts have been devoted to improve the data generation process through the development of methods that achieve lower detection limits, better specificity, precision, and sample throughput. Despite this, research on mathematical models used in analytical chemistry remains rather limited. In fact, most analytical methods assume a linear relationship between the signal and concentration. Furthermore, the suitability of the linear model is often assessed by a visual inspection of the calibration curve (1) and most scholars are satisfied with a coefficient of determination larger than, say, $R^2 = 0.99$.

In our recent study, we have shown that this approach can lead to overconfident measurement results even from well-designed measurements (2). As the use of a single measurement model can lead to biased results, we propose the application of calibration model averaging as a method to obtain more reliable measurement results.

THE PROBLEM

We illustrate the problem of unreliable measurement results in analytical chemistry using the following experiment. An aqueous standard solution of nitrate was gravimetrically prepared with known levels of nitrate, $w(NO_3^-)$ of 50.5 ± 0.2 mg kg^{-1} (95 % confidence interval). This solution was then treated as a sample and nitrate was determined using the method of standard additions. To achieve the best measurement uncertainty, nitrate was converted into volatile $EtONO_2$ by derivatization with triethyloxonium tetrafluoroborate and detected by headspace GC-MS using $^{15}NO_3^-$ as isotopic internal standard (3). Fig. 1 shows the measurements results. Although

these data are of high-precision (individual analytical signals are typically measured to within 0.5 % precision), with no obvious deviations from the linear relationship, ordinary least squares fitting gives a mass fraction of nitrate that is biased by 5 %, $w(NO_3^-) = 53.3 \pm 2.2$ mg kg^{-1}. *Why?*

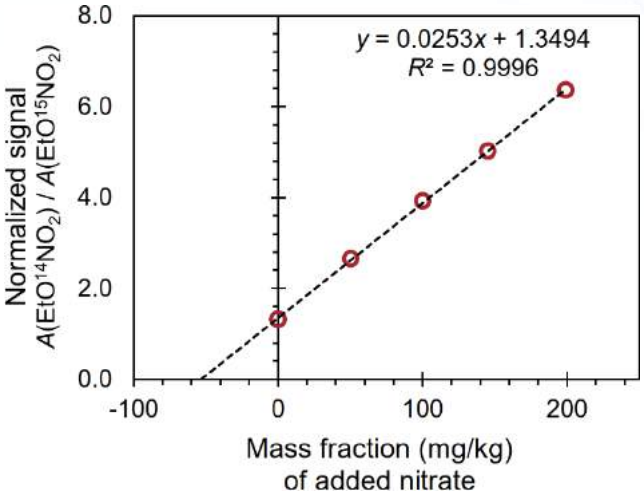

Figure 1. Determination of nitrate ions in aqueous standard solution using a conventional standard additions method. The linear calibration model (ordinary least squares fitting) provides a result, $w(NO_3^-) = 53.3 \pm 2.2$ mg kg^{-1}, that is inconsistent with the known value $w(NO_3^-) = 50.5 \pm 0.2$ mg kg^{-1} (95 % confidence intervals).

ROOT CAUSE AND NONLINEARITY

When an isotopically labeled analyte is used as an internal standard (i.e., isotope dilution mass spectrometry), a nonlinear response curve between the amount of the analyte and the measured isotope ratios is theoretically expected (4). Such nonlinearity arises from small overlaps of the signals of the natural analyte and enriched internal standard, with the magnitude of the nonlinear trend proportional to the extent of such overlap (5).

Although the nonlinear behavior is extremely low in the example shown in Fig. 1, the standard addition curve is, nevertheless, inherently nonlinear. Hence, one could expect an advantage when using a nonlinear calibration model. Indeed, a quadratic equation gives a result that is indistinguishable from the known gravimetric value: $w(NO_3^-) = 49.1 \pm 3.6$ mg kg^{-1} (95 % confidence interval). Thus, this experiment has shown that even an unnoticeable nonlinear trend can be a major source of bias and that the choice of the measurement model has a major influence on the reliability of the results. *What can we do about it?*

BAYESIAN INFORMATION CRITERION AND MODEL AVERAGING

Calibration models are often empirical meaning that they are chosen not because of some theoretical considerations but because of their performance. When several competing models are available, model comparison can be performed using the Bayesian information criterion (BIC or simply B) which provides a quantitative estimate of how well a particular model fits the data while considering the mathematical complexity of the calibration model as well (6). Assuming Gaussian measurement errors, B can be calculated for any given calibration model, $y = f_i(x)$, as follows:

$$B(f_i) = -2L_i + (1 + k_i)\ln(n) \qquad \text{Eq. 1}$$

$$L_i = -\frac{n}{2}\ln(2\pi e s_i^2) \qquad \text{Eq. 2}$$

$$s_i^2 = \frac{1}{n}\sum_{j=1}^{n}\left(y_i - f_i(x_j)\right)^2 \qquad \text{Eq. 3}$$

Here, k_i is the number of parameters in the measurement model f_i and n is the number of data points ($\{x_1, y_1\}, \ldots, \{x_n, y_n\}$).

A model with the lowest value of B is typically selected as the "best" measurement model while all other candidate models are simply discarded. However, when there is a doubt about the exact nature of the data generation process, discarding all other reasonable models might not be wise and averaging the results of all competing calibration models might be preferable. In fact, there are even examples with compelling reasons to favor model averaging: the radiocarbon dating of cultural artefacts from Machu Picchu is a well-known recent example where model averaging played a significant role in deciding when the Inca citadel was built (6-8).

When no sufficient theoretical reasons support the choice of a single model, results from all candidate models should be averaged, each with a probability (p_i) that is proportional to how well the model fits the data (x, y):

$$w = \sum_i p_i f_i(x, y) \qquad \text{Eq. 4}$$

Eq. 4 is the mathematical foundation of the model averaging in chemical analysis. When all candidate calibration models f_i are considered with equal prior probability, then the statistical model weights are given from B as follows (9):

$$p_i = \frac{e^{-B(f_i)/2}}{\sum_j e^{-B(f_j)/2}} \qquad \text{Eq. 5}$$

How this mathematical framework can be applied for experiments of standard additions?

MODEL AVERAGING APPLIED TO THE METHOD STANDARD ADDITIONS

For the method of standard additions, polynomial (10) and rational (11) calibration functions have been used when linear model has been deemed unsuitable. However, this has been always done in the framework of a single best calibration model. Here we show that the shortcomings of using a single empirical calibration model can be addressed by model averaging. In this proof-of-concept study, we have considered four calibration models for the interpretation of standard additions data:

linear	$x = a + by$
rational	$x = (a + by)/(1 + cy)$
quadratic	$x = a + by + cy^2$
reduced cubic	$x = a + by + cy^3$

The model fitting was performed using ordinary linear least squares (OLS) and the coordinate swapping method was employed to obtain the results from each calibration model (12). Thus, y was the analytical signal and x the amount of analyte added to the sample aliquots. Under such an approach, the standard additions result (mass fraction of the analyte, w) from any of these calibration models is the intercept coefficient (a). Thus, $w = a$ and $u(w) = u(a)$. All calculations were performed using R and the source code can be found here: https://github.com/meijaj/model-averaging-in-standard-additions (last visited on 2024-03-22).

When this set of calibration models was applied to the data of Fig. 1, the result obtained by model average is $w(NO_3^-)$ = 49.5 ± 3.4 mg kg^{-1}, in good agreement with the true gravimetric value of 50.5 ± 0.2 mg kg^{-1}.

linear	w = 53.3 ± 2.2 p = 5 %
rational	w = 49.0 ± 3.4 p = 50 %
quadratic	w = 49.1 ± 3.6 p = 28 %
reduced cubic	w = 50.4 ± 3.0 p = 17 %
model average	w = 49.5 ± 3.4

Worth noting is that the statistical weight of the rational model – which is, in this case, the true theoretical model describing the curvature of the calibration plot (4) – is the highest of all other calibration models. One may argue that the example shown in Fig. 1 uses a sophisticated analytical approach based on isotope dilution and therefore may not be representative of typical measurement. *Are there other examples where model average outperforms the linear model?*

MEASURING NITRITE IN SEAWATER

A standard solution of nitrite was gravimetrically prepared in a low-nutrient seawater (OSIL, UK) with a known mass fraction of $w(NO_2^-)$ = 0.3657 ± 0.0015 mg kg^{-1} (95 % confidence interval). This solution was treated as a sample and nitrite was determined using the method of standard additions (Fig. 2) by UV-Vis spectrophotometry at 541 nm wavelength using the well-known Griess method (13, 14). All absorbance readings were in the interval from 0.4 to 0.9 where the deviations from the Beer-Lambert law are considered negligible. Despite the seemingly linear response (Fig. 2), the standard additions result based on linear calibration model is biased by more than 10 %: $w(NO_2^-)$ = 0.4040 ± 0.0079 mg kg^{-1} (95 % confidence interval). However, the model averaging yields a result in an agreement with the gravimetric value (Fig. 2).

In our manuscript we provide a number of additional practical examples where model averaging provides more reliable results than the classical linear calibration model (2).

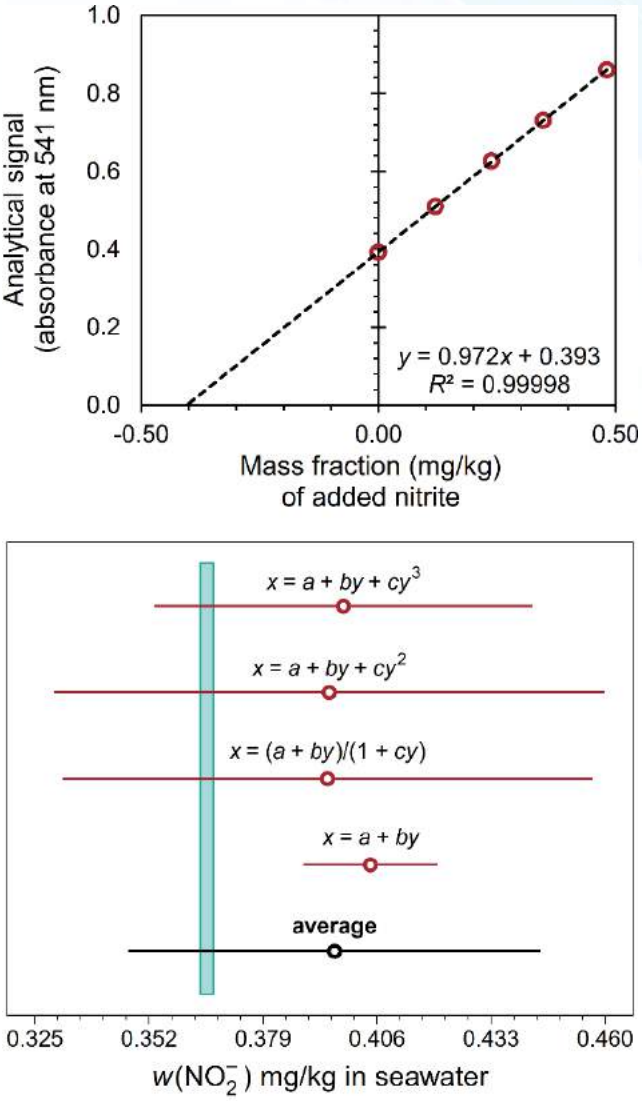

Figure 2. Determination of nitrite ions in seawater by spectrophotometry (Griess method) using the method of standard additions. Top: the standard additions calibration plot with the linear model. Bottom: comparing the performance of different measurement models. Error bars represent 95 % coverage intervals and the blue rectangle represents true value and its uncertainty.

CONCLUSIONS AND FUTURE PERSPECTIVE

The results of standard additions method can be strongly influenced by the choice of the calibration model. Indeed, even when the calibration plots appear linear, the traditional implementation of the linear calibration model can yield biased results with underestimated uncertainties. We have shown that this shortcoming can be remedied by taking the weighted average result from several reasonable calibration functions and we argue that this mathematical approach should be embraced in all cases where doubts exist regarding the true calibration model.

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CITAC BEST PAPER AWARD 2023

SUMMARY OF "CLINICALLY AND INDUSTRIALLY RELEVANT INCURRED REFERENCE MATERIALS TO IMPROVE ANALYSIS OF FOOD ALLERGENS, MILK, EGG, ALMOND, HAZELNUT AND WALNUT"

https://doi.org/10.1016/j.foodchem.2023.137391

Gill Holcombe[1], Michael J Walker[1,2*], Malvinder Singh[1], Kirstin Gray[1], Simon Cowen[1], Stephen L R Ellison[1], Adrian Rogers[3], Anuradha Balasundaram[4**], Malcolm Burns[1] and E. N. Clare Mills[4,5]

[1] LGC Ltd Queens Road Teddington Middlesex TW11 0LY, UK. E-mail: Gill.Holcombe@lgcgroup.com

[2] Institute for Global Food Security, Queen's University Belfast, BT9 5DL, UK. E-mail michael.walker@qub.ac.uk *Correspondence

[3] Romer Labs UK Ltd., The Heath Business & Technical Park, Runcorn, Cheshire WA7 4QX, UK, current address: Bio-Check (UK) Ltd., Spectrum House, Llys Edmund Prys, St Asaph Business Park, Denbighshire, LL17 0JA, UK

[4] Division of Infection, Immunity and Respiratory Medicine, School of Biological Sciences, Manchester Institute of Biotechnology, Manchester Academic Health Sciences Centre, The University of Manchester, 131 Princess Street Manchester M1 7DN, UK,

[5] School of Biosciences and Medicine, The University of Surrey, Guildford, Surrey, GU2 7XH, UK

**Author's photo was not provided at the time of publication

Gill Holcombe

Michael J Walker

Malvinder Singh

Kirstin Gray

Simon Cowen

Stephen L R Ellison

Adrian Rogers

Malcolm Burns

E. N. Clare Mills

INTRODUCTION

Food allergy is a well-recognised public health, business, and regulatory challenge.[1,2,3,4,5,6] Risk assessments calculate critical allergen protein concentrations ('Thresholds') against which analytical results can be interpreted by dividing an eliciting dose (amount (mg) of allergenic protein) by the amount of food consumed, e.g. the Voluntary Incidental Trace Allergen labelling scheme (VITAL®),[7,8,9,10] and the *Integrated Approaches to Food Allergen and Allergy Management* (iFAAM) consortium. Such an approach can improve the application of precautionary allergen labelling, well recognized as currently sub-optimal[11,12] and allergen risk management generally. However, measurement of critical allergen concentrations, whether by protein or DNA markers, suffers well known analytical problems.[3] The availability of well characterised calibrants and reference materials (RMs) would allow comparability and harmonisation of measurements between methods and laboratories, assist in method development and provide meaningful information for risk assessment. There have been many calls for RMs to support food allergen analysis; however, of the relatively few such RMs available, many raw ingredient RMs were not originally intended as allergen protein RMs, and the number of matrices represented is currently low.

Herein we describe the preparation and characterisation of the first industrially and clinically relevant multi-allergen natural matrix RM kit to quality assure analysis for five major allergens. Consensus information on priority allergens, the desired physical format of RMs and fortified concentrations were explored and agreed at a stakeholder meeting and consultation exercise. The RM kit is based on the matrix and allergens used for EuroPrevall clinical threshold studies.[14,15] The matrix is a polyphenol-containing chocolate paste prepared using commercial food ingredients, a processed food matrix of medium analytical difficulty. The RM kit contains the matrix (a) devoid of the five allergens, and (b) incurred with each of those allergens at the concentration of 10 mg kg^{-1} expressed as protein. This concentration is clinically relevant because it approximates to clinically derived eliciting doses and typical food intakes. In the context of this paper, unless otherwise indicated the term 'allergen protein(s)' means the total protein concentration of the allergen food, rather than the concentration of specific allergen protein molecules. The allergens, hens' egg white powder, skimmed cows' milk powder, almond powder (full fat), hazelnut powder (partially defatted), and walnut powder (partially defatted), are also available as RMs. This study is a further model for food allergen RM production, characterisation and curation building upon initial work on a peanut RM.

MATERIALS AND METHODS

Gravimetric preparation of RM mass fractions traceable to the International System of Units, (SI, Système International) was done by trained staff using calibrated balances to appropriate precision. Preparation was carried out to written protocols in a restricted access laboratory not previously used for food analysis.

Raw materials representing food allergens and matrix ingredients were obtained from reputable suppliers based on previous experience of sourcing for clinical challenge studies (EuroPrevall and iFAAM). Sourcing criteria included: traceability to the allergen plant or animal species without allergen cross-contamination; material representative of the allergen food as it is processed, eaten, and in allergen profile; capable of homogeneous distribution within a food matrix and manufacture at a multi-kg scale; stability with a shelf-life of 3-5 years and preferably stable in storage and shipping under ambient conditions. Prior to handling, laboratory surfaces were cleaned, and swab tested negative for all five allergens (Romer Ltd. AgraStrip® Lateral Flow Device (LFD).

The candidate RM raw materials were visually assessed and sieve tested. Skimmed milk and egg white powders passed 0.5 mm (>99.9 %), partially defatted hazelnut passed 1.0 mm (99.6 %) and 0.5 mm (94.5 %) and almond passed 1.0 mm (83.4 %) and 0.5 mm (29.1 %). Visually the partially defatted walnut powder appeared less homogenous and was milled to pass 0.5 mm (ultra-centrifugal high speed rotor mill, Retsch, Hope Valley, UK). Each material was separately tumble mixed under argon and filled (1.1 ± 0.1 g) into pre-labelled 2 mL amber glass vials under argon and closed. Each vial was sealed inside a metallised sachet (Polypouch UK Ltd, Watford, UK), and stored at (5 ± 4) °C.

Water was determined using an ISO/IEC 17025 (Calibration) accredited coulometric oven Karl Fischer procedure (Metrohm 744, Herisau, Switzerland).[16] Nitrogen as a proxy for total protein was determined using an ISO/IEC 17025 (Testing) accredited automated Dumas method[17] (AOAC International, 1992) Rapid N cube analyser, (Elementar UK Ltd, Stockport, UK) calibrated using an EDTA Organic Analytical Standard (Elemental Microanalysis Ltd, Okehampton, UK) with a certified value for nitrogen traceable to National Institute of Standards and Technology (NIST) NIST SRM 143d (Gaithersburg, USA), with metrological traceability to SI units through the NIST PS1 Primary Standard for quantitative NMR (Benzoic Acid). Total protein content was calculated using Equation 1:

$$P = f.N\,, \qquad (1)$$

where P = total protein (%), N = empirical nitrogen content (%) (Dumas) and f is the conversion factor.[18]

Enzyme Linked Immunosorbent Assay (ELISA) determinations used AgraQuant casein, egg white protein, hazelnut, and walnut kits (Romer Laboratories Ltd UK, Runcorn, UK), and Ridascreen FAST Milk, Egg protein, Almond, and Hazelnut kits (R-Biopharm AG, Darmstadt, Germany).

The matrix was prepared in line with the EuroPrevall standardized low-dose double-blind placebo-controlled challenge vehicle.[14] Trial batches optimised the composition. 20 kg was made and dispensed in (5.5 ± 0.5) g portions into 15 mL amber glass bottles, with tamper-evident screw caps and integral plugs. The 3000 units so produced were stored at (5 ± 4) °C.

A further 22 kg of blank matrix was prepared. The fortified material for inclusion in the RM kit was produced at 5000 mg kg^{-1} of each allergen respectively expressed as protein and serially diluted with 'blank' paste to 250 mg kg^{-1} and 10 mg kg^{-1} as (each) allergen protein. The 3000 units so produced were stored at (5 ± 4) °C.

Data were collected and saved in Microsoft Excel (2016) format. For homogeneity and stability studies, statistical analysis used the R statistical software environment.[19] Homogeneity and stability data were analysed using mixed effects modelling with restricted maximum likelihood (REML) to provide minimally biased variance estimates.[20,21] Significance tests for mixed effects models used the lmerTest package for R.[22] Tolerance interval calculations used the 'tolerance' package for R,[23] version 1.3.0, using the method of Howe.[24]

RESULTS

THE CANDIDATE RM RAW MATERIALS

Purified DNA was extracted from the almond, hazelnut, and walnut candidate reference materials. Pairs of species-specific oligonucleotides were used to direct the Polymerase Chain Reaction (PCR) amplification of polymorphic regions for the plastid *matK* and *rbcL* loci. Sanger DNA sequencing was performed on each of the amplified products in both forward and reverse directions. The resulting DNA sequence data were compared with annotated DNA sequence data using the Basic Local Alignment Search Tool BLASTn (v. 2.9.0) sequence alignment software[25] against DNA sequences accessible at the National Center for Biotechnology Information (NCBI) GenBank database.[26] The appropriate species identities were confirmed. Allergen profiles in the raw materials were characterised using a combination of immunoblotting using specific antibodies and discovery mass spectrometry using data dependent acquisition. Homologous allergens from the major plant food allergen families were identified across the plant species and shown to be present in an immunologically active form[27] These data demonstrated the suitability of the ingredients for the preparation of allergen reference materials.

For each material, fifteen units were analysed for water and nitrogen content in triplicate over three runs using a randomised block design, except for nitrogen in the skimmed milk powder where nine units were analysed in duplicate, three units on three different days, forming a nested design. Initial inspection suggested some run order effects for nitrogen. Following ISO Guide 35:2017,[28] run order was controlled for, where significant, by inclusion as an additional variable in the final fitted models for variance estimation. For materials that did not exhibit run-order trends, the models for estimating the between- and within-unit variances (s_b^2 and s_w^2) included only RM unit and run as random effects and the mean as a single fixed effect. Following variance estimation, a homogeneity contribution to the certified value uncertainty was calculated using Equation 2

$$u_{\text{hom}} = \max\left(\frac{s_w}{\sqrt{r}}, s_b\right), \quad (2)$$

where u_{hom} is the standard uncertainty associated with possible inhomogeneity, s_{w} the within-unit (residual) standard deviation, s_{b} the between-unit standard deviation and r the number of replicate observations per unit. For both analytes, the homogeneity of the ingredient materials was considered fit for purpose.

Deterioration is not expected over the lifetime of the dry ingredient materials when stored under the recommended conditions (unopened at (5 ± 4)°C); no pre-release stability study was considered necessary. The materials will be subjected to regular testing under LGC's stability monitoring programme during their sales lifetime.

Uncertainties for water and nitrogen content were evaluated following the principles of ISO Guide 35[28] and the ISO Guide to the Expression of Uncertainty in Measurement (GUM).[29,30] The general model used is shown in Equation 3:

$$u_{\text{CRM}} = \sqrt{u_{\text{char}}^2 + u_{\text{hom}}^2 + u_{\text{lts}}^2}, \quad (3)$$

where u_{CRM} is the (combined) standard uncertainty associated with the certified value and u_{char}, u_{hom} and u_{lts} the standard uncertainties for the characterisation measurements, the homogeneity, and long term stability, respectively. Noting the low risk of deterioration, u_{lts} was set to zero.

Expanded uncertainties were calculated using a coverage factor based on Student's t with effective degrees of freedom calculated using the Welch-Satterthwaite equation.[35] Coverage factors were set to 2.0 unless the effective degrees of freedom led to a higher value.

The mean water, nitrogen, and total protein contents of the allergen raw materials, with their expanded uncertainties, are shown in Table **1**. Protein contents corresponded with specification or literature values, though those for the partially defatted hazelnut and walnut powders were, as expected, higher reflecting the lower lipid content. While an expanded uncertainty is given for the water and nitrogen data this is not carried through to the calculated total protein content, which is provided on the statement of measurement[31] as information values[32] rather than certified values. This is because the nitrogen factor will vary according to the source of the ingredient and it is not currently possible fully to characterise the uncertainty arising from incomplete knowledge of the precise sources of nitrogen (which is known to be non-zero).[33] The factors used are, however, provided to users of the RMs to allow recalculation using plausible alternatives to support risk assessment or regulatory action.

Table 1. Mean water, nitrogen, and protein contents of the allergen raw materials

Material	Water content Mean ± *U* g/100 g	Nitrogen content Mean ± *U* g/100 g	Conversion factor N to total protein [a] (unitless)	Total Protein g/100 g	Specification or literature protein content g/100 g
Skimmed milk powder	4.22 ± 0.40	5.40 ± 0.17	6.38 6.21[b]	34.45 33.53	35.6[c] 36.1[d]
Egg white powder	6.01± 0.53	13.49 ± 0.41	6.25	84.31	85[c]
Almond powder	4.22 ± 0.54	4.19 ± 0.13	5.18	21.70	21.4[c] 21.1[e]
Hazelnut powder – partially defatted	8.6 ± 1.1	4.99 ± 0.16 *k* = 2.03	5.3	26.45	14.1[f]
Walnut powder – partially defatted	6.11 ± 0.65	6.15 ± 0.19	5.3	32.59	14.7[g]

U is the expanded uncertainty expressed as the half-width of the expanded uncertainty interval calculated using a coverage factor *k* which gives a level of confidence of approximately 95 %. Unless otherwise stated, *k* = 2.

a. See (McCance and Widowson 2002) and notes below. The abstraction of lipids from the partially defatted hazelnut and almond powders causes the expected increase in the overall protein content but may also have affected the conversion factor to some degree owing to the loss of nitrogen-containing lipids.
b. Based on the amino acid sequences of the milk proteins and their mass fractions in total cow's milk protein: Martinez-Esteso, M.J., O'Connor, G., Nørgaard, J., Breidbach, A., Brohée, M., Cubero-Leon, E., Nitride, C., Robouch, P. and Emons, H., 2020. A reference method for determining the total allergenic protein content in a processed food: The case of milk in cookies as proof of concept. *Analytical and Bioanalytical Chemistry*, *412*, pp.8249-8267.
c. Supplier information
d. McCance & Widdowson's 'The Composition of Foods' 5th Ed 1991, Record no. 200
e. McCance & Widdowson's 'The Composition of Foods' 5th Ed 1991, Record no. 972 (water 4.2g/100g, fat 55.8g/100g)
f. McCance & Widdowson's 'The Composition of Foods' 5th Ed 1991, Record no. 980, (water 4.6g/100g, fat 63.5g/100g, confirms partially defatted)
g. McCance & Widdowson's 'The Composition of Foods' 5th Ed 1991, Record no. 997, (water 2.8g/100g, fat 68.5g/100g, confirms partially defatted)

BLANK MATRIX PASTE

The blank paste material was prepared in a clean environment. To check the anti-cross-contamination precautions, the blank paste matrix was assessed by ELISA for casein, egg white protein and hazelnut. Thirty units were chosen by a random stratified selection for each analyte. Each unit was extracted once and plated in duplicate in a randomised run order. All absorbance data were below that of the lowest standard. Casein data were converted to milk protein using 80 % as the approximate casein content resulting in an (in buffer kit cited) LOD of <0.05 mg kg^{-1} milk protein. Hazelnut was reported as whole nut calibrated against commercially available raw (not roasted) whole hazelnut hence hazelnut protein was calculated assuming 14.95 % protein in the calibrant[34]. Milk protein and egg white protein were determined to be <0.05 mg kg^{-1} while hazelnut protein was found to be <0.04 mg kg^{-1} in the blank matrix paste.

FORTIFIED MATRIX PASTE

Almond and hazelnut flours were sieved (0.5 mm) immediately before incorporation in the fortified RM. Nitrogen determinations (Dumas) after sieving showed no significant difference from the homogeneity study means at the 95 % level of confidence.

Homogeneity assessments were carried out for all five allergen ingredients. For skimmed milk powder, egg white powder and hazelnut, fifteen units were analysed by ELISA in a randomised block design, each unit being measured once in each of three runs. For almond and walnut, for operational reasons, 16 units of each were measured in duplicate, with units randomly allocated to separate runs to form a nested design. For all ELISA measurements, each measurement consisted of the mean of two well readings.

Statistical analysis used plots for visual inspection and linear models to check for outlying observations, ELISA plate (run) effects, well location or run order effects, and fill or preparation order. There were no significant filling order effects, though one run for casein showed a significant run order trend (which was controlled for when determining the within- and between-unit standard deviations for casein) and one possible high outlier was noted in the hazelnut data. In addition, inspection of the hazelnut data (Figure 1) showed a substantially greater between-unit dispersion than for other allergens (about 26 % as a relative standard deviation). The within-unit dispersion for hazelnut was approximately proportional to the mean value for the unit and the distribution of mean values positively skewed, symmetry being improved on log transformation. Hazelnut was accordingly treated separately as explained below.

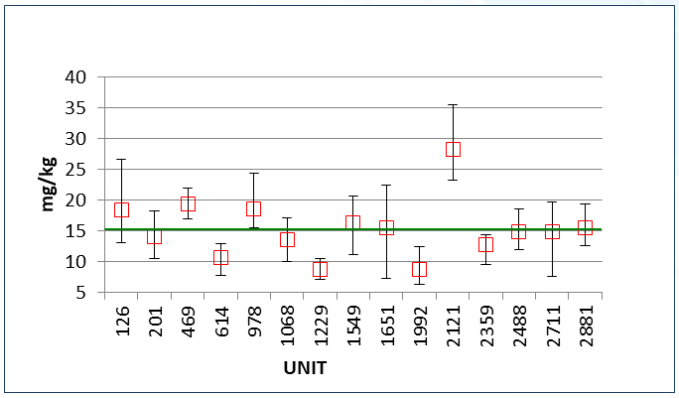


Figure 1. Hazelnut homogeneity. The figure shows the median (squares) and extremes (error bars) of the replicate results for hazelnut, mg/kg, for each unit.

For hazelnut, noting the large between-unit standard deviation, the increase in between-replicate difference with level and the approximately log-normal distribution of mean values, inhomogeneity was characterised in terms of a 95 %/95 % tolerance interval calculated from log-transformed data, instead of a simple between-unit standard deviation.

Assessment of short-term stability, also called transportation stability,[28] is important for determining shipping conditions and can assist decisions on instructions for storage and use at end-user premises. A stability assessment was carried out for the egg white protein, casein, and hazelnut content in the fortified matrix RM. The study was carried out as an isochronous study.[28] Statistical analysis used mixed-effects models with RM unit and run as random effects, and time and temperature treated as fixed effects. Broadly, the transportation stability results indicate that appreciable degradation in transit is likely at higher temperatures, particularly near 60 °C. Shipping chilled is accordingly recommended. Since the principal object of the short term stability test is to choose shipping conditions for negligible degradation, no uncertainty allowance for short term stability was made.

A brief accelerated longer-term stability study of the milk, egg and hazelnut protein ingredients was undertaken to confirm adequate stability under planned storage conditions (5 ± 4) °C. The study was carried out as an isochronous study.[28] Following inspection for anomalies, resulting in exclusion of three units for casein for which one or more values exceeded the upper limit for the test kit, mixed-effects models were used to fit separate straight-line models for each temperature assessed, with unit-to-unit and between-plate variation as random effects. Significance tests used degrees of freedom calculated using Satterthwaite's method.[35] There was no compelling evidence of instability at the temperatures studied over the timescale of the study. Given the stability evidence and the planned regular post-certification monitoring programme, no allowance for possible degradation was included in the uncertainty for the reference values.

Assigned values with expanded uncertainties or, for hazelnut, an expanded uncertainty interval, are summarised in Table 2. The uncertainty evaluations are described below.

Table 2. Assigned values and uncertainties for the fortified RM

	Assigned value (gravimetric), mg/kg	Standard uncertainty u_{RM}, mg/kg	k	Expanded uncertainty[a] U_{RM}, mg/kg
Milk protein	10.01	0.87	2.06	1.8
Egg white protein	10.03	0.70	2.04	1.5
Almond protein	9.71[b]	0.86	2.11	1.9
Walnut protein	10.00[b]	1.06	2.12	2.3
Hazelnut protein	9.8[b]	N/A[c]	2.97[c]	-5.1/+10.5[d]

a. Rounded upward on nonzero 3rd digit
b. Given as indicative value on certificate
c. Between-unit standard deviation calculated for log-transformed data; expansion factor k to provide symmetric 95%/95% tolerance interval after log-transformation. See text for details.
d. Lower and upper deviations from the assigned value. The interval is 4.7 – 20.3 mg/kg.

All characterisation values were based on the gravimetric preparation data. For protein content other than hazelnut, the uncertainty for each gravimetric value was calculated using the uncertainty for the weighings (allowing for correlation), uncertainties for measured nitrogen

content in the ingredients, and the homogeneity uncertainty for the nitrogen content of each allergenic food. Since the homogeneity uncertainty was derived from ELISA measurements, contributing uncertainties were combined in relative terms. The mass uncertainties proved to be negligible in all cases.

For hazelnut, the high relative uncertainty arising from inhomogeneity dominated the combined uncertainty, leaving the mass and nitrogen content uncertainty negligible. The distribution of values from the homogeneity study (above) also suggested an asymmetric distribution for the RM unit means. The tolerance interval derived from the homogeneity study was accordingly adopted as the expanded uncertainty interval for the assigned value, after proportional adjustment from the ELISA mean to the gravimetric value.

ELISA DATA FROM THE RM

The incurred RM was analysed by two ELISA platforms (Romer Laboratories Ltd AgraQuant and R-Biopharm Ridascreen FAST) in two laboratories. Results are shown in Figure 2 and illustrate the potential inadequacy of ELISA allergen protein results. Only for almond did the ELISA results approximate to the incurred value. Hazelnut and walnut protein were particularly poorly recovered from the RM fortified with the partially defatted nut flours. For egg white protein one of two ELISAs approximated to the incurred value. For milk protein the data confirm the advantage of applying two separate ELISAs and taking the mean of their results. A recent interlaboratory comparison with a well-defined milk protein measurand[36] confirmed significant scatter of milk protein results from commercially available ELISA test kits. This indicates the current impossibility of verifying the RM assigned values by ELISA nevertheless the ELISA results were also added to the statement of measurement.

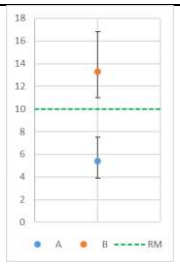
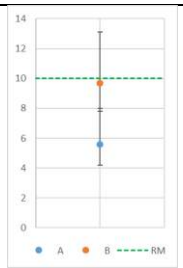
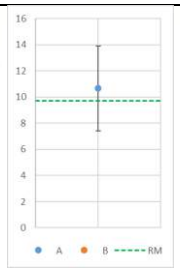
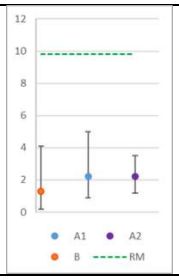
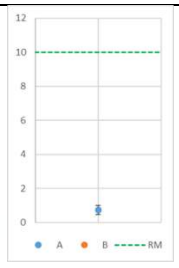
DISCUSSION

Peer reviewed publication of RM preparation is routine other than for allergens where it is scant with some notable exceptions. This is unfortunate as their use depends crucially on detailed knowledge of the origins and preparation of allergen RMs. Not only can there be biological variation in source material protein profiles but analytical responses in ELISAs depend on the protein profiles against which the detection antibodies were raised and the nature of the kit calibrators. This latter information may not be readily available to end users.

Homogeneity data for different allergens in the fortified RM are interesting. It is difficult to mill the nut flours smaller than 0.5 mm without potentially damaging heating, owing to their fat content. For milk, egg, almond and walnut, the data are satisfactory. For hazelnut the data are more dispersed. It is not possible to distinguish the inherent variability of the ELISA from within-unit variation perhaps caused by the raw material particle size and the partially defatted nature of the product.

The applications for the food allergen RM kit include validation and verification studies of analytical methods (ELISA, PCR, Liquid Chromatography tandem Mass Spectrometry, LC-MS/MS, LFD), and relevant ELISA and PCR kits,

Figure 2. Comparison of data on the RM kit from two ELISA platforms.

Raw material	Skimmed milk powder	Egg white powder	Almond powder	Partially defatted hazelnut powder	Partially defatted walnut powder
Incurred conc^{n}	Milk protein 10.0 mg kg^{-1}	Egg white protein 10.0 mg kg^{-1}	Almond protein 9.7 mg kg^{-1}	Hazelnut protein 9.8 mg kg^{-1}	Walnut protein 10.0 mg kg^{-1}
ELISA findings Y axis is the allergen protein mg kg^{-1}					

Dotted line is the gravimetric allergen protein concentration
A = ELISA platform A, B = ELISA platform B (where used), Numerals 1 and 2 in the hazelnut figure represent data from two different laboratories. Error bars represent the *range* of individual ELISA results obtained.

method and kit development, and competency assessments of analytical service providers and staff. The food allergen RM kit may be used to validate methods and verify in-house quality control materials. The raw material RMs can be used to generate kit calibrator extract solutions and generate external check calibrator extract solutions. The raw material RMs in this kit may be used to prepare fortified ("spiked") matrices either by way of an extract or, preferably, by addition of the raw material itself to assess recovery in real life situations. The blank matrix can be used as a 'no-template' control to provide assurance of absence of in-lab allergen cross contamination (either environmentally, from personnel or in reagents), and calculate method LoD or LoQ. The incurred matrix can be used to optimise analytical recovery from a chocolate-type matrix; to inform risk assessors of the possible 'true' estimate of allergen in a questioned product. For ELISA and PCR method quality control and assurance, the extraction and analysis of the RMs should be as recommended by the relevant kit or published method. Similarly, for LC-MS methods extraction and analysis procedures will need to be investigated, see for example[36] and references therein.

CONCLUSIONS

The process of preparation and characterisation of a food allergen reference material kit has been described. Traceability to the SI was achieved via gravimetric preparation, and homogeneity and stability of the RM have been demonstrated. The RM kit, which is on the market (Reference LGC746-KT, and LGC7421, -22, -24 to -26 for the raw materials), has been confirmed within the scope of ISO 17034 accreditation. A statement of measurement has been published[31] and assigned values have been compared with independently obtained data from two ELISA platforms.

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SPECIAL NEWS

JOIN US FOR BERM-16 IN HALIFAX, CANADA IN JUNE 2025!

BERM 16

International Symposium on Biological and Environmental Reference Materials

The 16th International Symposium on Biological and Environmental Reference Materials (BERM-16) will be held in Halifax, Canada during the first week of June 2025. Halifax is the capital city of Nova Scotia on the Atlantic Coast of Canada. It is a renowned university city with active research and technology but is also historic and walkable with numerous hotels and restaurants. BERM-15, the previous conference was hosted by BAM in Berlin, Germany in 2018.

For more than 40 years, the BERM symposia have been the premier meeting for discussions on needs for and production and use of reference materials. The symposium series originated in 1983 as a one-day session titled Biological Reference Materials (BRM) organized by Wayne R. Wolf (USDA) at the 10th annual meeting of the Federation of Analytical Chemistry and Spectroscopy Societies (FACSS) in Philadelphia, Pennsylvania in the USA with 25 participants. The next two BRM symposia were organized in Germany by Markus Stoeppler (KFA). In 1990, the BRM symposium broadened its scope to include environmental as well as biological reference materials and became BERM.

BERM has typically alternated between the United States and Europe every 2 to 3 years, with the exception of a trip to Japan in 2007. After the first decade, BERM symposia were hosted and chaired by various national metrology institutes (NMIs) involved in the production of certified reference materials. The only exception is BERM-14 that was held in 2015 in National Harbor, Maryland, USA, chaired by Dr Steve Wise from NIST and sponsored by Sigma-Aldrich, one of the biggest commercial reference material producers at the time. This conference opened the opportunity for closer collaboration between commercial and publicly funded reference material producers for future organisation of the conference.

BERM-16 promises to be an exciting opportunity to bring the reference material producer and user communities together again to facilitate technical exchange and deliberate on the new challenges facing the world after the COVID-19 pandemic. The demand for reference materials is ever increasing and pressure is also mounting to be able to respond to market needs for new types of reference materials faster. The traditional concept of a reference material is also changing in view of digitalisation and the growing adaptation of the use of artificial intelligence. In the future, more reference materials also in the form of devices will be required for point of use calibration in a diverse range of applications.

We would like to invite you to come and share your experiences and challenges with reference material production in your field of application at the conference. It will be a much-anticipated opportunity to exchange ideas and find solutions together. Please keep a look out for more information about the conference on the webpages of the National Research Council (NRC), Canada at https://nrc.canada.ca/en/.

We look forward to seeing you at BERM-16!

About CITAC

CITAC – Cooperation on International Traceability in Analytical Chemistry – arose out of an international workshop held in association with the Pittsburgh Conference in Atlanta in March 1993. The aim of this workshop was to discuss how analytical activities could be developed to meet the needs of the 21st century, and it identified a wide variety of issues to be addressed to ensure that analytical measurements made in different countries or at different times are comparable. These range from the development of traceable reference materials and methods to the harmonisation of analytical quality practices.

The CITAC initiative aims to foster collaboration between existing organisation to improve the international comparability of chemical measurement. A Working Group takes matters forward and its initial activities have centered on a few specific high priority activities. The first tasks included the compilation of a directory of certified reference materials under development; preparation of quality system guidelines for the production of reference materials; preparation of a directory of international chemical metrology activities; defining criteria for establishing traceability to the mole; and the preparation of an international guide to quality in analytical chemistry.

Many of these activities are of a strategic nature, laying the ground for the improvement of international analytical measurement. This reflects the added geographical complexities associated with a world-wide organisation, such as greater diversity in culture and in technical approach, and frequently long timescales associated with its activities. Nevertheless, if the full benefits of improved analytical measurement are to be realised internationally, a truly global approach is needed, and there is a clear role for CITAC to play in this respect.

CITAC
Cooperation on International Traceability in Analytical Chemistry

For enquiries, contact the CITAC SECRETARY
Prof Dr. Felipe R. Lourenço
Tel.: +551 13 0913667
E-mail: feliperl@usp.br