Abstract

Introduction: Standardization is the ability to obtain interchangeable results leading to same medical interpretation. External quality assessment (EQA) is the main support of the on-going harmonization initiatives. Aim of study was to evaluate results obtained from two years category 1 EQA program experience in Spain and determine the impact of applying this type of EQA program on the analytical standardization.

Materials and methods: According to the analytical method, traceability and instrument different groups were established which results were evaluated by calculating mean, coefficient of variation and percent of deviation to the reference value. Analytical performance specifications used to the results’ evaluation were derived from biological variation for bias and from the inter-laboratory coefficients of variation found in a previous pilot study.

Results: Only creatinine measured by enzymatic methods gave excellent results, although few laboratories used this method. Creatine kinase and GGT gave good precision and bias in all, but one instrument studied. For the remaining analytes (ALT, ALP, AST, bilirubin, calcium, chloride, glucose, magnesium, potassium, sodium, total protein and urate) some improvement is still necessary to achieve satisfactory standardization in our setting.

Conclusions: The two years of category 1 EQA program experience in Spain have manifested a lack of standardization of 17 most frequent biochemistry tests used in our laboratories. The impact of the information obtained on the lack of standardization is to recommend abandoning methods such as ALT, AST without exogenous pyridoxal phosphate, Jaffe method for creatinine, and do not use non-commutable calibrators, such as aqueous solutions for calcium and sodium.

Keywords: standardization; external quality assessment; traceability; bias

Received: July 31, 2018 Accepted: September 25, 2018

Introduction

The main objective of clinical laboratory is to provide clear, reliable and useful information for clinical decision-making. Current healthcare systems imply performing laboratory tests in different lo-
cations, so standardization among laboratories become one of the cornerstones of the quality patient’s care. Standardization can be defined as the ability to obtain interchangeable results (within certain analytical quality uncertainty) in order to achieve the same medical decision, regardless of the analytical procedure (method, traceability and instrument), measurement units and reference intervals.

The standardization should be based on six basic pillars, which include in vitro diagnostic companies, reference materials, reference methods, reference laboratories, medical laboratories and external quality assessment (EQA) organizations (1). Recently, Greaves noted that EQA is not just a pillar but the central support for on-going harmonization (2). Discordance in results between laboratories and methods should become a practice no longer accepted.

It is widely accepted that the best strategy to organize an EQA scheme is to use fresh frozen commutable control samples with values assigned by reference laboratories using reference methods, which can be found on www.harmonization.net (3,4).

Spanish Society of Laboratory Medicine (SEQCML) is a non-profit scientific organization that has been providing EQA schemes in Spain since 1980 by using stabilized control materials. Since 2013 a category 1 program has been organized for basic biochemistry analytes. According to Miller et al. this kind of program distributes commutable control materials with reference-measurement procedure (RMP) assigned values and replicate samples in surveys are tested (3). Accuracy of individual laboratories is assessed by comparison with the RMP, while reproducibility is checked both intra- and inter-laboratory, and standardization is assessed by comparison of measurement procedure calibration traceability with RMP. Two initial surveys were performed in 2013 and 2014, as preliminary experiences and regular annual surveys have been organized since 2015. For a proper assessment of bias, having adequate information of measurement’s traceability is therefore a crucial point (5,6).

Another important aspect to consider is the analytical performance specification (APS) or acceptability limits selected for the evaluation of the derived results. When APS are based on biological variation (BV), it is highly recommended to use the gradual classification of APS according to its strictness: optimal, desirable and minimal (7). It should be noted that the APS grade could be selected according to the limitations of the current state of the art, being defined as the performance achieved by about 80% of laboratories. According to this criterion, in this study the minimal BV-based APS grade was selected for electrolytes evaluation, while desirable BV APS were chosen for enzymes and substrates.

In this regard, a performance worse than the minimum APS should alert the laboratory that its results could be at risk and clinical decision-making might be detrimentally affected. Likewise, a performance reaching the minimal grade suggest that further improvement may be beneficial for patients (8,9).

The aim of this work is to evaluate the results obtained from two years category 1 EQA program, 2015 and 2016 surveys, performed in our country and to assess the impact of applying this kind of EQA program over the analytical standardization. Evaluation is based on the inter-laboratory imprecision and the bias of the peer group means compared with the reference method values.

**Materials and methods**

Commutable control materials were purchased from MCA laboratory (Queen Beatrix Hospital, Winterswijk, The Netherlands) by means of the Stichting Kwaliteitsbewaking Medische Laboratorium Diagnostiek (SKML). According to Cobbaert et al. controls had been prepared from fresh anonymized left-over sera of routine laboratory with exclusion of lipemic, icteric, positive hepatitis B surface antigen (HBsAG), human immunodeficiency virus (HIV) and hepatitis C virus (HCV) samples, and stored frozen at – 84 ºC in aliquots. Pathological concentration ranges were created by adequately mixing pools and by spiking with minerals, recombinant human enzymes and human al-
bumin (10). Commutability had been verified by SKML, as explained by Baadenhuijsen et al. and Jansen et al. (11,12). Throughout the years commutability has been monitored by including a native, single donation spy-sample (10,12).

Six vials of fresh frozen human serum pools at different concentrations were distributed once per year in a single express shipment at – 80 °C and delivered within 24 hours to laboratories all over Spain. Different lots at different concentrations were provided for each of the two surveys. Participant laboratories were requested to maintain samples at – 20 °C until analysis, which had to be performed within the following 14 days. Each vial had to be analysed in duplicate, one vial per day, for 6 consecutive days whenever possible. Results were registered on the SEQCML-EQA website, in order to be either individually and globally evaluated.

A preliminary 2013 survey was carried out in 19 laboratories and was addressed to ascertain whether the logistics of managing a non-stabilized set of control materials was operative in our country. No incidents were observed with temperature maintenance during the time between deliveries of control materials from the provider to the laboratory analysis.

Another point of interest of this preliminary survey was to explore whether laboratories could adequately inform about their analytical traceability to standards. Important difficulties were perceived that impelled holding a meeting between EQAs organization and providers, claiming for clear and complete information on calibrators’ traceability.

In 2014 first survey was performed, as part of a pilot European study (INPUTs) (Italy, The Netherlands, Portugal, Spain and The United Kingdom), with a total of 20 laboratories participants and whose results has been already published (12,13). Only about 45% of participants were able to correctly inform about its traceability, so results are not shown in this study. This survey was then considered as a pilot to identify the problems that could impact on the EQA participation and further interpretation of results. For both surveys as well as for those performed in 2015 and 2016, same sample management protocol was applied.

The 2015 and 2016 surveys were exclusively run in Spain and included 17 analytes. The number of registered participants was 93 and 105, respectively. The target values of distributed control materials were assigned by the reference methods and laboratories (Table 1).

Table 1. Analytes, reference methods and laboratories used to assign values

| Analytes | Reference method | Reference laboratory |
|----------|------------------|----------------------|
| **Electrolytes** | | |
| Calcium | Atomic Absorption Spectrometry | INSTAND eV. Düsseldorf, Germany |
| Chloride | | |
| Magnesium | ICP-IDMS | |
| Potassium | | |
| Sodium | | |
| **Substrates** | | |
| Bilirubin | Doumas method | DGKL, Hannover, Germany |
| Creatinine | IDMS | DGKL, Bonn, Germany |
| Glucose | GC-IDMS | INSTAND eV. Düsseldorf, Germany |
| Protein | Modified Biuret | |
| Urate | HPLC | Erasmus Medical Centre, Rotterdam, Netherlands |
| **Enzymes** | | |
| ALP | | Unknown |
| α-Amylase | | |
| AST | | |
| ALT | IFCC | Haga Hospital, The Netherlands |
| CK | | |
| GGT | | |
| LD | | |

The Doumas method according to Rainer et al. (14). ICP-IDMS - Inductively Coupled Plasma-Isotope Dilution Mass Spectrometry. DGKL - German Society for Clinical Chemistry and Laboratory medicine. IDMS - Isotope Dilution Mass Spectrometry. GC-IDMS - Gas Chromatography - Isotope Dilution Mass Spectrometry. HPLC - High Performance Liquid Chromatography. ALP: Alkaline phosphatase. ALT - alanine aminotransferase. AST - aspartate aminotransferase. CK - creatine kinase. GGT – gamma glutamyl transferase. LD - lactate dehydrogenase. IFCC - International Federation of Clinical Chemistry.
Results were categorized by measurement procedure, traceability and instrument. The description of standard materials used by participants for calibration traceability is shown in Table 2. Participant laboratories using the same combination of these three elements were considered as a peer group. The peer groups and the number of laboratories included for each analyte are shown in Figures 1-17. Compared to 2015, a new instrument was incorporated in 2016 survey (Bio-systems BA 400), with only 6 participating laboratories. The overall evaluation of the 2015 survey was published on the SE-QCML website and was presented at the 2016 EQALM annual meeting (13,15). Only groups formed by 5 or more final laboratories were considered in this study.

Inter-laboratory imprecision was calculated by averaging the coefficient of variation (CV) obtained from the six controls distributed on the 2016 and 2015 surveys and compared with the best (Dutch) inter-laboratory CV derived from the 2014 pilot study, which used similar six commutable control materials (16).

Bias was calculated by the percent difference between the peer group mean (same measurement procedure, traceability and instrument) and the reference value. The analytical performance specification to apply for bias evaluation was based on the BV data collected on the online 2014 database, which had been elaborated as detailed by Ricós et al., applying the minimum level of requirement for electrolytes and the desirable level for substrates and enzymes (17-19).

The results of this study were examined with the particular focus on the most common analytical procedures used in Spain and its repercussion on non-comparable results, detected throughout participation on level 1 EQA schemes.

Standardization is defined by the attainment of inter-laboratory imprecision within the predefined APS and peer group bias (% mean deviation to the reference value) below the allowed bias derived from BV.

**Table 2.** Description of standards used by participating laboratories

| Standard            | Traceability                                      |
|---------------------|---------------------------------------------------|
| ERM-AD 452 / IFCC   | Animal tissue. Non commutable                     |
| ERM-AD 455 / IFCC   | Lyophilized human serum. Commutability not proven |
| ERM-AD 453 / IFCC   | Animal tissue. Non commutable                     |
| IRMM / IFCC 456     | Human tissue. Commutability not proven             |
| NIST SRM 909 a,b    | Lyophilized human serum. Commutability not proven |
| NIST-SRM 915        | Calcium carbonate                                 |
| NIST SRM 918b       | Potassium chloride                                |
| NIST SRM 919b       | Sodium chloride                                   |
| NIST SRM 929        | Magnesium gluconate                               |
| NISTSRM 956, 965    | Frozen human serum. Commutability not proven       |
| NIST SRM 967        | Sodium chloride in aqueous solution                |
| NIST SRM 2201       | Potassium chloride in aqueous solution             |

Reference materials and analytes (involved in this study) associated: ERM-AD 452 / IFCC: gamma glutamyl transferase. ERM-AD 455 / IFCC: creatine kinase. ERM-AD 453 / IFCC: lactate dehydrogenase. NIST SRM 909 a,b: calcium, chloride, creatinine, magnesium, potassium, sodium, urate. NIST SRM 915: calcium. NIST SRM 918b: potassium. NIST SRM 919b: sodium. NIST SRM 929: magnesium. NIST SRM 956: calcium, magnesium, potassium, sodium. NIST SRM 965: glucose. NIST SRM 967: creatinine. NIST SRM 2201: sodium. NISTSRM 2202: potassium. IRMM - Institute for Reference Materials and Measurements. IFCC - International Federation of Clinical Chemistry.

**Results**

All results exceeding the mean ± 3 standard deviation of each group were rejected as outliers. The number of rejected participant laboratories was 5 for the 2015 survey and 10 for the 2016 survey. Moreover, 30 results for lactate dehydrogenase (LD) which were 100% higher than the others due to the different substrate (pyruvate instead of lactate) were also excluded from the study. Results for bias are presented in Figures 1-17. Results for the inter-laboratory imprecision of each peer group for electrolytes, enzymes and substrates are presented in Tables 3-5 and compared with the APS for inter-laboratory imprecision (APSIL) from the pilot 2014 survey (16). An overview of the
Ricós C. et al. Standardization with category 1 EQA

**Figure 1.** Calcium. Percentage deviation (Dev%) of peer group means from the reference value. X axis shows reference values of the six control materials. Y axis shows percent deviation of peer group mean versus the reference value. Numbers in brackets mean the number of participant laboratories. Lim Bias (m): acceptability limit for bias based on BV, minimum grade. NM-BAPTA: calcium specific amino-polycarboxylic acid.

**Figure 2.** Chloride. Percentage deviation (Dev%) of peer group means from the reference value. X axis shows reference values of the six control materials. Y axis shows percent deviation of peer group mean versus the reference value. Lim Bias (m): acceptability limit for bias based on BV, minimum grade. ISE - ion selective electrode. Numbers in brackets indicate the laboratories participating for each instrument.

**Figure 3.** Magnesium. Percentage deviation (Dev%) of peer group mean from the reference value. X axis shows reference values of the six control materials. Y axis shows percent deviation of peer group mean versus the reference value. Lim Bias (m): acceptability limit for bias based on BV, minimum grade. Xil - Xilidil blue. Numbers in brackets indicate the laboratories participating for each instrument.
Ricós C. et al. Standardization with category 1 EQA

**Figure 4.** Potassium. Percentage deviation (Dev%) of peer group mean from the reference value. X axis shows reference values of the six control materials. Y axis shows percent deviation of peer group mean versus the reference value. Lim Bias (m): acceptability limit for bias based on BV, minimum grade. ISE - ion selective electrode. Numbers in brackets indicate the laboratories participating for each instrument.

**Figure 5.** Sodium. Percentage deviation (Dev%) of peer group mean from the reference value. X axis shows reference values of the six control materials. Y axis shows percent deviation of peer group mean versus the reference value. Lim Bias (m): acceptability limit for bias based on BV, minimum grade. ISE - ion selective electrode. Numbers in brackets indicate the laboratories participating for each instrument.

**Figure 6.** Alkaline phosphatase. Percentage deviation (Dev%) of peer group means from the reference value. X axis shows reference values of the six control materials. Y axis shows percent deviation of peer group mean versus the reference value. Lim Bias (d): acceptability limit for bias based on BV, desirable grade. AMP - 2-amino-2-methyl-1-propanol. Numbers in brackets indicate the laboratories participating for each instrument.
Figure 7. Amylase. Percentage deviation (Dev%) of peer group mean from the reference value. X axis shows reference values of the six control materials. Y axis shows percent deviation of peer group mean versus the reference value. Lim Bias (d): acceptability limit for bias based on BV, desirable grade. G3 - malto trioside. G7 - malto-heptaoside. Numbers in brackets indicate the laboratories participating for each instrument.

Figure 8. ALT. Percentage deviation (Dev%) of peer group means from the reference value. X axis shows reference values of the six control materials. Y axis shows percent deviation of peer group mean versus the reference value. Lim Bias (d): acceptability limit for bias based on BV, desirable grade. Numbers in brackets indicate the laboratories participating for each instrument.

Figure 9. AST. Percentage deviation (Dev%) of peer group means from the reference value. X axis shows reference values of the six control materials. Y axis shows percent deviation of peer group mean versus the reference value. Lim Bias (d): acceptability limit for bias based on BV, desirable grade. PSP - pyridoxal-5-phosphate. Numbers in brackets indicate the laboratories participating for each instrument.
Figure 10. Creatine kinase. Percentage deviation (Dev%) of peer group mean from the reference value. X axis shows reference values of the six control materials. Y axis shows percent deviation of peer group mean versus the reference value. Lim Bias (d): acceptability limit for bias based on BV, desirable grade. NAC - N-acetyl-cysteine. Numbers in brackets indicate the laboratories participating for each instrument.

Figure 11. Gamma glutamyl transferase. Percentage deviation (Dev%) of peer group mean from the reference value. X axis shows reference values of the six control materials. Y axis shows percent deviation of peer group mean versus the reference value. Lim Bias (d): acceptability limit for bias based on BV, desirable grade. All groups use substrate: γ-glutamyl-3-carboxy-4-nitroanilide > 4 mmol/L. The exception is: Siemens Dimension, Vista that uses substrate < 4 mmol/L. Numbers in brackets indicate the laboratories participating for each instrument.

Figure 12. Lactate dehydrogenase. Percentage deviation (Dev%) of peer group mean from the reference value. X axis shows reference values of the six control materials. Y axis shows percent deviation of peer group mean versus the reference value. Lim Bias (d): acceptability limit for bias based on BV, desirable grade. NMG - N-methyl-D-glucamine. DEA - diethanolamine. TRIS - hydroxymethylaminomethane. Numbers in brackets indicate the laboratories participating for each instrument.
Ricós C. et al. Standardization with category 1 EQA

Figure 13. Bilirubin. Percentage deviation (Dev%) of peer group mean from the reference value. X axis shows reference values of the six control materials. Y axis shows percent deviation of peer group mean versus the reference value. Lim Bias (d): acceptability limit for bias based on BV, desirable grade. DPD - 3,5-dichlorophenyl-diazonium-tetrafluoroborate. Numbers in brackets indicate the laboratories participating for each instrument.

Figure 14. Creatinine. Percentage deviation (Dev%) of peer group mean from the reference value. Methods in figure appearing according to the following order: enzymatic, compensated and non-compensated. X axis shows reference values of the six control materials. Y axis shows percent deviation of peer group mean versus the reference value. Lim Bias (d): acceptability limit for bias based on BV, desirable grade. Numbers in brackets indicate the laboratories participating for each instrument.

Figure 15. Glucose. Percentage deviation (Dev%) of peer group mean from the reference value. X axis shows reference values of the six control materials. Y axis shows percent deviation of peer group mean versus the reference value. Lim Bias (d): acceptability limit for bias based on BV, desirable grade. GOD - glucose oxidase. HK - hexokinase. Numbers in brackets indicate the laboratories participating for each instrument.
standardization achieved in our setting, according to the bias and the imprecision calculated for instruments, is presented in Table 6.

**Discussion**

The percentage of laboratories excluded was higher in 2016 than in 2015 due to better knowledge of the traceability-instrument, so groups were more specific in 2016. This cannot be considered a disadvantage. The results in this study are discussed form the light of their impact on the aims proposed. These are: positive, negative and needed to be dialogued with providers.

Main positive impacts, which imply an adequate standardization not needing for further improvements, apply to potassium and creatine kinase (CK). Potassium shows inter-laboratory imprecision and bias (Figure 4) within the allowable limits for almost all peer groups. For the remaining electrolytes good inter-laboratory imprecision can also be seen, well in agreement with the 2014 survey (performed in collaboration with other European countries) where all participant laboratories and manufacturers fulfilled the APS for total analytical error at the minimum performance level (20). Creatine kinase show good inter-laboratory imprecision and bias (Figure 10), except for the new group...
**Table 3. Inter-laboratory imprecision for electrolytes**

| Electrolyte | 2015, CV (%) | 2016, CV (%) | APS_{IL} |
|-------------|--------------|--------------|----------|
| **Calcium** |              |              |          |
| Arsenazo, SRM 909b - Beckman Coulter AU | 1.2 | 4.4 |         |
| Arsenazo, SRM 909b - Siemens Advia | 1.6 | 6.2 |         |
| Arsenazo, SRM 915a - Abbott Architect | 1.2 | / |         |
| Arsenazo, SRM 956c - Abbott Architect | 2.4 | 5.9* | 3.5 |
| NM BAPTA, SRM 956 - Roche Cobas | 1.7 | 1.6 |         |
| Cresolfalein, SRM 915 - Siemens Dimension, Vista | 2.7 | 2.4 |         |
| Arsenazo, SRM 956c – Bio-systems BA | / | 2.6 |         |
| **Chloride** |              |              |          |
| ISE, SRM2202 – Abbott Architect | 0.6 | 1.4 |         |
| ISE, Gravimetry–Roche Cobas | 2.1 | 1.5 |         |
| ISE, SRM 919 – Siemens Advia | 0.7 | 0.6 |         |
| ISE, SRM 2201 – Siemens Vista | 0.6 | 2.3* | 1.4 |
| ISE, SRM 919 - Beckman Coulter AU | 1.4 | 0.7 |         |
| DSE, SRM 956c-Bio-systems BA400 | / | 0.6 |         |
| **Magnesium** |              |              |          |
| Xilidil blue, SRM 929 - Siemens Advia | 11.7* | 1.0 |         |
| Enzymatic, SRM 956 - Abbott Architect | 4.1 | 2.1 |         |
| Xilidil blue, SRM 909 - Beckman Coulter AU | 5.3 | 2.5 | 4.5 |
| Xilidil blue, Atomic Absorption -Roche Cobas | 8.5 | 3.0 |         |
| Xilidil blue, SRM929 - Siemens Dimension, Vista | 6.2 | 2.2 |         |
| **Potassium** |              |              |          |
| ISE, SRM 956-Abbott Architect | 0.9 | 1.2 |         |
| ISE, SRM 2202-Beckman Coulter AU | 0.6 | 0.8 |         |
| ISE, Gravimetry-Roche Cobas | 0.9 | 1.0 |         |
| ISE, SRM909b-Siemens Advia | 0.7 | 0.3 |         |
| ISE, SRM 909-Siemens Vista | 0.8 | 0.3 |         |
| DSE, SRM 956c-Bio-systems BA400 | / | 1.7 |         |
| **Sodium** |              |              |          |
| ISE,SRM 956-Abbott Architect | 0.4 | 0.9 |         |
| ISE, SRM 32202-Beckman Coulter AU | 0.6 | 0.7 |         |
| ISE,Gravimetry-Roche Cobas | 1.7 | 0.9 |         |
| ISE, SRM 909b - Siemens Advia | 0.5 | 0.3 |         |
| ISE,SRM 909-Siemens Vista | 0.5 | 0.6 |         |
| DSE, SRM 956c-Bio--systems BA400 | / | 0.4 |         |

*exceeding APS_{IL}*. The coefficient of variation (CV) is presented as the group’s average for six controls. DSE - direct selective electrode. ISE - indirect selective electrode. APS_{IL} - analytical performance specifications for inter-laboratory imprecision.
| Table 4. Inter-laboratory imprecision for enzymes |
|-----------------------------------------------|
| **ALP**                                      |
| 2015, CV (%)       | 2016, CV (%)       | APS_{IL}  |
| 4PNP-AMP, IFCC-A     | 1.9                | /         |
| 4PNP-AMP, IFCC-Beck       | 2.5                | 5.8       |
| 4PNP-AMP, IFCC-Roche     | 1.2                | 3.7       |
| 4PNP-AMP, IFCC-Sin         | 3.3                | 4.2       |
| 4PNP-AMP, IFCC-Sin Adv     | 3.0                | 5.5       |
| AMP, IFCC- Bio-systems BA | /                  | 11.7*     |
| **ALT**                                     |
| IFCC with PSP, IRMM/IFCC 454- Siemens Advia | 14.1*              | 15.7*     |
| IFCC without PSP, "IFCC"- Abbott Architect | 14.3*              | 6.7       |
| IFCC with PSP, IFCC- Beckman Coulter AU  | 13.2*              | 9.5       |
| IFCC without PSP, other- Roche Cobas 6000,8000 | 15.0*              | 3.4       |
| IFCC with PSP, IFCC- Siemens Vista | 17.0*              | 8.1       |
| IFCC with PSP- IFCC Bio-systems BA | /                  | 10.4      |
| **Amylase**                                 |
| G3, IFCC- Abbott Architect | 2.5                | 6.3       |
| G7 ethilidene, IFCC- Roche Cobas | 3.7                | 5.6       |
| G7 ethilidene, IFCC-Siemens Advia | 9.7                | 0.6       |
| G7 ethilidene, IFCC-Beckman Coulter AU | 2.5                | 3.2       |
| G3, IFCC-Siemens Dimension, Vista | 6.2                | 4.6       |
| G3, not declared-Bio-systems BA | /                  | 8.8       |
| **AST**                                     |
| IFCC with PSP, IRMM/IFCC 454- Siemens Advia | 6.4                | 4.2       |
| IFCC without PSP, "IFCC"- Abbott Architect | 3.0                | 3.2       |
| IFCC with PSP, IFCC- Beckman Coulter AU  | 1.4                | 2.1       |
| IFCC without PSP,other- Roche Cobas 6000,8000 | 4.5                | 8.7       |
| IFCC with PSP, IFCC- Siemens Vista | 6.0                | 5.6       |
| IFCC with PSP, IFCC- Bio-systems BA | /                  | 4.0       |
| **CK**                                      |
| NAC, IFCC - Abbott Architect | 2.2                | 3.7       |
| NAC, IFCC – Beckman Coulter AU | 3.9                | 2.6       |
| NAC, IFCC - Roche Cobas 6000,8000 | 7.4                | 4.5       |
| NAC, IFCC - Siemens Advia | 2.6                | 2.8       |
| NAC, IFCC - Siemens Dimension, Vista | 3.7                | 2.7       |
| NAC, IFCC - Bio-systems | /                  | 2.6       |
GGT

| IFCC- Abbott Architect | 1.1 | 4.2 |
|------------------------|-----|-----|
| IFCC- Beckman Coulter AU | 1.2 | 2.2 |
| IFCC- Roche Cobas | 3.6 | 2.2 |
| IFCC- Siemens Advia | 10.0 | 4.3 |
| IFCC- Siemens Dimension, Vista | 2.9 | 1.3 |
| IFCC- Bio-systems BA 400 | / | 6.5 |

LD

| L-P, DEA, IFCC - Abbott Architect | 2.6 | 6.0 |
| L-P, NMG, IFCC - Beckman Coulter AU | / | 10.0* |
| L-P, NMG, IFCC - Roche Cobas | 2.4 | 9.4* |
| L-P, TRIS, IFCC - Siemens Advia | 5.7 | 7.3* |
| L-P, NMG, IFCC - Siemens Dimension, Vista | 3.6 | 7.6* |

*exceeding APSIL. The coefficient of variation (CV) is presented as the group’s average for six controls. ALP - Alkaline phosphatase. ALT - Alanine aminotransferase. AST - Aspartate aminotransferase. CK - Creatine kinase. GGT – Gamma glutamyl transferase (substrate > 4 mmol/L only). LD - Lactate dehydrogenase (substrate lactate to pyruvate only). APSIL - Analytical performance specifications for inter-laboratory imprecision. 4PNP – 4-p-nitrophenyl phosphate. AMP - 2-amino-2-methyl-1-propanol. P5P - Pyridoxal-5-phosphate. IRMM - Institute for Reference Materials and Measurements. NAC - N-acetyl-cysteine. L-P - Lactate to pyruvate. DEA – Diethanolamine. NMG - N-methyl-D-glucamine. TRIS - Hydroxymethyl-aminomethane.

Table 5. Inter-laboratory imprecision for substrates

| Bilirubin | 2015, CV (%) | 2016, CV (%) | APSIL |
|-----------|--------------|--------------|-------|
| DPD, SRM 916-Abbott Architect | 3.8 | 4.7 |
| DPD, SRM 916-Beckman Coulter AU | 2.3 | 4.8 |
| DPD, SRM 916-Roche Cobas 6000, 8000 | 1.8 | 15.7* |
| Vanadate, SRM 916-Siemens Advia | 5.1 | 1.1 |
| Sulfanilic, SRM 916- Siemens Dimension, Vista | 5.3 | 2.5 |
| Sulfanilic, SRM 916-Biosystems BA | / | 6.5 |

Creatinine

| Jaf nc, SRM 967-Abbott Architect | 1.4 | 2.0 |
| Jaf nc, SRM 967-Beckman-Couler AU | 7.7 | 5.8 |
| Jaf c, IDMS – Roche Cobas6000, 8000 | 2.4 | 3.6 |
| Jaf c, SRM 967-Roche Cobas 6000, 8000 | 4.0 | / |
| Jaf c, SRM 967-Siemens Advia | 3.0 | 1.2 |
| Jaf c, NIST SRM 914a – Dimension | / | 1.4 |
| Enz, NIST SRM 967a – Coulter AU | / | 2.9 |
| Enz, NIST 967a – Bio-systems | / | 4.0 |
| Enz, IDMS-Cobas 8000 | / | 3.1 |
### Glucose

| Test          | Abbott Architect | Beckman Coulter AU | Roche Cobas 6000,8000 | Siemens Advia | Siemens Dimension, Vista | Bio-systems BA 400 |
|---------------|------------------|--------------------|-----------------------|--------------|-------------------------|-------------------|
| HK, SRM 965   | 5.4              | 5.3                | 5.9                   | 5.9          | 5.9                     | /                 |
| HK, SRM 965-Beckman Coulter AU | 2.4              | 2.4                | 2.4                   | 2.4          | 2.4                     | /                 |
| HK, SRM 965-IDMS-Roche Cobas 6000,8000 | 8.1*             | 8.1*               | 8.1*                  | 8.1*         | 8.1*                    | /                 |
| HK, SRM 965-Siemens Advia | 3.8              | 3.8                | 3.8                   | 3.8          | 3.8                     | /                 |
| HK, SRM 917-Siemens Dimension, Vista | 7.2*             | 7.2*               | 7.2*                  | 7.2*         | 7.2*                    | /                 |
| GOD, SRM 965-Bio-systems BA 400 | /                | /                  | /                     | /            | /                       | /                 |

### Total protein

| Test          | Abbott Architect | Beckman Coulter AU | Roche Cobas 6000,8000 | Siemens Advia | Siemens Vista | Bio-systems BA 400 |
|---------------|------------------|--------------------|-----------------------|--------------|--------------|-------------------|
| B, SRM 927    | 3.2              | 3.2                | 3.2                   | 3.2          | 3.2          | /                 |
| B, SRM 927-Beckman Coulter AU | 4.9              | 4.9                | 4.9                   | 4.9          | 4.9          | /                 |
| B, SRM 927-Roche Cobas 6000,8000 | 4.6*             | 4.6*               | 4.6*                  | 4.6*         | 4.6*         | /                 |
| B, SRM 927-Siemens Advia | 8.8*             | 8.8*               | 8.8*                  | 8.8*         | 8.8*         | /                 |
| B, SRM 927-Siemens Vista | 4.2              | 4.2                | 4.2                   | 4.2          | 4.2          | /                 |
| B, SRM 927-Bio-systems BA 400 | /                | /                  | /                     | /            | /            | /                 |

### Urate

| Test          | Abbott Architect | Beckman Coulter AU | Roche Cobas 6000,8000 | Siemens Advia | Siemens Vista | Bio-systems BA 400 |
|---------------|------------------|--------------------|-----------------------|--------------|--------------|-------------------|
| Uricase-POD, SRM 913-Abbott Architect | 3.0              | 3.0                | 3.0                   | 3.0          | 3.0          | /                 |
| Uricase-POD, IDMS-Beckman Coulter AU | 3.5              | 3.5                | 3.5                   | 3.5          | 3.5          | /                 |
| Uricase-POD, IDMS-Roche Cobas 6000,8000 | 3.5              | 3.5                | 3.5                   | 3.5          | 3.5          | /                 |
| Uricase-POD, SRM 909-Siemens Advia | 2.2              | 2.2                | 2.2                   | 2.2          | 2.2          | /                 |
| Uricase-POD, SRM 913-Siemens Dimension, Vista | 1.1              | 1.1                | 1.1                   | 1.1          | 1.1          | /                 |
| Uricase-POD, SRM 909c-Bio-systems BA400 | /                | /                  | /                     | /            | /            | /                 |

*exceeding APS\textsubscript{IL}. The coefficient of variation (CV) is presented as the group's average for six controls. Only instruments with more than 5 participating laboratories are shown in this table. APS\textsubscript{IL} - analytical performance specifications for inter-laboratory imprecision. B – Biuret. DPD - 3,5-dicholorophenyl-diazoniumtetrafluoroborate. Enz – enzymatic. Jaf – Jaffe. Jaf c – Jaffe compensated. Jaf nc - Jaffe non compensated. HK – hexokinase. POD – peroxidase.

enrolled in the 2016 survey (BA400). So it may be expected a well standardized measurements soon. Negative impacts may be due to several reasons. The aqueous matrix of SRM 915 and 918 used for calcium and sodium, respectively (Figures 1 and 5), produces low results. Lack of commutability of calibration traceability materials was described to be a crucial factor to assure standardization in medical laboratories by Panteghini and Ambruster (21,22).

Instrument dependent problems can be seen in this study for alkaline phosphatase (ALP) with low results for Roche users (Figure 6), whereas all participants use same method and traceability; this event causes an important lack of standardization in our country because it is the greatest group. Same results had been seen by Braga et al., and Aloisio et al. who observed discrepancies among Abbott Architect users related to an “experimental” calibration factor provided by the manufacturer (23,24). Non-standardized ALP results could have a great impact in some clinical scenarios such as hypophosphatemia diagnosis, so an improvement in the results’ traceability becomes a crucial objective (25). Method dependent troubles are seen in four cases.

Firstly, amylase, were all groups using malto-hep-taoside (G7) substrate, as well as the malto-trioside (G3) of Abbott Architect show harmonized results. The remaining G3 groups have unacceptable neg-
Table 6. Overview of achieved results toward standardization in our setting

| Analytes              | Architect | AU | BA400* | Cobas 6000 and 8000 | Advia | Dimension Vista |
|-----------------------|-----------|----|--------|---------------------|-------|-----------------|
| ALP                   | TI        | OK | TI     | TI                  | TI    | OK              |
| ALT                   | TI        | TI | TI     | TI                  | TI    | OK              |
| Amylase               | OK        | OK | TI     | OK                  | TI    | TI              |
| AST                   | TI        | TI | TI     | TI                  | TI    | TI              |
| Bilirubin             | TI        | TI | TI     | TI                  | TI    | TI              |
| Calcium               | TI        | TI | TI     | TI                  | TI    | TI              |
| Chloride              | OK        | TI | TI     | TI                  | TI    | OK              |
| CK                    | OK        | OK | TI     | OK                  | TI    | OK              |
| Creatinine, enzymatic | -         | -  | -      | OK                  | -     | OK              |
| Creatinine, Jaffe     | TI        | TI | TI     | TI                  | TI    | TI              |
| GGT                   | OK        | OK | OK     | OK                  | OK    | TI              |
| Glucose               | TI        | TI | TI     | TI                  | TI    | TI              |
| LD                    | OK        | TI | -      | TI                  | TI    | TI              |
| Magnesium             | TI        | TI | TI     | TI                  | TI    | TI              |
| Potassium             | OK        | OK | TI     | OK                  | OK    | OK              |
| Total protein         | TI        | TI | TI     | TI                  | TI    | TI              |
| Sodium                | TI        | TI | TI     | TI                  | TI    | TI              |
| Urate                 | OK        | TI | TI     | OK                  | TI    | OK              |

TI: To improve because either bias or inter-laboratory imprecision does not reach the APS in both or in one of the two surveys evaluated. *BA400 group (Bio-systems) began its participation in the 2016 survey. Only instruments with more than 5 participating laboratories are shown in this table. ALP - alkaline phosphatase. ALT - alanine aminotransferase. AST - aspartate aminotransferase. CK - creatine kinase. GGT – gamma glutamyl transferase. LD - lactate dehydrogenase. OK: Bias and inter-laboratory imprecision achieve the APS.

This lack of standardization affects one third of the participants of this study, thus producing a considerable impact on the healthcare in our country. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) testing show unacceptable inter-laboratory imprecision and bias (low results) (Figures 8 and 9) for laboratories that did not add pyridoxal-5-phosphate (P5P) in its measurement procedure. Infusino et al. and Jansen et al. reported that when reagent is supplemented with P5P the ratio of preformed holoenzyme to apoenzyme differs among specimens (12,26). Gamma glutamyl transferase (GGT), were all groups using substrate of γ-glutamyl-3carboxy-4nitroanilide > 4mmol/L have good precision and bias; however, the Siemens Dimension Vista group that uses a different concentration of substrate (< 4 mmol/L) produces unacceptable high results (Figure 11). Lastly, creatinine shows good inter-laboratory CV. However, only enzymatic methods have good bias at the entire concentration range studied, whereas most of the Jaffe based measurements produce unacceptable high results at low-normal concentrations (≤ 50 mmol/L) and some of them show inconsistent bias along the two surveys evaluated (Figure 14). Part of the 2015 results had been previously published and is in accordance with the 2016 survey, as well as with Jassam et al. that observed as Abbott compensated and Jaffe methods were most af-
fected by glucose interferences, resulting in either under- or over-estimation of GFR and may also lead to errors in the classification of chronically kidney disease (20,27,28). Likewise, data reported by Panteghini showed an 18 μmol/L positive bias derived from the Jaffe-based method on a Beckman AU 2710 instrument (29). These results are especially relevant for paediatric population. Our results evidences that for consecutive years the Jaffe method produces false high results at low-normal concentration values, in all the instruments used in our country. Consequently, creatinine is not standardized in our setting and considering the clinical implications associated, Jaffe method should be abandoned. Dialogue with providers is of upmost necessity in several cases. The main negative issue is the lack of adequate information about the calibration traceability of the measurement procedure; this circumstance was observed to affect the 55% of participating laboratories in 2015. In order to address and minimize this issue, the SEQCML-Analytical Quality Commission promoted regular and specific meetings with providers and holding educational communications and workshops in national laboratory congresses (5,6). This effort seems to have been worthy, observing a decrease in the percentage of wrong-coding traceability from 55% to 20% in 2016.

Some in vitro diagnostic medical device providers reported their methods for ALT and AST as “IFCC traceable” when no PSP was added; this created a high incidence of wrong codifications by laboratory workers that was solved and recorded by SEQCML after informing of this circumstance to providers and users.

Lactate dehydrogenase measurements gave good inter-laboratory CV in the 2015 survey but not in 2016; the reason for this remains unknown and should be discussed with providers. Bias showed an interesting improvement, resulting in satisfactory results for all users of the lactate to pyruvate based measurement in the 2016 survey (Figure 12).

Our findings for bilirubin, chloride, glucose, magnesium (irregular inter-laboratory CV and bias), as well as total protein and urate (good inter-laboratory imprecision, but irregular bias) led us to the opinion that a dialogue with providers would be necessary for improving standardization in our country.

A limitation of this study would be the reduced number of participants in certain groups, due to the fact that this program is still poorly known by many Spanish laboratories. Consequently, one symposium, various workshops in the national congress and specific meetings were organized in 2017, a book has been written in 2018 and other educational activities are planned for the future to overcome this limitation.

Another drawback might be that there is a single exercise per year; this could be not enough to guarantee the trueness for the rest of the year. Because the economic difficulty to make more distributions of these controls materials along the year, laboratories in Spain could use our regular EQA schemes (stabilized materials, peer group evaluation, one sample per month) to verify if their analytical performance is maintained along the year.

Conclusions

The two years of category 1 EQA program experience in our country have manifested a lack of standardization of the 17 more frequent general biochemistry tests used in our laboratories. The application of this kind of EQA program allows estimating measurement procedure-traceability-instrument bias in a way that can be expanded to what happens with real patient samples. The impact of the information obtained by category 1 EQA program on the lack of standardization is: to recommend abandoning methods such as for ALT, AST without exogenous pyridoxal phosphate, Jaffe method for creatinine, pyruvate-lactate for LD, and do not use non-commutable calibrators, such as aqueous solutions for calcium and sodium.

Potential conflict of interest

None declared.
References

1. Braga F, Panteghini M. Verification of in vitro medical diagnostics (IVD) metrological traceability: Responsibilities and strategies. Clin Chim Acta. 2014;432:55-61. https://doi.org/10.1016/j.cca.2013.11.022

2. Greaves RF. The central role of external quality assurance in harmonization and standardization for laboratory medicine. Clin Chem Lab Med. 2017;55:471-3. https://doi.org/10.1515/cclm-2016-0782

3. Miller WG, Jones GRD, Horowitz GL, Weykamp C. Proficiency testing/external quality assessment. Current challenges and future directions. Clin Chem. 2011;57:1670-80. https://doi.org/10.1373/clinchem.2011.168641

4. Myers GL, Miller WG. The roadmap for harmonization: status of the International Consortium on Harmonization of Clinical Laboratory Results. Clin Chem Lab Med. 2018;56:1667-72. https://doi.org/10.1515/cclm-2017-0907

5. González-Lao E, Díaz-Garzón J, Ricós C, Álvarez V, Fernández-Calle P, et al. Category 1 External Quality Assurance Program for serum Alanine Aminotransferase and Aspartate Aminotransferase. European Congress of Clinical Chemistry and Laboratory Medicine. Athens 2017. Available at: https://www.degruyter.com/view/j/cclm.2017.55.issue-55.55.issue-55.55.issue-s1.xml. Accessed September 19th 2018.

6. González-Lao E, Fernández-Calle P, Perich C, Ricós C, Álvarez V, Minchinela J, et al. Category 1 External Quality Assurance Program for serum Creatinine. 4th Joint EFLM-UEMS Congress “Laboratory Medicine at the Clinical Interface”. Warsaw 2016. https://www.degruyter.com/downloadpdf/j/cclm.2016.54.issue-10/cclm.2016-0657/cclm.2016-0657.pdf. Accessed September 19th 2018.

7. Fraser CG, Hyltoft Petersen P, Libeer JC, Ricós C. Proposals for setting generally applicable quality goals solely based on biology. Ann Clin Biochem. 1997;34:8-12. https://doi.org/10.1177/000456329703400103

8. Jones GRD, Albarede S, Kesseler D, MacKenzie F, Mammen J, Pedersen M, et al; EFLM Task Finish Group – Analytical Performance Specifications for EQAs (TFG-APSEQA). Analytical performance specifications for external quality assessment - definitions and descriptions. Clin Chem Lab Med. 2017;55:949-55. https://doi.org/10.1515/cclm-2017-0151

9. Dallas Jones GR. Analytical performance specifications for EQA schemes need for harmonization. Clin Chem Lab Med. 2015;53:919-24.

10. Cobbaert C, Weykamp C, Franck P, De Jorge R, Kuipers A, Setigtra H, et al. Systematic monitoring of standardization and harmonization status with commutable EQA-samples -five years’ experience from the Netherlands. Clin Chim Acta. 2012;414:234-40. https://doi.org/10.1016/j.cca.2012.09.027

11. Baadenhuijzen H, Steistra X, Cobaert C, Kuipers A, Weykamp C, Jansen R. Commutability assessment of potential reference materials using a multicenter split-patient-sample between-filed-methods (twin-study) design: study within the framework of the Dutch project “Calibration 2000”. Clin Chem. 2002;48:1520-5.

12. Jansen R, Jassam N, Thomas A, Perich C, Fernández-Calle P, Faria AP, et al. A category 1 EQA scheme for comparison of laboratory performance and method performance: An international pilot study in the framework of Calibration 2000 project. Clin Chim Acta. 2014;432:90-8. https://doi.org/10.1016/j.cca.2013.11.003

13. Cortés M, Alsina MJ, Álvarez V, Boned B, Biosca C, Bullich S, et al. Programa Piloto de Garantía Externa de la Calidad de Sero Conmutable con Valores de Referencia de la Sociedad Española de Bioquímica Clínica y Patología Molecular (2015). Available at: http://www.contcal.org/qcweb/Documents/90%20Analizar%20Sero%20Conmutable.pdf. Accessed September 19th 2017.

14. Klauke R, Hans-Joachim K, Friederike W, Denis G-K, Kornbainan B, Gerhard S. Reference measurement procedure for total bilirubin in serum re-evaluated and measurement uncertainty determined. Clin Chim Acta. 2018;481:115-20. https://doi.org/10.1016/j.cca.2018.02.037

15. Boned B. SEQC external quality assurance programs. Commutable serum with reference values. Impact on standardization. Available at: http://www.eqalm.org/site/event/Symposium2016.php. Accessed September 14th 2018.

16. Perich C, ricós C, Alvarez V, Biosca C, Boned B, Cava F, et al. External quality assurance programs as a tool for verifying standardization of measurement procedures: Pilot collaboration in Europe. Clin Chim Acta. 2014;432:82-9. https://doi.org/10.1016/j.cca.2013.11.005

17. Minchinela J, ricós C, Perich C, Fernández-Calle P, Álvarez V, Domenech MV, et al. Biological variation database and quality specifications for imprecision, bias and total error (desirable and minimum). The 2014 update. Available at: http://www.westgard.com/biodatabase-2014-update.htm. Accessed September 19th 2018.

18. ricós C, Alvarez V, Perich C, Fernández-Calle P, Minchinela J, Cava F, et al. Rationale for using data on biological variation. Clin Chem Lab Med. 2015;53:863-70.

19. ricós C, álverez V, Minchinela J, Fernández-Calle P, Perich P, Boned B, et al. Biological variation approach to daily laboratory. Clin Lab. 2017;37:47-56. https://doi.org/10.1016/j.cll.2016.09.005

20. Weykamp C, Secchiero S, Plebani M, Thelen M, Cobbaert C, Thomas A, et al. Analytical performance of 17 general chemistry analytes across countries and across manufacturers in the INPUtS project of EQA organizers in Italy, the Netherlands, Portugal, United Kingdom and Spain. Clin Chem Lab Med. 2017;55:203-11. https://doi.org/10.1515/cclm-2016-0220

21. Panteghini M. Traceability as a unique tool to improve standardization in laboratory medicine. Clin Biochem. 2009;42:236-40. https://doi.org/10.1016/j.clinbiochem.2008.09.098

22. Armbrustner D, Miller RR. The Joint Committee for Traceability in Laboratory Medicine (JCTLM): a global approach to promote the standardisation of Clinical Laboratory Test Results. Clin Biochem Rev. 2007;28:105-13.
23. Braga F, Frusciante E, Infusino I, Aloisio E, Guerra E, Ceriotti F, Panteghini M. Evaluation of the trueness of serum alkaline phosphatase measurement in a group of Italian laboratories. Clin Chem Lab Med. 2017;55:e47-e50. https://doi.org/10.1515/cclm-2016-0605

24. Aloisio E, Frusciante E, Pasqualetti S, Querciolli M, Panteghini M. Traceability of alkaline phosphatase measurement may also vary considerably using the same analytical system: the case of Abbott Architect. Clin Chem Lab Med. 2018;56:e135-e137. https://doi.org/10.1515/cclm-2017-1007

25. Deeb A, Elfatih A. Could alerting physicians for low alkaline phosphatase levels be helpful in early diagnosis of hypophosphatasia? J Clin Res Pediatr Endocrinol. 2018;10:19-24. https://doi.org/10.4274/jcrpe.4426

26. Infusino I, Frusciante E, Braga F, Panteghini M. Progress and impact of enzyme measurement standardization. Clin Chem Lab Med. 2017;55:334-40. https://doi.org/10.1515/cclm-2016-0661

27. González-Lao E, Díaz-Garzón J, Corte Z, Ricós C, Perich C, Álvarez V, et al. Category 1 External Quality Assessment Program for serum creatinine. Ann Transl Med. 2017;5:133. https://doi.org/10.21037/atm.2017.03.70

28. Jassam N, Weykamp C, Secchiro S, Plebani M, Sciacciotti L, Thelen M, et al. Post standardization of routine creatinine assays- are they suitable for clinical applications? Ann Clin Biochem. 2017;54:386-94. https://doi.org/10.1177/0004563216664541

29. Panteghini M. Enzymatic assays for creatinine: time for action. Clin Chem Lab Med. 2008;46:567-72. https://doi.org/10.1080/00365510802149978