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EDITORIAL

What is novel about certified reference materials?

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Reference materials play a critical role in validating analytical methods and assessing accuracy and comparability of results among different laboratories and over time. A reference material is defined as a "material, sufficiently homogeneous and stable with respect to one or more specified properties, which has been established to be fit for its intended use in a measurement process" [1]. A certified reference material (CRM) is defined as a "reference material characterized by a metrologically valid procedure for one or more specified properties, accompanied by a reference material certificate that provides the value of the specified property, its associated uncertainty, and a statement of metrological traceability" [1]. Most CRMs are produced by National Metrology Institutes (NMIs) such as the National Institute of Standards and Technology (NIST) in the United States; the European Commission Joint Research Centre for Directorate F, Health Consumers and Reference Materials (JRC) (Geel, Belgium); Federal Institute for Materials Research and Testing (BAM) in Germany; National Measurement Institute Australia (NMIA); National Research Council of Canada (NRCC); National Institute of Metrology China (NIMC); National Metrology Institute of Japan (NMIJ); Korea Research Institute for Science and Standards (KRISS); and others.

During my career as an analytical chemist at NIST, many of my activities focused on the development of Standard Reference Materials (SRMs), which are CRMs issued by NIST. I started at NIST just as the first natural-matrix SRMs for trace organic analysis were being developed, which was a decade later than similar SRMs for trace element content had been first issued at NIST. Since the SRMs for organic analysis were "firsts", it was easy to justify publishing papers in analytical chemistry journals describing their development and the analytical methods used to assign the certified values. Over the years, I have had numerous discussions with colleagues at NIST and elsewhere (including ABC editors) concerning the merits of publishing CRM papers and specifically what are the criteria for analytical novelty regarding CRMs. This Editorial will address the question of analytical novelty associated with CRM papers by highlighting some recent examples published in ABC and other analytical chemistry journals.

Analytical and Bioanalytical Chemistry (ABC) has had a long tradition of publishing papers related to the development of reference materials. Beginning with its predecessors, *Fresenius' Zeitschrift für Analytische Chemie* and continuing with *Fresenius' Journal of Analytical Chemistry*, ABC has published topical collections of papers related to reference materials, with the most recent collection assembled in 2015 [2]. One important criterion for papers published in ABC, as well as in other analytical journals, is "analytical novelty", which is typically directed to the methods reported in the paper. For papers describing CRM development, the scope of analytical novelty extends beyond just the analytical methods used to characterize the CRMs to include also: (1) new or unique CRMs, e.g., new matrices or analytes, and (2) novel approaches for preparing reference materials. Finally, many CRM papers describe the complete CRM development process including preparation, homogeneity and stability assessment, and assignment of certified values. In my opinion (which may be biased because of my CRM career), the development process for CRMs, because of its rigor and high metrological approach, represents analytical novelty.

Why do NMIs publish papers describing CRMs? CRM producers are required to publish a "certification report" describing the preparation, homogeneity testing, stability assessment, analytical measurements, and value assignment approach for each CRM. Although this requirement is satisfied in different ways and to different degrees by the various CRM producers, one way to fulfill the requirement is to publish a peer-reviewed paper describing the CRM development process. The rigorous details of the CRM development may be overwhelming for most analytical journals. However, supplemental material now provides an option to include many of these details. Another

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reason to publish CRM papers is to promote their availability and use. Why should ABC's readers be interested in papers describing CRMs? Analytical chemists should be aware of what CRMs are available to support their analytical measurements, and they should understand and appreciate the rigor and exactness involved in their production.

Novel matrix CRMs

Many of the recent CRM papers describe new (first time) and unique materials, and thereby qualify as novel even if the analytical methods used to characterize the CRMs may be well-established. NIST has a long history of producing synthetic greenhouse gas mixture SRMs (CO_2, CH_4, and N_2O) based on gravimetric preparation. Recently, Rhoderick et al. [3, 4] reported the first CRMs for whole natural air with values assigned for greenhouse gases, denoted as SRM 1720 Northern Hemisphere Air [3] and SRM 1721 Southern Oceanic Air [4]. These unique SRMs consist of batches of 30 cylinders of pristine ambient air collected at two remote locations: (1) Niwot Ridge (CO, USA) for SRM 1720 and Baring Head, New Zealand for SRM 1721. Rhoderick et al. [3, 4] described the process of assigning certified values to each individual cylinder based on calibrating the instrumentation using a suite of gravimetrically-prepared NIST primary reference gas mixtures. The nominal values for the three gases were nearly identical for CO_2 (388 μmol/mol) and N_2O (323 μmol/mol) in both SRMs, and the value for CH_4 in SRM 1720 is 5.6% higher (1860 μmol/mol). The relative uncertainties associated with the certified values were <0.06%, representing the smallest uncertainties on any NIST gas SRM.

Another first-time CRM matrix, cigarette tobacco filler, was introduced by Sander et al. [5]. SRM 3222 Cigarette Tobacco Filler has certified values assigned for nicotine, two tobacco-specific nitrosamines [N-nitrosonornicotine (NNN) and 4-(methylnitrosomino)-1-(3-pridyl)-1-butanone (NNK)]. The certified values for nicotine, NNN, and NNK were assigned by combining the results from several different sample preparation approaches followed by isotope dilution (ID) liquid chromatography – tandem mass spectrometry (LC-MS/MS) analysis.

Not all CRM papers describe new or first-time matrices or analytes to qualify as novel. For example, Phinney et al. [6] do not describe a new CRM but instead satisfy the novelty requirement by offering a significantly "improved" material. In 2012 Phinney et al. [7] reported the first serum-based CRM with values assigned for three vitamin D metabolites, i.e., 25-hydroxyvitamin D_2 [25(OH)D_2], 25-hydroxyvitamin D_3 [25(OH)D_3], and 3-epi-25-hydroxvitamin D_3 [3-epi-25(OH)D_3]. SRM 972 Vitamin D in Human Serum was a huge success and the inventory was rapidly exhausted. In 2017 Phinney et al. [6] introduced a new, improved SRM 972a Vitamin D Metabolites in Human Serum to replace SRM 972. What were the significant improvements to satisfy the analytical novelty criteria? The original material was designed to provide typical analytical challenges encountered in the determination of total 25-hydroxyvitamin D [i.e., the sum of 25(OH)D_2 and 25(OH)D_3] in clinical patient samples. SRM 972 consisted of four levels of vitamin D metabolites representing normal and low levels of 25(OH)D_3, high levels of 25(OH)D_2, and high levels of 3-epi-25(OH)D_3, which must be chromatographically separated from 25(OH)D_3 for accurate measurement of total 25(OH)D. To achieve target levels, the normal level was diluted with horse serum, which contains no 25(OH)D, to provide a low level, and the remaining two levels were fortified with either 25(OH)D_2 or 3-epi-25(OH)D_3. Even though SRM 972 was widely used, a major criticism was that the use of modified serum and/or the presence of non-endogenous metabolites in three of the four levels produced inaccurate results using some assays, particularly immunoassays.

In SRM 972a only one of four levels contained non-endogenous metabolites, i.e., only addition of 3-epi-25(OH)D_3. The low level and normal levels of 25(OH)D_3 were from donor pools that were screened to provide target levels, and the high level of 25(OH)D_2 was achieved through donors supplemented with vitamin D_2. Another significant improvement for SRM 972a was the use of only LC-MS/MS methods that separated the 25(OH)D_3 and 3-epi-25(OH)D_3 isomers to assign the certified values. Finally, the paper also contained results addressing the commutability of SRM 972a, i.e., does it behave the same as clinical patient samples, which is an important aspect for clinical CRMs. Tai et al. [8] described a related new SRM with a high level of 25(OH)D_3 (25% higher than in SRM 972a), SRM 2973 Vitamin D Metabolites in Serum (High Level). The paper's novelty was based on the first certified values in both SRM 2973 and SRM 972a for another important metabolite, 24R,25-dihydroxyvitamin D_3 [24,25(OH)$_2$$D_3$], using an ID LC-MS/MS reference measurement procedure.

Novel CRM production

NIST and JRC have invested considerable effort in developing CRMs to support measurements of trace elements and organic pollutants in fine particulate matter (PM). Two of the earliest and most widely-used NIST environmental-matrix SRMs are based on PM, i.e., SRM 1648 Urban Particulate Matter and SRM 1649 Urban Dust, which were issued in 1978 and 1982, respectively. Both SRMs were prepared from large quantities of total suspended particulate (TSP) matter (< 63 μm) collected over 18-month periods. Because of the significant quantities collected, these two SRMs are still available today after nearly 40 years. However, because of the need for contemporary PM and the potential hazards and regulations for PM with

aerodynamic diameter < 10 μm (PM_{10}) and <2.5 μm ($PM_{2.5}$), Schantz et al. [9] reported the development of the first natural fine PM CRMs, i.e., SRM 2786 Fine Particulate Matter (< 4 μm) and SRM 2787 Fine Particulate Matter (< 10 μm), for determination of polycyclic aromatic hydrocarbons (PAHs), nitro-substituted PAHs, brominated diphenyl ether congeners, and trace elements. Schantz et al. [9] described the multiple analytical methods used (e.g., different extraction techniques and gas chromatography/mass spectrometry (GC/MS) on columns of different selectivity for the organic contaminants). Obtaining a sufficient quantity of fine PM to produce a CRM is a significant challenge. After attempts to collect ambient $PM_{2.5}$, NIST ultimately resorted to resuspending TSP using a particle suspension unit and ultra-high-volume sampler to collect the size-fractionated PM.

JRC has also devoted substantial time and thought to obtaining the required quantity of $PM_{2.5}$ to produce a CRM. Initial efforts at JRC produced two CRMs for PM_{10}-like materials, ERM-CZ100 [10] and ERM-CZ120 [11], which are certified for PAHs and toxic heavy metals, respectively. Both materials were prepared by jet-milling dust collected from a road tunnel, hence the designation as "PM_{10}-like" materials. As the next step in the quest for producing $PM_{2.5}$ CRMs Emteborg and coworkers [12] describe a novel process for preparing $PM_{2.5}$-like material intended for the determination of anions (SO_4^{2-}, NO_3^-, Cl^-,) and cations (Ca^+, K^+, NH_4^+, Ca^{2+}, and Mg^{2+}). Starting with the TSP used for ERM-CZ100 and ERM CZ-120, a PM_{10}-like material was produced by jet-milling and then suspended in water. After sedimentation of the larger particles, 90% by volume was siphoned off, and the remaining solution was spiked with the anions and cations prior to drop-wise shock freezing in liquid nitrogen. The resulting ice kernels were then freeze-dried. Using this process, it was possible to produce 500 g of $PM_{2.5}$-like material. The authors envision using this process to produce future $PM_{2.5}$-like materials for PAHs and trace elements.

Novel methods

Obviously, a CRM paper can be considered as novel based on the methods developed and used to certify the CRM. Phillips et al. [13] described the use of two novel independent methods for the determination of isoflavones in four different soy-based dietary supplement SRMs. Different sample preparation approaches were optimized followed by analysis using LC with absorbance detection and ID LC-MS for the determination of six isoflavones in soy flour, soy protein isolate, soy protein concentrate, and soy-containing oral solid dosage form. Phillips et al. [13] compared the results from the two analytical methods, which also contributes to the analytical novelty, and described the combination of results to obtain the certified values.

The complete CRM development process

My final classification is papers describing the complete CRM development process, i.e., production, homogeneity and stability assessment, and value assignment approach. Several excellent examples of these "complete process" papers are highlighted below. Since the early 1990s, NRCC Halifax, the premier NMI for marine biotoxin research, has produced both calibration solution and shellfish tissue-matrix CRMs for a wide variety of biotoxins. Recently, two companion papers from NRCC reviewed developments in the production of CRMs for diarrhetic shellfish poisoning toxins (DSPs). Beach et al. [14] summarized the current approach for production of calibration solution CRMs for DSPs, i.e., isolation and purification of sufficient quantities of DSPs from algal cultures and characterization and purity assessment using quantitative nuclear magnetic resonance (NMR). After stability and homogeneity assessments, final quantification was performed by LC-MS/MS. In the companion paper, McCarron et al. [15] described the development of a new mussel tissue CRM with naturally-incurred levels of DSPs.

A series of papers describing development of a new freeze-dried mussel tissue material (CRM-FDMT1) for multiple phycotoxins was published by McCarron and coworkers [16–19]. The papers described the various aspects of CRM development including design and preparation [17]; LC-MS/MS, extraction, and quantification approaches [18]; homogeneity and stability [16] and certification [19]. Prior to the development of CRM-FDMT1, mussel tissue CRMs were available only for single toxin groups. CRM-FDMT1 was prepared as a mixture of five batches of mussels from different locations and contaminated with six different toxin groups, i.e., domoic acid, DSPs, azapiracids, pectenotoxins, yessotoxinsm, and spirolides. For the certification measurements, LC-MS/MS methods, which were optimized for each toxin group, were employed following exhaustive extraction procedures [19]. To compensate for LC-MS/MS matrix effects, a variety of quantification approaches were used including standard addition, dilution, or matrix-matched calibration. CRM-FDMT1 is the first shellfish-tissue matrix CRM with values assigned for 10 toxins from multiple groups, and the first CRM with values assigned for yessotoxins, pectenotoxins, and spirolides.

Papers describing development of CRMs for the determination of trace elements may be criticized as having limited analytical novelty, since the analytical techniques used are often well-established or even routine methods. A recent paper by Yu et al. [20] describing a kelp powder SRM is, in my opinion, a excellent model for a trace element CRM paper. SRM 3232 Kelp Powder (*Thallus laminariae*) was developed to support measurements for compliance with food and dietary supplement regulations. Kelp was selected because it is a rich source of dietary iodine and vitamin K_1, and it contains high levels of total arsenic, including both toxic inorganic

species and less toxic organic species. Yu et al. [20] explained the need for the kelp material and compared the elemental content of the kelp powder SRM to similar seaweed-matrix CRMs from other NMIs. Using multiple analytical techniques, certified values for 15 nutritional and toxic elements were assigned. Most importantly regarding analytical novelty, values are assigned for arsenic species, including arsenic acid, dimethylarsinic acid, and three arsenosugars using a novel combination of LC with instrumental neutron activation analysis (INAA) and LC with inductively coupled plasma/mass spectrometry (ICP/MS). SRM 3232 is the first seaweed-matrix CRM with values assigned for iodine, arsenic species (including arsenosugars), and *cis*- and *trans*-isomers of vitamin K_1, which were determined using ID LC-MS/MS.

Another example of the rigor of the CRM development process is reported by Merrick and coworkers [21] from NMIA describing the production of a coastal seawater CRM (NMIA MX014) with certified values for 12 elements, which were added to the natural sea water sample to provide levels relevant to the Australian environmental regulations. Values were assigned using different sample preparation, ID and standard addition quantification approaches, and various ICP/MS techniques.

NIST and JRC have the longest history and broadest scope of CRMs for chemical composition, and they have worldwide distribution and CRM brand recognition. Some NMIs choose to focus on only a limited chemical measurement area (e.g., the case of marine biotoxins at NRCC Halifax described above). Other NMIs with more recently-established chemical metrology programs (e.g., NMIJ, KRISS, NIMC) are making significant contributions to CRM production; however, in most cases, these CRMs are intended for use only in the country of origin. For example, recent papers from NIMC report the production of CRMs for the determination of clinical markers in human serum. L. Feng et al. [22] and Y. Lui et al. [23] described the certification of human serum-based CRMs for the determination of electrolytes and total homocysteine, respectively, using higher-order methods, which were validated through international comparisons with other NMIs and through comparisons with existing CRMs. The CRMs reported in these papers are not new or novel materials (other NMIs have produced similar materials); however, these CRMs are significant in that they demonstrate the high level of chemical metrology practiced at NIM China.

Finally, it is noteworthy that many of the papers highlighted in this Editorial have an extensive list of coauthors; most papers have more than six coauthors and two papers have 15 coauthors. The development of a natural-matrix CRM is a massive undertaking that generally involves multiple analytical techniques, numerous analysts, statisticians, and often collaborators with unique capabilities from other institutions.

I hope that this Editorial has stimulated your interest in papers describing CRMs and has emphasized the various aspects of novelty associated with these valuable analytical tools. I will continue to encourage my colleagues at NMIs worldwide to submit high-quality papers to ABC describing their CRM development activities, and I hope that ABC readers will continue to find these papers to be beneficial.

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