A category 1 EQA scheme for comparison of laboratory performance and method performance: An international pilot study in the framework of the Calibration 2000 project

Rob Jansen a,⁎, Nuthar Jassam b, Annette Thomas c, Carmen Perich d, Pilar Fernandez-Calle d, Ana Paula Faria e, Helena Correia e, Julian H. Barth f, Cas Weykamp a, Christa Cobbaert g, Marc Thelen h, Carmen Ricós d

a Dutch Foundation for Quality Assessment in Medical Laboratories (SKML), Nijmegen, The Netherlands
b Department of Clinical Biochemistry, Harrogate District Foundation Trust, Harrogate, UK
c WEQAS Quality Laboratory, Cardiff and Vale University Health Board, Cardiff, UK
d Spanish Society of Clinical Chemistry and Molecular Pathology (SEQC), Analytical Quality Commission, Spain
e Instituto Nacional de Saúde, Portugal
f Department of Clinical Chemistry, Leiden University Medical Center, Leiden, The Netherlands
g Department of Clinical Chemistry and Hematology, Amphia Hospital, Breda, The Netherlands
h WEQAS Quality Laboratory, Cardiff and Vale University Health Board, Cardiff, UK

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ABSTRACT

Introduction: In the modern healthcare service, patients receive care at multiple hospitals and healthcare settings. Therefore, harmonization of results from different methods and instruments, both between and within laboratories, is of the utmost importance. The present pilot study aims to test the use of a Category 1 EQA scheme across four European countries by assessing the current level of equivalence of test results.

Method: This work was led by the Dutch External Quality Assurance Scheme SKML and involved 28 laboratories from three regions in the UK, Spain and Portugal, and 120 laboratories from The Netherlands. A set of six commutable samples, targeted with reference methods, were circulated and 18 biochemistry analytes were tested.

Results and conclusions: The Total Error (TE) score, defined as the probability (%) that results are within the Total Error Acceptable (TEA) limits, for the eighteen analytes was calculated. Our data show that there is a need for further harmonization of laboratory data, in particular for electrolytes (calcium, chloride, magnesium, sodium), enzymes (ALT, amylase, AST, LDH), lipids (HDL-cholesterol), and for substrates (creatinine, total protein). Lack of performance consistency between instruments was seen for most analytes. The lack of harmonization is still present, despite manufacturer claims of established traceability.

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1. Introduction

Most efforts in the management of analytical quality in clinical chemistry and laboratory medicine have focused on the reduction of within-laboratory variation and the assessment of between-laboratory variation. In recent years the importance of minimizing bias, both between laboratories and within laboratory, has become paramount. Patients are frequently treated by a team of physicians rather than one, often extending across several healthcare settings and making use of information from several laboratories. In monitoring patients during treatment, the absence of bias from one measurement to the next, together with minimum imprecision is essential. Calibration and harmonization of results from different analyzers, both between and within laboratories, and the continuity of such harmonization in time are, therefore, of the utmost importance. Small assay biases may have a large impact on patient classification and on the number of patients to be treated, particularly for assays for which cut-off values are used. This is true, for example, in lipid and lipoprotein analyses, in which stringent cut-off values are used throughout the world for the prevention and treatment of cardiovascular diseases. It is also true for creatinine in the estimation of renal function or for human growth hormone in HGH deficiency.

The American Association for Clinical Chemistry (AACC) conference in October 2010 focused on the roadmap [1] to reach harmonization for analytes for which no reference system is defined. However, even for analytes for which such systems exist, standardization is often lacking. The process is defined as standardization if the analyte is clearly defined and reference method and standards exist. Harmonization is confined to describe processes where one or more of these elements are missing. External Quality Assessment schemes should play a central role in achieving harmonization and in trueness verification. It is widely
accepted that we need commutable materials [2,3], reference method target values and tolerance limits based on the biological variation concept [4–6]. EQA schemes having these characteristics have been denoted as Category 1 schemes [7]. The importance of using this concept in EQA schemes was stressed recently in several sessions during the Biorad Convocation of Experts on Laboratory Quality 2010 in Bardolino, Italy [8] and 2011 in Salzburg, Austria. In the Calibration 2000 project of the Dutch NEQAS organizer SKML, this was achieved for several analytes [9–15]. The In vitro Diagnostic medical Devices Directive (IVDD) requires traceability to reference systems [16]. These systems are defined for a number of analytes. For these analytes trueness verification is possible and harmonization is within reach.

The Calibration 2000 project in The Netherlands produces materials [9–15] for general clinical chemistry, proven to be commutable in conformity with the Clinical and Laboratory Standards Institute (CLSI) C53A [17]. The samples are targeted with reference methods, undertaken in either The Joint Committee for Traceability in Laboratory Medicine (JCTLM) listed Reference laboratories or in International Federation of Clinical Chemistry and Laboratory medicine (IFCC) network laboratories, if available, and results are processed in the Combi scheme [11–14] in which participating laboratories assay several samples covering the clinically interesting concentration range. The scheme uses the biological variation based Total Error allowable (TEa) at the desirable level as tolerance limit. Harmonization of minimal acceptable performance criteria among EQA organizers is desirable [18].

The present study is a pilot study. It aims to test the use of a Category 1 EQA scheme across the countries, UK, Spain, Portugal and The Netherlands, and to compare in a pilot study the performance of the participating laboratories and the methods used. The results of the pilot study are seen as a preliminary view of the role of category 1 EQA to improve harmonization in Europe.

2. Methods

In the SKML Combi scheme 24 samples are analyzed for general chemistry parameters in the course of a year, i.e. at a frequency of one sample per 2 weeks. For the lipids a separate dedicated batch of 24 samples is used. The samples are prepared according to exactly the same protocol as previously prepared samples which were proven to be commutable [9–15]. In short, two master samples are prepared, one from pooled normal human left over sera and one from pooled normal human sera, spiked with abnormal pools, minerals, recombinant human enzymes and human albumin. The master pools are mixed in ten proportions thus obtaining 12 concentration levels. After dispensing, vials are frozen at −84 °C. Previously prepared samples according to this procedure were repeatedly proven to be commutable, both master samples and spiked and mixed samples. Throughout the years commutability has been monitored by including a native, single donation spy-sample that is prepared according to CLSI C37-A2. Concentrations cover the range of clinical interest. The samples are targeted by JCTLM listed laboratories for electrolytes and substrates, and by IFCC or CDC network laboratories for enzymes and lipids. Information on reference methods and laboratories used is provided as supplementary data. Biological variation based tolerance limits are used (TEa desirable).

Thirty laboratories from three European countries participated in this study in addition to 120 regularly participating Dutch SKML EQA Combi scheme. Ten laboratories each participated from the UK, Spain (one lab with two procedures for all analytes, except for lipids) and Portugal. The UK laboratories’ inclusion in this study was solely based on expression of interest from laboratories that have received an invitation for participation. The authors have had no previous knowledge of the analytical performance for the participating laboratories.

The Spanish and the Portuguese laboratories were selected from the laboratories falling within the 20th percentile of the target deviation of their national EQA schemes. However, the participating laboratories range from small independent health care laboratories to large laboratories serving teaching hospitals, which is a mix of size and analytical platforms, which reflects the same distribution in each country.

A set of six samples for general chemistry and a set of six samples for lipids frozen at −80 °C, were transported on dry ice to a central laboratory in each of the three countries, and stored at −80 °C. The frozen samples were distributed on dry ice from the central laboratory to the participating laboratories. Samples arrived thawed in two Portuguese laboratories and these were discarded. Since most of the laboratories...
did not have a −80 °C freezer available, the laboratories were asked to analyze the samples as soon as possible after receipt or to store the samples at −20 °C and analyze them within 1 week (the period of stability, as determined by the sample provider). The Dutch participants received their sets of samples at the start of the year and kept these at −80 °C until analysis.

Laboratories were asked to analyze 18 analytes for which target values were obtained from reference labs using internationally recognized reference methods and reference materials:

- 5 electrolytes: calcium, chloride, magnesium, potassium, sodium;
- 6 enzymes: ALT, amylase, AST, CK, Gamma-GT, LDH;
- 2 lipids: cholesterol, HDL-cholesterol; and
- 4 substrates and a formula: creatinine, eGFR (F, 55y, Caucasian), glucose, total protein, uric acid.

The laboratories were asked to use their routine methods with no adaptations compared to routine practice. The laboratories reported their results, methods, and the instruments used. The laboratories in the UK and Spain mostly reported SI units, while the laboratories in Portugal mostly reported conventional units. Conventional units were converted to SI units by the organizer of the pilot study. In a few cases the reported results were not in agreement with the reported units and corrections were made. One Spanish laboratory reported creatinine in SI units after converting its conventional units (mg/dL), however using a wrong converting factor. This mistake did not affect the eGFR results, because a formula for creatinine values in mg/dL was used. Results for creatinine of this laboratory were discarded. Four Spanish laboratories used a pancreatic amylase assay instead of total amylase and their results were discarded.

2.1. Statistical methods

In the Combi scheme in each round and for each analyte the individual lab data is reported, displayed as a difference plot of the six results compared with the reference method value (Fig. 1). A tolerance area is constructed around the reference values based on the TEA (desirable) limit [4,5]. In the Combi scheme the desirable TEA limit (TEA = 1.65 × 0.5 × CVw + 0.25 √(CVw^2 + CVb^2)) is used rather than the minimal or optimal limits as alternative approaches suggested by Fraser [6]. Linear regression is calculated from the laboratory results against the consensus method group mean value. As the samples are
measured on different days, the residual SD of the regression line represents the within-laboratory SDWL. The difference between the mean of the six laboratory results and the average of the six reference values is the bias. Using SDWL at the average concentration of the six samples the probability is estimated that the laboratory results will be within the TEA tolerance area. This probability is the percentage of the density function (the broadness of which is defined by SDWL) around the laboratory bias that is within the TEA area. The TE score equals this percentage. By definition TE includes bias and imprecision. Causes of lower TE scores could be significant positive or negative bias, or a large within-laboratory SD. Increasingly, minimal acceptable performance criteria based on the biological TEA concept are being utilized within laboratories. The level of acceptance is defined by Fraser [6] as minimal, desirable or optimal. In the SKML scheme performance is considered to be acceptable if the results of a laboratory are within the desirable TEA area with a probability of 95%.

For each analyte the TE scores of the individual laboratories of the UK, Spain and Portugal were plotted against and compared to the average TE score of the Dutch laboratories. For each analyte, the individual laboratory results sorted by instrument were also plotted. Average TE-scores and the percentage TE scores >95% were calculated for the four countries and the percentage of laboratories that had a TE score >95%. The Netherlands’ TE score was the highest at 81%, followed by the UK’s at 77%, Spain’s at 75% and Portugal’s at 67%.

Table 1 presents the TEA values [5], average TE scores of the four countries and the percentage of laboratories that had a TE score ≥95%. The Netherlands’ TE score was the highest at 81%, followed by the UK’s at 77%, Spain’s at 75% and Portugal’s at 67%.

TE scores for all electrolytes, except potassium, in all of the four countries are low (Table 1, Fig. 3). Urgent improvement in harmonization is needed particularly for calcium, chloride, magnesium and sodium where less than 30% of the TE scores were above the 95% criterion. The same observation was maintained for the enzymes. The highest TE score has been seen for CK and GGT. However, a wide variation in TE score within and between countries has been recorded for ALT, AST and amylase. For amylase, laboratories show two types of TE scores, either TE above 95% or a very low score often zero. Calibration to a method different from the reference method is the main cause of low TE scores. Four Spanish labs analyzed pancreatic amylase instead of total amylase. The results of these laboratories were removed as they were testing a different analyte.

With the exception of The Netherlands, Portugal, Spain and the UK showed poor TE score for LDH with many scores of zero obtained and 19 out of 28 laboratories having scores below 10% (Fig. 4). This is due to the fact that a number of laboratories are using a pyruvate to lactate substrate. These methods vary by a factor of 2 and will therefore have a profound effect on bias, which explains the poor TE score for these laboratories.

In general the TE scores for enzymes in The Netherlands are higher, and a larger percentage of the laboratories score above the 95% limit, as compared to the other countries.

For cholesterol the average TE scores were above 90% and over 85% of the laboratories satisfied the criterion of TE score ≥95% (Table 1 and Fig. 5). However, the HDL method has not matched the consistent high performance for cholesterol. While the Portuguese achieved a TE score of 100% for all the participating laboratories, other countries demonstrated a wider variation in performance (Fig. 5).

For creatinine (Table 1 and Fig. 6) low average scores were obtained for creatinine (Table 1 and Fig. 6) low average scores were obtained as well as low percentages of TE scores ≥95%, indicating that many

Table 1

<table>
<thead>
<tr>
<th>Analyte</th>
<th>TEA</th>
<th>NL %</th>
<th>NL &gt;95%</th>
<th>PT %</th>
<th>PT &gt;95%</th>
<th>ES %</th>
<th>ES &gt;95%</th>
<th>UK %</th>
<th>UK &gt;95%</th>
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<td>18</td>
<td>65</td>
<td>0</td>
<td>64</td>
<td>27</td>
<td>73</td>
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<tr>
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<td>16</td>
<td>39</td>
<td>0</td>
<td>81</td>
<td>30</td>
<td>72</td>
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<td>28</td>
<td>57</td>
<td>13</td>
<td>67</td>
<td>22</td>
<td>79</td>
<td>30</td>
</tr>
<tr>
<td>Potassium</td>
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<td>94</td>
<td>77</td>
<td>89</td>
<td>63</td>
<td>97</td>
<td>82</td>
<td>97</td>
<td>70</td>
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<tr>
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<td>5</td>
<td>26</td>
<td>0</td>
<td>42</td>
<td>9</td>
<td>47</td>
<td>20</td>
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<td>ALT</td>
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<td>84</td>
<td>80</td>
<td>63</td>
<td>83</td>
<td>45</td>
<td>87</td>
<td>40</td>
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<tr>
<td>Amylase</td>
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<td>85</td>
<td>77</td>
<td>53</td>
<td>43</td>
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<td>88</td>
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<td>90</td>
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<td>HDL-cholesterol</td>
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<td>55</td>
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<td>100</td>
<td>74</td>
<td>60</td>
<td>82</td>
<td>60</td>
</tr>
<tr>
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<td>52</td>
<td>13</td>
<td>33</td>
<td>0</td>
<td>65</td>
<td>20</td>
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<td>eGFR (F, 55y, Caucasian)</td>
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<td>66</td>
<td>47</td>
<td>62</td>
<td>33</td>
<td>64</td>
<td>27</td>
<td>57</td>
<td>25</td>
</tr>
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<td>67</td>
<td>88</td>
<td>63</td>
<td>92</td>
<td>73</td>
<td>96</td>
<td>90</td>
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<tr>
<td>Total protein</td>
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<td>58</td>
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<td>53</td>
<td>13</td>
<td>77</td>
<td>36</td>
<td>64</td>
<td>30</td>
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<td>98</td>
<td>96</td>
<td>93</td>
<td>63</td>
<td>99</td>
<td>91</td>
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<td>Overall</td>
<td>81</td>
<td>67</td>
<td>75</td>
<td>77</td>
<td>10</td>
<td>72</td>
<td>30</td>
<td>90</td>
<td>90</td>
</tr>
</tbody>
</table>

Fig. 1 shows an example of a difference plot of the six results of a single laboratory for creatinine. The green area represents the TEA area around the reference values. The blue area is the state of the art agreement with previously reported results [19].

laboratories failed to achieve minimal acceptable performance. Instruments show widely varying results. The Jaffé methods had the lowest score (data not shown), which is in agreement with previously reported results [20].

This has a consequence for eGFR, which was calculated using different formulae by 23 participating laboratories. Average TE scores were below 70% and less than half of the laboratories attained a TE score of 95%.

Glucose and uric acid met the acceptable performance criterion of a TE score >95% for the majority of participating laboratories in the four countries.

The data for Total Protein indicated unsatisfactory performance, with average TE scores well below 95% and more than 70% of the laboratories failing the 95% criterion. Fig. 6 illustrates widely varying individual scores.

**Fig. 3.** Electrolytes, TE scores of individual laboratories per country (region). The blue line represents the average TE score for The Netherlands laboratories.
4. Discussion

In the European Union, the IVDD 98/79/EC [21] demands traceability of test results to a higher order reference material. This means that the results for each instrument type should be comparable with reference method results. However, this pilot study shows considerable within instrument and between laboratory variations in TE scores. Although the number of participant laboratories from outside The Netherlands is small they may be considered as representative of countries because they are positioned within the 20th percentile of the target deviation distribution in their national EQA schemes (Spain, Portugal) or are representative for a whole region (Yorkshire, UK).

Fig. 4. Enzyme TE scores of individual laboratories per country (region). The blue line represents the average TE score for The Netherlands laboratories.
Jansen et al. showed in 2006 [19] that large variation between methods and analytical platforms exist for the enzymes and that in many cases there is a lack of traceability and harmonization despite the IVDD requirements. This study shows little improvement with enzymatic methods and analytical platforms exist for the enzymes and that in be corrected by calibration. Despite the IFCC recommendations [24,25], and marked effect on transaminase activity especially AST which cannot pyridoxal phosphate in the reagent pack. The co-enzyme has a variable to the use of ALT/AST methods lacking the addition of the co-enzyme users of the same instrument. In our view, this methods e.g. ALT/AST, variation in TE score has been seen within the users of the same instrument. In our view, this finding may be attributed to the use of ALT/AST methods lacking the addition of the co-enzyme pyridoxal phosphate in the reagent pack. The co-enzyme has a variable and marked effect on transaminase activity especially AST which cannot be corrected by calibration. Despite the IFCC recommendations [24,25], manufacturers still present the market with method versions lacking pyridoxal phosphate. Methods that do not contain the co-enzyme cannot be considered traceable. Another source of discrepant (biased) results has been observed for a Spanish laboratory (data not shown) for AST and gamma-GT when a routine calibrator was traceable to a non commutable reference material, whereas results were correct when correctly calibrated and traceable to a reference method. In these examples, the manufacturers can play a pivotal role in paving the road to harmonization, by simply removing undesired methods from the market.

The variation in the TE scores in UK, Spain and Portugal cannot be explained by the different analytical platforms. Fig. 2 shows examples of TE ranges for instruments used. Within the same instrument TE scores vary greatly, in some cases from 0% to 100%. One explanation for this is the production by manufacturers of more than one assay on the same platform for some analytes, e.g. LDH lactate to pyruvate and pyruvate to lactate, and AST/ALT with and without P5P, whilst traceability demands the IFCC recommendations. Other reasons could be bias due to the use of different factors, different calibrators, and varying within-laboratory SD. The bias could be proportional, constant or mixed i.e. varying across the concentration area. Inspection of the data shows that in many cases all of these errors are present. E.g. for creatinine many labs use the non-compensated Jaffé kinetic method, giving a positive bias at low concentration level. Laboratories need to show acceptable precision as well as bias to attain a TE score of 95%. Lack of commutability of the reference material used for routine calibrator traceability has been seen as a major reason for biased results in the Spanish group. The same happens for magnesium and sodium. Our data shows that urgent improvement in harmonization is needed particularly for calcium, chloride, magnesium and sodium where less than 30% of the TE scores were above the 95% criterion. Harmonization of analytes that have a narrow biological variability can be improved by sharing a common but clinically relevant analytical goal [26]. Examples of different kinds of errors made are provided as supplementary data.

All the analytes in this study have a well-defined reference measurement procedure and traceability chain, yet considerable analytical variation has been seen. This suggests that standardization alone is not sufficient to guarantee production of comparable results. Traceability of a method to higher order reference measurement methods does not necessarily mean that the field method results are identical to the reference method results. It requires a functional relationship between the method and the reference method and reference material. From a patient’s perspective, results from different laboratories should not only be traceable to the reference method, i.e. show a defined functional relationship to the reference method, but should in addition be standardised, i.e. give equivalent results as the reference method. The Calibration 2000 project [9–11,15] and the present results show that harmonization is achievable for some analytes as shown in the Category 1 EQA scheme. The Combi scheme in its present form, using commutable samples, value assigned with reference methods, and having biological variance based tolerance limits, has been operational in The Netherlands for over 7 years. In an attempt to replicate The Netherlands experience, with larger numbers of laboratories, the Portuguese, the Spanish and the UK EQA scheme organizers are considering collaboration in at least one round per year in the SKML Combi scheme.

Since 2005, the Spanish Society of Clinical Chemistry (SEQC) have undertaken an educational task in recommending the use of biological variation as tolerance limits and these criteria are included in the participants’ reports. Despite this, the group’s results are not as satisfactory as they should be. This is mainly due to the lack of method harmonization and traceability and not to a different culture in quality monitoring practices.

5. Conclusion

The IVDD 98/79/EC demands traceability of test results to a reference system, if available. Our data show that there is a need for further harmonization of laboratory data, in particular for electrolytes (calcium, chloride magnesium, sodium), enzymes (ALT, amylase, AST, LD), lipids (HDL-cholesterol), and for substrates (creatine, total protein). Lack of performance consistency between instruments was seen for most analytes. The variation in the TE scores cannot be explained by the
different analytical platforms. Within the same instrument TE scores vary greatly, in some cases from 0% to 100%. Lack of harmonization is still present, despite manufacturer claims of established traceability. Current data shows that the standardization of methods is insufficient to result in complete consistency in reporting of laboratory results and needs to be followed by harmonization of the methods and practices.

**Fig. 6.** Substrate TE scores of individual laboratories per country (region). The blue line represents the average TE score for The Netherlands laboratories.

**Declarations**

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Guarantor: Dr Rob Jansen
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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.cca.2013.11.003.

References