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RECENT DEVELOPMENTS IN THE PRODUCTION AND USE OF REFERENCE MATERIALS AND MEASUREMENT STANDARDS IN ROMANIA

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Abstract: Reliable, traceable and comparable measurements provide the rational basis for evaluation of the quality of a result and the starting point for laboratory accreditation. Nowadays good measurements are essential not only for manufacturing, science and trade, they also underpin a wide range of aspects of the quality of live, from healthcare to sport. Starting from the role of the National Institute of Metrology (INM) in assuring the traceability of all measurements, regardless their end-use and level of accuracy, the paper depicts some aspects related to the present state-of-art in the field of metrology in chemistry in Romania. The paper reviews locally available reference materials (RMs) and certified reference materials (CRMs) in quality of life related measurements, the experience of the INM in developing new measurement standards and aspects of certification of RMs issued in Romania. Practical examples on the use of CRMs for calibration, traceability and measurement uncertainty evaluation are discussed.

Keywords: reference materials, traceability, metrology in chemistry

1. INTRODUCTION

The implementation of the European Directives and Regulations in environmental, public health and food safety and control, the increasing number of accredited laboratories in these fields and the need to assure the traceability of results reported by these laboratories are few challenges facing at present the Romanian metrology.

Therefore, it is necessary to permanently discuss what metrological tasks have to be carried out by the National Institute of Metrology (INM) regarding the assurance of uniformity, consistency and traceability of above mentioned measurement results.

The issues related to the physico-chemical quantities represented a distinct field of activity within the INM following closely the customers' needs on calibration, testing and metrological verification. Under different names, the present laboratory 'Physico-chemical Quantities' dealing with these quantities, developed several measurement standards, reference materials and certified reference materials in order to meet its main mission. Mainly, these developments represented the outcomes of different research and development projects self-supported or financed by Romanian authorities. For instance, at present, the laboratory is involved in a complex research project (51-084) supported by the National Center for Project Management (CNMP) aiming at developing primary methods of measurement traceable to SI units and at evaluating the capability of testing food samples. Beside the metrology activities (such as calibration, verification etc.), the laboratory was involved in different PT schemes organized in Romania mainly in the stage of certification of the reference materials used during the proficiency testing.

Within this framework a broad representation of the principal metrological activities required at national level regarding RMs and CRMs is given. Also, the effort of the INM towards updating the development and certification of CRMs of physical and chemical properties is presented.

The interests and requirements of many users of RMs and CRMs are not limited to the regional scene, as they are engaged in international trade, international co-manufacture of products and in QS implementation / accreditation. For quality assurance, the RMs are generally used for method validation, method development, internal quality control and external quality assessment purposes. Practical examples of how RMs and CRMs for chemical properties are used for method and instrument validation in Romanian testing laboratories are given.

2. RMs AND MEASUREMENT STANDADRS USED FOR CHEMICAL MEASUREMENTS

Within the INM, since its foundation in 1951, a small group related to physico-chemical quantities was developed. The present Physico-chemical Quantities Laboratory was set up on 2002, by merging three separate Groups on Reference Materials (set up in 1981), on Physico-chemical Quantities (set up in 1955) and on Gas Concentration (set up in 1978), respectively. Note that each Group followed different developments in accordance with the national needs. For instance, the Reference Materials Group started its activity as a laboratory whose first aims were to carry out the calibration and the periodical verification of the spectro(photo)meters (for UV-VIZ mainly) used, at that time, in the most diverse industrial and analytical chemistry activities. For these purposes, in the beginnings, the Group

elaborated the first legal regulations on these subjects and developed few absorbance reference materials containing cobalt nitrate, nickel nitrate and potassium dichromate and used to calibrate/verify the absorbance scale of spectrophotometers. Later on, as the need for certified reference materials used in connection with other types of spectrometers, such as atomic emission and absorption spectrometers, has grown more and more, the work of the Group took into consideration some other aspects regarding the production (in small quantities) and the certification of such specific Reference Materials. Starting 1989, the Reference Materials Group was involved in the certification of some certain kinds of national CRMs (matrix form) and some spectrometric standards, according to the ISO Guide 35. Since 1992 the Group was involved in different metrological activities related to environment, health and food issues,

The main methods of measurement used at present in the INM are presented in Table 1.

Table 1. Methods of measurement developed in the field

Quantity	**Method of measurement**	**Measure-ment range**	**Expanded uncertainty**
Cinematic viscosity	Flow due to gravity	(0,03 ...100) mm^2s^{-2}	(0,15 ... 0,50) %
Density (of liquids)	Hydrostatic weight	(0,6…1,8) $g{\cdot}cm^{-3}$	$5{\cdot}10^{-5}$ $g{\cdot}cm^{-3}$
pH	Comparative method	0 ... 14	0,02
Mass fraction (in liquid/solid materials)	Gravimetry Spectro (photo)metry	0,001...1,000	2 % (rel)
Amount concentration	Coulometry Titrimetry	(10^{-5}...10^{-1}) $mol{\cdot}L^{-1}$	2 % (rel)
Humidity (in solids and gases)	Gravimetry / Two pressure standard generated	0 ... 100	5 % (rel)
Volume fraction	Gravimetric preparation of gas mixtures	$40{\cdot}10^{-6}$ ...$20{\cdot}10^{-2}$	($2{\cdot}10^{6}$… $0{,}2{\cdot}10^{2}$)

2.1. Present demands on measurement standards for physico-chemical quantities

The present development of different measurement standards and RMs for physico-chemical quantities aiming at ensuring the traceability, accuracy and comparability of reported analytical measurement results was mainly influenced by:

- implementation of a quality system in an increasing number of testing laboratories; more than 150 analytical laboratories working in environment protection field, water quality testing, food safety testing or clinical laboratories have been accredited or are in the process of accreditation;

- increased awareness for accuracy, traceability and comparability of measurement results (due to the implementation of European regulations in the Romanian legislation);

- legal metrological control of the instruments and the measurements.

2.2 Types of reference standards and materials for physico-chemical measurements

In parallel with the development of the measurement techniques, a constant effort was put to extend the necessary measurement standards needed to materialize and disseminate units related to physico-chemical quantities. Main Reference Materials developed by the INM are illustrated in table 2.

Table 2. Measurement RMs provided by the INM for metrological purpose

Measurement standard / RMs provided by the INM	Measurement range	Expanded uncertainty (k =2)
Working standard solution for electrolytic conductivity	(40…5000) $\mu S{\cdot}cm^{-1}$	1 % (rel)
Electrolytic conductivity cells	(0,60 … 1,50) cm^{-1}	2 %
pH working standard solution (t=20°C)	4,00; 6,88; 9,00	0,02
Spectrometric monoelemental solutions (Na, Cu, Pb, Zn, Ca etc.)	(0,500 ... 1,000) $g{\cdot}dm^{-3}$	1.5 % (rel)
Reference materials of amount concentration containing glu-cose, calcium, urea, creatinine, magnesium, Na and K	Similar to the normal and pathologic sera range	(1 … 10) %
Gas mixtures working standards (CO, CO_2, H_2, O_2) in air or in N_2	$88{\cdot}10^{-6}$ … $9{\cdot}10^{-2}$	$2{\cdot}10^{-6}$… $0{,}1{\cdot}10^{-2}$
Gas mixtures of hydrocarbons in air at the lower limit of explosion	$3{,}1{\cdot}10^{-3}$…$3{,}7{\cdot}10^{-2}$	$30{\cdot}10^{-6}$ … $4{\cdot}10^{-4}$

2.3 On the participation of the INM in relevant comparisons for CIPM – MRA process

Since 2000, the Laboratory for physico-chemical quantities has participated in different relevant comparisons, mainly in the field of viscosity and metrology in chemistry. Thus, starting 1971, the INM has constantly participated in the ASTM cooperation program where the viscosity of a number of materials was measured at different temperatures. The results obtained during the past years constitute a useful database reflecting the state-of-art, allowing us to take the necessary corrective actions for improving the measurement technique. The INM has participated in the EUROMET Project M.V-S3 (415) and in the Project CCM.V-k1. The results of these comparisons have already been published [1].

In the field of metrology in chemistry, the INM participated in comparisons related to: matrix material measurement (such as EUROMET 568, CCQM P12.1, CCQM K30, CCQM P76), water measurement (EUROMET

924) and elemental solutions (EUROMET 763 and CCQM P46), the results of which are published [2, 3] or under publication. The experience of the INM in measurement uncertainty estimation was reported in [4].

3. ROLE OF THE INM IN ENSURING THE TRACEABILITY OF ASSIGNED VALUES IN PT SCHEMES

Proficiency testing (PT) schemes are widely used by Accreditation Bodies as part of the assessment process to assess the ability of laboratories to perform competently tests for which accreditation is held. Several national PT schemes are organized on regular basis in fields like environmental and clinical chemistry. In food safety there are certain difficulties in organizing such schemes, mainly due to the lack of appropriate RMs and the wide range of matrices being tested.

As a result of a R&D Project no.156, financed by the National Accreditation Body (RENAR) within the framework of INFRAS Program during 2002–2005, the INM was involved in assigning traceable values of mass fraction of Cd, Cu, Pb, Zn in food matrices, further used in a PT scheme. Three materials were prepared by the Research Institute for Chemistry - ICECHIM by spiking natural materials - wine, vodka and vinegar with metal solution. The homogeneity study was conducted on bottles randomly selected using ICP-OES. ANOVA was used to analyze the measurement results. The materials' stability was studied for several months using control charts and further on during the interlaboratory comparisons.

The certification of the reference materials was performed by the INM, taking into consideration the ISO Guide 35 requirements. The interlaboatory results, homogeneity and stability studies were taken into account accordingly. The certified values of the reference materials are presented in table 3.

Table 3. Certified values of the RMs developed by the ICECHIM

CRM description	Heavy metal	Certified mass fraction, $mg \cdot kg^{-1}$	Confidence limits	
			lower	upper
Wine; alcohol concentration of 11.5 %, density of 0,9923 $g \cdot cm^{-3}$ (20 °C), bottled in dark bottles of 500 mL	Cd	0.262	0.237	0.287
	Cu	1.02	0.91	1.13
	Zn	2.11	2.04	2.18
	Pb	1.52	1.44	1.60
Vodka; alcohol concentration of 37.5 %, density of 0,9532 $g \cdot cm^{-3}$ (20 °C), bottled in dark bottles of 500 mL	Cd	0.044	0.039	0.049
	Cu	2.04	1.86	2.22
	Zn	0.711	0.618	0.804
	Pb	1.23	1.06	1.40
Vinegar, total acidity of 9,27 g / 100 mL, density of 1,0123 $g \cdot cm^{-3}$ (20 °C), bottled in dark bottles of 500 mL	Cd	0.402	0.355	0.449
	Cu	11.91	11.21	12.61
	Zn	2.37	2.21	2.53
	Pb	3.27	3.05	3.49

The RMs, certified by the INM, were used in a PT scheme aiming at the evaluation of the present state-of-art in food control at working level. The scheme was conducted in two steps with the participation of 11 laboratories. In the first step, three CRMs (BCR-IRMM) – flour, black bread and milk powder have been tested. In the second step the three above mentioned CRMs have been analyzed.

The PT was conducted by the ICECHIM and the Z score was calculated. The results showed the same spread both in the case of BCR-IRMM samples and in the case of ICECHIM CRMs.

During the research project 51-084, within its second stage, this experience will be further developed by expanding the types of reference materials to be produced by the ICECHIM, certified by the INM and used within a PT scheme involving private and public analytical accredited laboratories specialized on food measurements.

4. SOME ASPECTS ON THE USE OF CRMs IN CALIBRATION AND VALIDATION

In laboratory medicine there is a growing demand to perform analyses in a manner that allows comparisons of results among laboratories. Also, there is an increasing awareness among analysts of the need to produce results which are accurate and precise. However, acceptable limits of accuracy and precision are poorly defined in this field. In Romanian community of clinical chemistry laboratories certain maximum errors are set for the target values. For instance, a maximum 10 % is allowed in the case of calcium, 15 % is accepted in the case of glucose and 24 % is allowed in the case of urea measurements.

To measure the mass concentration of the different analytes commonly required for medical diagnostics several types of instruments are in use. Among them the automated analyzers based on photometric methods have been subject to a study aiming at assessing their actual accuracy and long term behavior. The study, performed by the INM over a period of 10 years, was conducted on more than 30 types of analyzers in use in different clinical public and private laboratories. Thus, more then 150 of such instruments were tested.

Note that each type of automated analyzer is calibrated in the laboratory using the type of calibrator indicated by the manufacturer of the analyzer. Daily, the calibration of analyzers is checked using some control sera, also as indicated by each manufacturer. Multi-sera can be supplied as an assayed serum for control of accuracy or as a precision serum for control of reproducibility. Constituent concentrations are in the low, normal and elevated ranges. The values of each batch of assayed control serum are assigned from a consensus mean. With each batch, a control range is provided for individual parameters and each

parameter method and, when available, a value for automated analysers. Usually, the control range is equivalent to the assigned mean ± 2 standard deviation. In many cases, the control range is stated as the assigned mean ± 3 standard deviation.

In this respect, it is interesting to note the control range associated with the control sera being in use on the Romanian market, as illustrated in the figures 1 … 4 in the case of calcium and glucose at normal and pathologic levels. In each figure the type of the control sera was indicated with the parameter method indicated by the manufacturer.

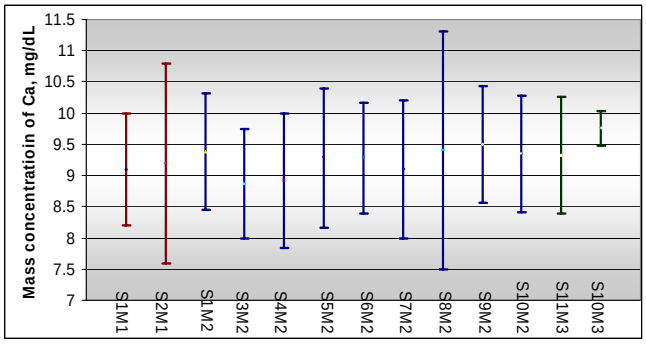

Figure 1 Control range associated with calcium normal level in 11 control sera in use supplied by different suppliers

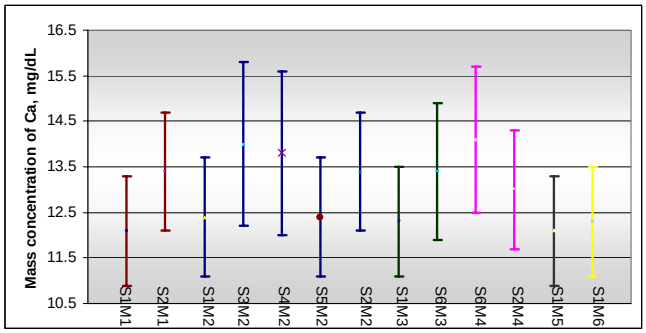

Figure 2 Control range associated with calcium pathologic level in 11 control sera in use supplied by different suppliers

For calcium measurements M1 denotes arsenazo III method, M2 – o-cresolftaleine method and M3 – AAS, M4 – reference method, M5 – dry chemistry and M6 - methylthymol blue method.

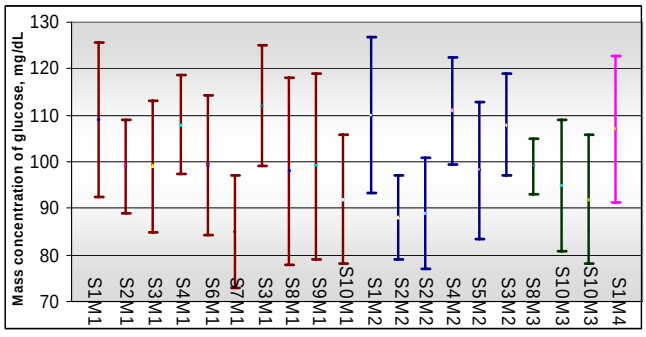

Figure 3 Control range associated with glucose normal level in 10 control sera in use supplied by different suppliers

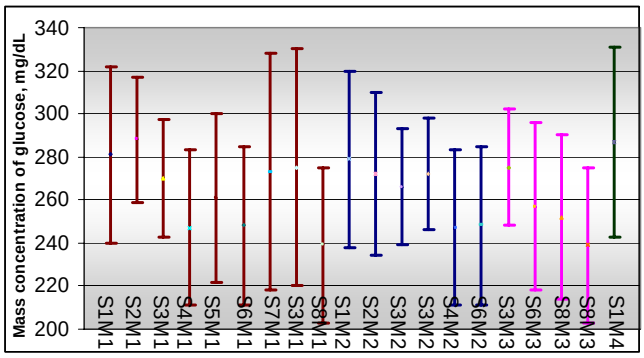

Figure 4 Control range associated with glucose pathologic level in 10 control sera in use supplied by different suppliers

For glucose measurements, M1 denotes hexockinase method, M2 – GOD/PAP method, M3 – IDMS and M4 – dry chemistry.

One may note that certain control sera being in used by the clinical chemistry laboratories has a control range exceeding 10 % from the target value in the case of calcium and 15 % from the target value in the case of glucose, both at normal and pathologic levels. As expected, the target values vary with the parameter method (for instance, arsenazo III, o-cresolftalein, AAS, dry chemistry in the case of calcium, and hexockinase, GOD/PAP, IDMS and dry chemistry in the case of glucose, respectively).

"Calibrating" an instrument is a common expression in most clinical chemistry laboratories used with quite different meanings at some extends. In order to check the correct calibration of a automated analyzers based on photometric methods the INM developed a set of reference materials control sera type as described in table 2. The CRMs are gravimetrically prepared, in batches, in accordance with a written procedure starting from high purity chemical reagents. The actual values assigned to a certain batch are verified against NIST SRM 909 b and BCR CRM 573, 574, 575 and 304 using photometric methods and AAS in the case of Ca and Mg.

Typical certified values of the CRMs developed by the INM are presented in table 4.

Table 4. Certified values of the CRM 14.01 and 14.02 (batch 1/2008)

	Analyte	Certified mass concen-tration, mg/dL	Expanded uncertainty, (*k*=2)	
			mg/dl	% (rel)
CRM 14.01 (normal level)	calcium	9.1	0.9	10.0
	glucose	88.6	12.4	14.0
	urea	33.2	7.9	23.8
	creatinine	2.28	0.24	10.5
	magnesium	2.14	0.21	9.8
CRM 14.02 (pathologic level)	calcium	5.96	0.60	10.0
	glucose	297.4	44.6	15.0
	urea	108.4	24.6	22.7
	creatinine	6.9	0.7	10.1
	magnesium	4.1	0.5	12.2

The results obtained since 2000 clearly pointed out the advantages related to the wide applicability, commutability, stability and the traceability of the assigned values. The main disadvantage is related to the synthetic matrix, different from the calibrators, control sera and samples being usually analyzed in a clinical chemistry laboratory. A systematic virtual exercise of comparability of the measurement results obtained with different types of calibrated clinical chemistry analyzers was performed for the past years. A typical example of the spread of the results in the case of calcium al normal level is presented in figure 5.

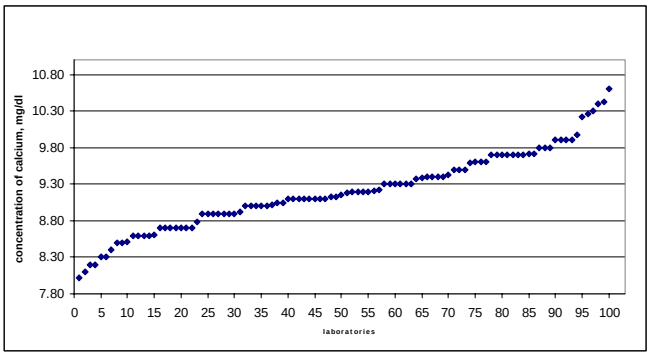

Figure 5. Spread of amount concentration of Ca (normal level)

Similar spreads were obtained for urea (figures 6 and 7), glucose, creatinine and magnesium measurements using different methods and instruments.

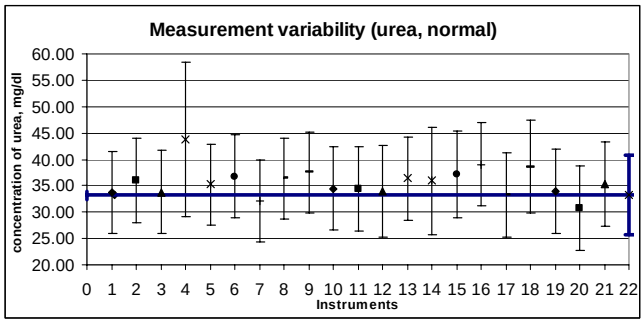

Figure 6. Measurement variability obtained in the case of urea, normal level

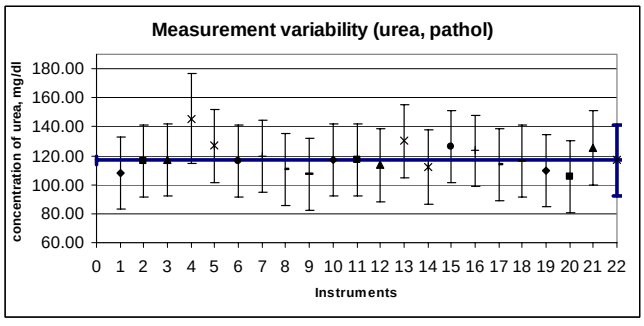

Figure 7. Measurement variability obtained in the case of urea, pathologic level

It may be noted that the spread of results lied within the typical control range. Also, the measurement uncertainty evaluated in each case took into account the short term variability, long term variability, the uncertainty of the CRMs and the bias, in accordance with [5, 6].

5. CONCLUSION

Main activities needed to assure and demonstrate the traceability of chemical results and of values of measurement standards are:

- recognition of new national measurement standards;
- diversification and development of the existing RMs to enable them to meet all the requirements of society;
- expanding the production of CRMs covered by the ISO Guide 34 requirements;
- pro-active participation of the INM in the CIPM - MRA process.

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