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A Multilaboratory Commutability Evaluation of Proficiency Testing Material for Carbamazepine and Valproic Acid: A Study Within the Framework of the Dutch Calibration 2000 Project

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Background: Medical laboratories are required to participate in interlaboratory comparisons of the analyses they perform. The materials used in these comparisons need to be of sufficient quality so that the comparison provides a picture of the performances. One of the main characteristics of the testing material is commutability, which is the ability of a material to yield the same numerical relationships between results of measurements as those relationships obtained when the same procedures are applied to patient samples. The aim of this study was to assess the commutability of 3 different matrices for the preparation of proficiency testing material (PTM) for the analysis of carbamazepine and valproic acid.

Methods: Patient samples and PTM containing various concentrations of carbamazepine and valproic acid were collected, prepared, and shipped to different laboratories for analysis. Reported results for patient samples from each laboratory were plotted against results for patient samples of each of the other laboratories, and the corresponding regression line was calculated. The distance of results from PTM to the regression line is a measure for commutability. The distance is expressed as a multiple of the SD_{wl} (average within-laboratory SD as calculated from external quality assessment scheme results) and referred to as relative residual. A commutability decision limit of 2 SD_{wl} was set.

Results: For carbamazepine and valproic acid, a total of 78 and 105 laboratory couples respectively could be formed. The number of relative residuals for liquid human serum outside the commutability decision limit was 1, 4, and 0 for low, medium, and high concentrations of carbamazepine, respectively and 3, 1, and 0 for low, medium, and high concentrations of valproic acid, respectively. In both liquid and lyophilized bovine sera, the number of relative residuals outside the commutability decision limit was between 2 and 15 and between 6 and 21 for carbamazepine and valproic acid, respectively.

Conclusions: Although not all results for PTM with carbamazepine and valproic acid are within the commutability decision limits, a preference for human serum can be seen.

Key Words: commutability, external quality assessment scheme, proficiency testing material, immunoassay

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INTRODUCTION

According to the International Organization for Standardization, all medical laboratories are required to participate in interlaboratory comparisons or proficiency testing for quality assessment of their analysis.[1] These external quality assessment schemes (EQAS) preferably contain proficiency testing material (PTM) that closely resembles patient samples,[2,3] a characteristic that is also known as commutability, to evaluate the results reported by participants. Commutability is defined by the Clinical and Laboratory Standards Institute (CLSI) as "the ability of a material to yield the same numerical relationships between results of measurements by a given set of measurement procedures, purporting to measure the same quantity, as those between the expectations of the relationships obtained when the same procedures are applied to other relevant types of material."[4]

Commutability of PTM is of great importance in the comparison of participants' results among different methods of analysis, because erroneous results for PTM from participants can result from analytical errors, the use of unsuitable samples and/or erroneous distribution of samples. With the use of (correctly distributed) commutable proficiency-test samples, the problem of unsuitable samples can be eliminated, and observed biases can be assigned to used analytical methods.[5,6] The importance of the use of commutable samples in EQAS was recently shown in a study by Jansen et al and Perich et al.[7,8] In this study, 10 of 26 analytes did not show comparable results

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to the reference measurement. These incomparability's were caused by different items including the use of reference materials without established commutability, the use of noncommutable reference material to assign values to the routine calibrator and the use of nonstandard methods (eg, the Jaffé kinetic method instead of an enzymatic method).

The Association for Quality Assessment in Therapeutic Drug Monitoring and Clinical Toxicology (KKGT), a section of the Dutch EQAS organizer SKML (Dutch Foundation for Quality Assessment in Medical Laboratories), has developed its own PTM. The samples are based on lyophilized bovine serum, with exception of the samples for drugs of abuse testing that are prepared with human urine and the samples for the antiretroviral and antifungal drugs programs that are validated and used to be prepared with human plasma[9] and are currently prepared with human serum.

In 2004, the KKGT joined the Dutch project "Calibration 2000," an initiative of the SKML that started in 1998. The goal of this working group is harmonization of results reported by all different kinds of laboratories in the medical field.[10] Because of the trend where patients are treated in more than 1 hospital, harmonization will improve the continuity of laboratory results and will provide better patient care.

The assessment of commutability of all components in the KKGT EQAS will take several years based on the number of programs and drugs tested. Therefore, commutability studies were started for (1) components from which results in proficiency rounds have shown to be nonrobust, according to the six sigma methodology (see "study design"); (2) new components added to existing programs; (3) new programs added to the EQAS; or (4) components that are analyzed by an immunoassay-based method.

MATERIALS AND METHODS

Study Design

Before a commutability study was performed, a retrospective data analysis of the averages of results reported by participants per round for anti-epileptic drugs was performed for a period of 10 years in which various PTMs were used, which were not tested for commutability. Results reported by participants as a concentration were converted by KKGT to the percentage of the weighed-in concentration of the analyte. Participant results per round were averaged after removal of outliers.

The process capability index (C_{pk}) was calculated according to the six sigma methodology.[11] The C_{pk} value is a statistic that indicates the variability of process characteristics and indicates if a process is able to perform according to the specification limits and is calculated as the minimal difference between the average result and the lower or upper specification limit, divided by 3 times the standard deviation.[11] The higher the C_{pk} value, the more a process performs according to the specified limits.

For therapeutic drug monitoring, lower and upper specification limits of 95% and 105%, respectively were chosen, based on the specifications for the assay of the active ingredient in medicines that is usually set at 90%–110%.

From the results of the retrospective analysis, 2 anti-epileptic drugs were selected for the assessment of commutability; carbamazepine with the lowest C_{pk} value and valproic acid with the highest C_{pk} value (Table 1). These components were chosen to see whether there is any difference in commutability results for robust (valproic acid) versus less robust (carbamazepine) processes.

The second step in the approach was to evaluate the commutability of different matrices used for the preparation of PTM. Results from candidate PTM matrices were compared with patient material results to determine the (non) commutability of the PTM matrices (see "data analysis").

Candidate Matrices

The matrices tested in this commutability study are human and bovine sera. Bovine serum was chosen because of costs associated with human serum. Because of the preference to distribute PTM samples by mail, the consequences of lyophilization of samples containing bovine serum were also studied.

Gamma irradiated newborn calf serum was purchased from Invitrogen (Paisley, Scotland, United Kingdom, www.invitrogen.com). This material was frozen at −20°C until use. The bottles were slowly brought to room temperature before sample preparation.

Human serum was collected during routine analysis in a hepatitis screening program for healthy adults in the first-line treatment in 1 laboratory. The sera were collected within 1 month before the study sample preparation and stored in laboratory tubes at −20°C. After slowly defrosting the samples, the sera of approximately 70 healthy persons were pooled. The serum-pool was tested negative for HIV-1/2 antibody, hepatitis B surface antigen, hepatitis C virus antibody, and the presence of carbamazepine and valproic acid. A search for other possible drug substance contaminants, tested on a high-performance liquid chromatography (HPLC)–diode array detection toxicological screening system (I-Tox; Agilent Technologies, Amstelveen, the Netherlands, www.home.agilent.com) with the ability to identify at least 1000 relevant drugs compounds, also yielded negative results.

Candidate Matrices Sample Preparation

PTM samples were prepared by adding a volumetric quantity of a stock solution of carbamazepine and valproic acid to the matrices. Both human and bovine sera were spiked with

TABLE 1. C_{pk} Values of All Anti-Epileptic Drugs in a 10-Year Period

Component	C_{pk}
Carbamazepine	0.63
10,11-epoxide-carbamazepine	0.64
Ethosuximide	0.90
Phenobarbital	1.00
Phenytoin	0.89
Lamotrigine	0.98
Monohydroxycarbazepine	1.09
Valproic acid	1.35

volumetric amounts of carbamazepine and valproic acid stock solutions creating low, medium, and high concentrations of both components (Table 2). The bovine matrix was divided in aliquots of 2 mL. Half of the bovine serum batch was lyophilized according to a process, which is regularly used in the preparation of Dutch drug PTM. Before shipment, the freeze dried samples were stored in the refrigerator, the liquid bovine serum samples were frozen at −20°C. The human serum was dispensed in laboratory vials in volumes of 250 μL for immunoassay methods and 2 mL for HPLC-methods and gas chromatography (GC)-methods. The vials were stored at −20°C until dispatch.

Patient Material Preparation

Carbamazepine and valproic acid patient serum samples, with concentrations spanning the clinically relevant concentration range leftover from routine clinical analysis, were collected during 3 months before the study. Hemolytic and icteric samples were discarded. Samples were stored at −20°C and slowly defrosted at room temperature before pool preparation.

For both carbamazepine and valproic acid, 5 sera with concentrations evenly distributed along the clinically relevant concentration range were created by pooling these leftover serum samples. The concentrations were 3.1, 6.3, 8.9, 10.7, and 12.4 mg/L for carbamazepine and 22.9, 51.1, 70.8, 102.5, and 126.6 mg/L for valproic acid (all laboratory means of the results reported by participants). The pooled sera were tested negative for HIV-1/2 antibody, hepatitis B surface antigen, and hepatitis C virus antibody. The pooled sera were divided into aliquots of 3 mL in laboratory vials and stored at −20°C until dispatch.

The influence of 3 freeze–thaw cycles on patient samples was investigated and had no effect on the measured values of the studied drugs (data not shown).

Analysis

All Dutch EQAS participants were asked about their method and reagent specifications for the analysis of carbamazepine and valproic acid and their willingness to perform analyses for this study. A selection of laboratories was made to obtain the greatest diversity in the types of analytical methods and reagent kits (in case of an immunoassay) so that the candidate matrices for PTM were analyzed with all methods used in the analysis of the components in the EQAS for anti-epileptic drugs. Participating laboratories received written instructions about dispatch, storage conditions, preparation of the lyophilized samples, and minimum/maximum time before analysis. Both patient material and PTM were dispatched on dry ice and delivered within 24 hours to the participating laboratories. Laboratories were asked to test the samples preferably on the day of receipt, but at least within 3 days. In case the samples were not analyzed the same day, participants were instructed to store the samples at −20°C. All laboratories were asked to analyze every sample in duplicate in a single run to improve statistical power and the possibility of detecting a matrix effect.

TABLE 2. Carbamazepine and Valproic Acid Concentrations in PTM

	Human Serum (Liquid), mg/L	Bovine Serum (Liquid), mg/L	Bovine Serum (Lyophilized), mg/L
Carbamazepine			
Low	3.0	3.2	3.1
Medium	7.0	8.0	7.6
High	12.8	12.9	12.3
Valproic acid			
Low	51.6	54.9	52.2
Medium	76.6	86.7	82.5
High	101.0	109.6	104.2

Data Analysis

Data analysis was performed according to the CLSI guidelines EP14-A2 and EP30-A.[4,12] Results from patient material reported by each laboratory were plotted against results from patient material of each of the other laboratories ($X_{lab\ 1}$ versus $Y_{lab\ 2}$, $X_{lab\ 1}$ versus $Y_{lab\ 3}$, $X_{lab\ 1}$ versus $Y_{lab\ 4}$, $X_{lab\ 2}$ versus $Y_{lab\ 3}$ etc). In EP14-A2, linear regression is used that supposes the absence of variance in the comparative method plotted on the *x* axis. In this study for all laboratory couples, the Passing and Bablok regression analysis[13,14] was performed according to EP30-A because of the absence of an error-free method. In addition, EP14-A2 uses the 95% prediction interval to test individual results. However, a large residual variance would lead to a disputable conclusion of commutability, and on the other hand, a very small variance could lead to a disputable conclusion of noncommutability. Therefore in this study, the average within-laboratory SD (SD_{wl}) as calculated from EQAS results over a period of 3 years was used as a fixed and robust preset criterion.

To compare the results obtained from candidate matrices to the patient material results, the orthogonal residuals between all $X_{lab\ A}$, $Y_{lab\ B}$ coordinates for candidate materials and the Passing and Bablok regression line for patient material results were calculated for all laboratory couples. For comparison, each orthogonal residual is divided by the SD_{wl} for each concentration, resulting in a relative residual. The commutability decision limit was chosen at 3 SD_{wl} rather than 2 SD_{wl} in the CLSI protocol because of the more strict requirements as defined above.

RESULTS

Fifteen laboratories participated in this commutability study. Carbamazepine was analyzed with 9 immunoassays and 4 HPLC-methods. One laboratory was not able to report results for carbamazepine because of failure of the assay, and 1 laboratory did not perform the carbamazepine analysis. Valproic acid was analyzed by 12 immunoassays, 2 GC-method, and 1 HPLC-method. All laboratories followed the instructions regarding the preferred time of analysis or storage if applicable.

Carbamazepine

With 13 laboratories performing the carbamazepine analysis in this study, a total of 78 laboratory couples could

be formed. The results were inspected for outliers. One of the laboratories reported an outlying result for the lowest carbamazepine concentration in patient material, therefore this result was discarded. Two other laboratories did not report a result for the 2 highest concentrations in the patient sera, therefore the corresponding relative residuals in the candidate matrices were not evaluated because of the lack of data of the patient regression line in these parts of the concentration range. One laboratory reported an outlying result for the medium concentration in lyophilized bovine serum, this result was also discarded.

Calculated relative residuals of the candidate materials to the regression line of the patient materials are depicted in Figure 1A. For human serum, 1 and 4 couples produced results outside the 3 SD_{wl} cutoff for low and medium concentrations, respectively. None of the relative residuals for the highest concentration in human serum exceeded this commutability decision limit. The analysis of carbamazepine in liquid bovine serum produced 5, 19, and 12 relative residuals outside the cutoff range for low, medium, and high concentrations in liquid bovine serum, respectively and 1, 13, and 12 relative residuals outside the cutoff range for low, medium, and high concentrations in lyophilized bovine serum, respectively.

The relative residuals outside the cutoff range in human serum were produced by the combination of FPIA and EMIT (n = 1) methods for the lowest concentration and CEDIA/PETINIA (n = 1), EMIT/PETINIA (n = 1), and HPLC/PETINIA (n = 2) for the medium concentration.

When a stricter commutability decision limit of 2 SD_{wl} was chosen, the number of relative residuals for liquid human serum, liquid bovine serum, and lyophilized bovine serum is greater (Fig. 1 and Table 3); however, the relative residuals in human serum remain the lowest. All relative residuals for liquid human serum outside the 2 SD_{wl} decision limit were produced by combinations of methods containing at least 1 immunoassay.

Valproic Acid

For valproic acid, a total of 105 laboratory couples could be formed. All calculated relative residuals are depicted in Figure 1B. For human serum, 3 and 1 couples produced results outside the 3 SD_{wl} cutoff range for low and medium concentrations, respectively. For the highest concentration, none of relative residuals exceeded the cutoff range. The analysis of valproic acid in liquid bovine serum produced 18, 8, and 6 relative residuals outside the cutoff range for low, medium, and high concentrations in liquid bovine serum, respectively and 21, 16, and 18 relative residuals outside the cutoff range for low, medium, and high concentrations in lyophilized bovine serum, respectively.

The relative residuals outside the cutoff range in human serum were produced by the combination of GC/immunoturbidimetric assay (n = 2) and EMIT/immunoturbidimetric assay (n = 1) for the lowest concentration and HPLC/immunoturbidimetric assay (n = 1) for the medium concentration of valproic acid.

For valproic acid, the same result for the relative residuals is seen as for carbamazepine when a stricter commutability decision limit of 2 SD_{wl} was chosen. The number of relative residuals is greater, but the relative residuals in human serum remain the lowest. All relative residuals for liquid human serum outside the 2 SD_{wl} decision limit were produced by combinations of methods containing at least 1 immunoassay.

DISCUSSION

The method for assessment of commutability described in the CLSI EP14-A2 guideline is a time-consuming and expensive way for assessing commutability.[4] This study was designed comparable with the study presented by Baadenhuijsen et al,[15] but here an X-ling design was chosen in which the results from all laboratories are compared with every other laboratory because of the large amount of different methods available for the analysis of carbamazepine and valproic acid. With this design, the results produced by all methods can be compared with each other. Because of this design, there was a need for pooling of patient sera. This pooling has the advantage of diluting interfering substances, possibly present in an individual patient sample, to a sufficiently low level that has no influence on the analysis. However, the pooled serum will not be a representative for patient samples in which the interfering substance is present, but the risk of an individual patient sample that shows different behavior because of the interfering substance in any method will always exist, and these samples with interfering substances are uncommon in EQAS.

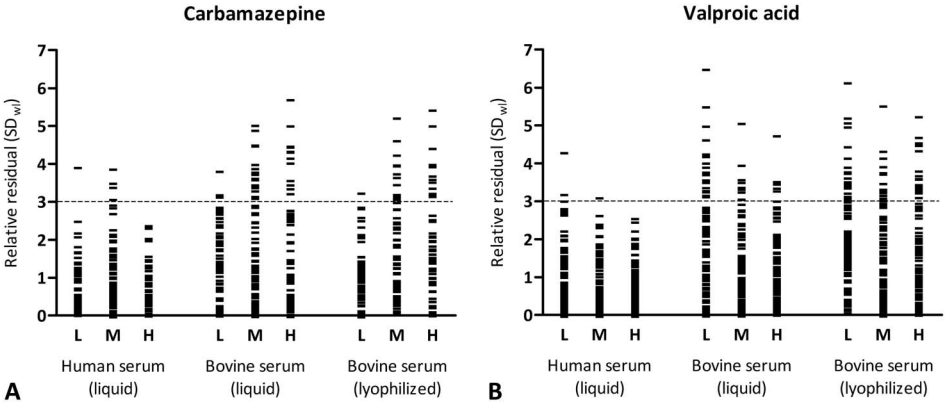

FIGURE 1. Relative residuals for carbamazepine (A) and valproic acid (B) for liquid human, liquid bovine, and lyophilized bovine sera spiked with low (L), medium (M), and high (H) concentrations of carbamazepine and valproic acid. Dashed line (——) at 3 SD_{wl} indicates the commutability decision limit.

TABLE 3. Number of Relative Residuals for Carbamazepine and Valproic Acid Violating Commutability Decision Limits at 2 SD_{wl} and 3 SD_{wl}, Respectively

	Human Serum (Liquid)		Bovine Serum (Liquid)		Bovine Serum (Lyophilized)	
	2 SD_{wl}	3 SD_{wl}	2 SD_{wl}	3 SD_{wl}	2 SD_{wl}	3 SD_{wl}
Carbamazepine						
Low	4	1	21	5	12	1
Medium	11	4	30	19	24	13
High	4	0	22	12	20	12
Valproic acid						
Low	13	3	42	18	44	21
Medium	9	1	24	8	32	16
High	6	0	22	6	34	18

The use of the standard error of regression ($S_{y\text{-}x}$), as described in the CLSI EP30-A guideline for calculation of the relative residuals resulted in unrealistic residuals above 20 $S_{y\text{-}x}$ because of a very small $S_{y\text{-}x}$. Therefore, the state-of-the-art within-laboratory SD (SD_{wl}) was used, the same as was done in the study by Baadenhuijsen et al.[15]

The hypothesis was that the results from the retrospective validation would give an indication about the commutability of the used material. A sample of carbamazepine in bovine serum would then be noncommutable because it appeared in the retrospective validation as a less robust process. Results for valproic acid, however, did show a more robust process, suggesting a sample of valproic acid in bovine serum would be commutable. The less robust process was considered the worst-case scenario and the robust process as the optimal scenario, assuming to reveal noncommutability and commutability, respectively. If this hypothesis would have been true, the results from the retrospective validation could have been a helpful tool for prioritizing the components for commutability studies, but no difference can be seen between the results for carbamazepine and valproic acid. For both components, the numbers of relative residuals for both liquid and lyophilized bovine sera are higher than the number of relative residuals in human serum and a preference for this type of serum can be seen, although not all calculated relative residuals in human serum are below the commutability decision limit of 3 SD_{wl}. The same conclusion can be drawn when a commutability decision limit of 2 SD_{wl} would have been chosen. All relative residuals for human serum that exceeded these limits were produced by method combinations that consist of at least 1 immunoassay–based method. For carbamazepine, there is literature that describes the deviation of results for carbamazepine in patient material analyzed with immunoassays compared with HPLC and/or liquid chromatography mass spectrometry.[16–20] These deviations are attributed to the existence of cross-reactivity of the immunoassays for carbamazepine-10,11-epoxide, the pharmacologically active and structurally similar metabolite of carbamazepine. For valproic acid, no articles regarding cross-reactivity were found. Giving the structural formula of valproic acid and the absence of metabolites, no cross-reactivity will be expected.

One of the shortcomings of this study is the absence of carbamazepine-10,11-epoxide in the candidate materials, although the carbamazepine metabolite is present in the patient material. The relative residuals that exceed the commutability decision limit could be the result of this dissimilarity. A new commutability study for the analysis of carbamazepine should indicate whether the current preference for human serum remains when the metabolite is added to the candidate materials. The expectation is that the number of relative residuals for both human and bovine sera might decrease, because the candidate materials will be more like the patient sera. The preference for human serum would probably hold, because the only difference between the currently used candidate materials is the origin of the matrix. Thus, it can be concluded that a matrix effect is probably present in the bovine serum samples.

Because of the preference to send PTM by mail, a lyophilized PTM in bovine serum was included in this study, but unfortunately no lyophilized sample with human serum was included. Although the results between liquid and lyophilized bovine sera do not differ that much, no conclusions can be drawn regarding the commutability of lyophilized human serum for the analysis of carbamazepine and valproic acid. A future commutability study should provide more clarity.

CONCLUSIONS

This first commutability study in the field of therapeutic drug monitoring shows a preference for the use of human serum for PTM for the analysis of carbamazepine and valproic acid. For human serum, not all methods produce results within the limits of commutability, but this could be a result of unsound analytical methods used, as is also seen in other external quality surveys.[21]

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