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Analytical performance of eight enzymatic assays for ethanol in serum evaluated by data from the Belgian external quality assessment scheme

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Abstract

Objectives: Fast and reliable ethanol assays analysis are used in a clinical context for patients suspected of ethanol intoxication. Mostly, automated systems using an enzymatic reaction based on ethanol dehydrogenase are used. The manuscript focusses on the evaluation of the performance of these assays.

Methods: Data included 30 serum samples used in the Belgian EQA scheme from 2019 to 2021 and concentrations ranged from 0.13 to 3.70 g/L. A regression line between target concentrations and reported values was calculated to evaluate outliers, bias, variability and measurement uncertainty.

Results: A total of 1,611 results were taken into account. Bias was the highest for Alinity c over the whole concentration range and the lowest for Vitros for low concentrations and Cobas 8000 using the c702 module for high concentrations. The Architect and Cobas c501/c502 systems showed the lowest variability over the whole concentration range. Highest variability was observed for Cobas 8000 using the 702 module, Thermo Scientific and Alinity c. Cobas 8000 using the c702 module showed the highest measurement uncertainty for lower concentrations. For higher concentrations, Alinity c, Thermo Scientific and Vitros were the methods with the highest measurement uncertainty.

Conclusions: The bias of the enzymatic techniques is nearly negligible for all methods except Alinity c. Variability differs strongly between measurement procedures. This study shows that the Alinity c has a worse measurement uncertainty than other systems for concentrations above 0.5 g/L. Overall, we found the differences in measurement uncertainty to be mainly influenced by the differences in variability.

Keywords: ethanol; external quality assessment; method evaluation.

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Introduction

Ethanol in blood is a frequently requested test in forensic and clinical laboratories, in particular in connection with emergency testing with, for example in Belgium, more than 100,000 tests performed every year. In emergency testing facilities, fast and reliable methods of ethanol analysis are needed whenever a patient with impaired/altered consciousness is admitted with a suspicion of ethanol involvement, because it is important to distinguish between gross intoxication or e.g. head trauma or other traumas, or both. Moreover, consumption of ethanol needs to be quickly distinguished from intoxication with more dangerous substances, like methanol or ethylene glycol, more particularly in case of increased osmolar gap. In addition, ethanol determination is also important in the context of enforcing drinking and driving legislation. A variety of methods is applied for assaying ethanol in blood samples, in a clinical setting mostly automated systems using an enzymatic reaction based on ethanol dehydrogenase and in forensic cases most often gas chromatography [1, 2]. Enzymatic methods are preferred in a clinical

setting because they give a fast result, which is essential in an emergency care situation. It should be noted however that the osmolar gap and clinical background should be taken into account for staying alert for intoxications with other alcohols, which can only be confirmed by non-enzymatic methods, like chromatographic methods [3]. Even in case of a non-ethanol ethanol intoxication, ethanol assays play a role for following up the ethanol concentration, which is in that case the most commonly used treatment.

This manuscript focusses on the evaluation of the performance of the enzymatic assays in the Belgian medical laboratories and more generally in the Western world. Assays of various manufacturers are available and they are all based on the oxidation of ethanol to acetaldehyde, catalyzed by ethanol dehydrogenase (ADH), and resulting in the production of the reduced form of nicotinamide-adenine-dinucleotide (NADH) [2, 4]. NADH is measured spectrophotometrically by all methods used in Belgium except for Vitros, that uses reflectometry.

The performance evaluation was realized using results from the Belgian external quality assessment (EQA) scheme for ethanol in blood testing. Information on analyzers and reagents was collected, allowing to identify measurement procedures up to a detailed level. Methods to derive measurement uncertainty from EQA data have been described before [5].

Materials and methods

Data included commutable samples used in one EQA round from 2019 and two from 2020, and samples with unknown commutability from four rounds in 2021. In each round in 2019 and 2020, six samples were sent to the laboratories. During the four rounds in 2021, three samples were sent to the laboratories. Commutable serum samples were taken from healthy volunteers. Samples with unknown commutability status used in 2021 were collected as frozen plasma bags from the Belgian blood bank. Samples were converted to serum and spiked with predefined ethanol concentrations ranging from 0.13 to 3.70 g/L, followed by preparing 2 mL aliquots in glass containers, which were hermetically sealed and frozen at −18 °C. The day of sending, samples were thawed at room temperature and distributed to the laboratories by overnight mail at ambient temperature. Samples were tested for homogeneity [6] and the stability of ethanol in the samples was assessed by evaluating the change of mean reported value in function of day of analysis.

Laboratories were requested to keep the samples refrigerated before analysis and to report the concentrations of ethanol within 2 weeks to the EQA organizer. A total of 18 commutable samples were sent to the participants in three distinct EQA rounds in 2019 and 2020. Since several laboratories reported a maximum limit of quantification of 3 g/L and start diluting from that concentration on, only the 16 commutable samples having a concentration below 3 g/L were included in the study. Only laboratories that used the same measurement procedure for the three EQA rounds were taken into account. In 2021, 12 samples were sent, of which 10 were taken into account because they had a concentration below 3 g/L. The analysis of the 2021 data was based on the same group of laboratories that was taken into account for the 2019–2020 data.

A total of 25 laboratories analyzed the samples with gas chromatographic methods. The median of the 25 results obtained with chromatographic methods was taken as the target concentration. For the first round of 2020, two participants had not responded and hence only 23 values were taken into account.

Data from commutable samples and samples with unknown commutability status were analyzed separately.

In order to identify laboratories with gross variability or bias, outliers were identified on an individual laboratory bias: for each individual laboratory, a regression line between target concentrations and reported values was calculated and outliers against the regression model were discarded for further analysis [7].

After excluding outliers, data were grouped and analyzed for each method separately. Measurement uncertainty was assessed via a top-down approach using a linear mixed effects analysis based on a regression model between the reported value and the target concentration. The factors sample and laboratory were modelled as two crossed random effects and differences in variability for the different target concentrations were taken into account by weighting the observations inversely proportionally to the observed variability for each sample [8]. Subsequently, the fixed effects coefficients of the linear mixed model were used to represent the bias between each measurement procedure and the target concentrations by the following equation:

$$B_c = a + (b - 1) \times c \tag{1}$$

with B_c the concentration-dependent bias, a the intercept, b the slope, as obtained by the linear mixed model, and c the target concentration of each sample.

The variance-covariance matrix of the linear mixed effects model was used to calculate the variability for each sample, expressed as a standard deviation. A nonlinear regression analysis was applied to estimate the coefficients of the characteristic function that describes the relation between the target concentration and the variability observed for each sample by the following equation [9]:

$$S_c = \sqrt{d + e \times c^2} \tag{2}$$

with S_c the concentration-dependent variability of each sample, d and e respectively the concentration-independent and the concentration-dependent parameter of the characteristic function, and c the target concentration of each sample.

The relation between the target concentration and the bias on the one hand, expressed by Eq. (1) and the relation between target concentration and the variability on the other hand, expressed by Eq. (2), were combined to obtain a measure of measurement uncertainty [5]:

$$MU_c = \sqrt{B_c^2 + S_c^2} \tag{3}$$

with MU_c the concentration-dependent measurement uncertainty, B_c the concentration-dependent bias and S_c the concentration-dependent estimation of variability. Plugging in Eq.(1) and Eq. (2) gives:

$$MU(c) = \sqrt{(a + (b - 1).c)^2 + d + e \times c^2} \tag{4}$$

where c is the concentration of interest, a and b are the fixed effects coefficients of the linear mixed model, and d and e are the coefficients

of the characteristic function. Standard errors on *MU(c)* were calculated by taking the square root from the sum of the squared standard errors bias and variability. Expanded uncertainties were obtained by multiplying *MU(c)* by a coverage factor of 2.

Calculations were performed for methods used by at least six laboratories in the 2019–2020 period and at least four laboratories in 2021. Bias and measurement uncertainty were only considered for the commutable samples and hence, are only available for the 2019–2020 period.

In order to draw a link between the observed method performance and the performance declared by the manufacturers, inserts from kits that are currently in use were obtained from the participating laboratories and the measuring range and intermediate imprecision were listed. For reasons of comparability, the intermediate imprecision was considered at a concentration of 0.5 and 1.5 g/L. Intermediate imprecisions not available for these concentrations were interpolated or extrapolated from imprecisions of nearby concentrations. Extra- and interpolations were realized using variances after which a square root was taken from the extra- or interpolated result. The R code performing these analyses can be found in the on line supplement.

At last, vigilance warnings for each of the assays were requested to the national authorities responsible for IVD vigilance.

Results

A total of 1,611 results were initially taken into account, of which 51 (3.2%) were identified as outlier and not included in the subsequent analysis.

Table 1 lists the measurement procedures and their manufacturer, the number of laboratories included in the study, the regression coefficients that reflect bias and the coefficients of the characteristic function for every method group that describe variability. Table 2 shows the functions of the characteristic function for each measurement procedure for the non-commutable samples.

The number of laboratories per measurement procedure ranges from five for Thermo Scientific to 44 for Cobas 6000 with the c501 module from Roche.

Figure 1 shows the relation between the target concentration and the mean value as calculated from the regression coefficients in Table 1. At the point of the highest investigated concentration of 2.41 g/L, the bias ranges

Table 2: Overview of measurement procedures, number of laboratories and coefficients that describe the relation between target value and variability (d, e) for the samples with unknown commutability. The meaning of the coefficients can be derived from from Eq. (4). Values shown are parameter estimates, their standard errors are between parentheses.

Measurement procedure	Number of laboratories	d	e
Alinity c	8	8e-04(0.0014)	0.0014(7e-04)
Architect c 8000	4	9e-04(0.0014)	6e-04(6e-04)
Cobas 6000 (c 501)	28	7e-04(3e-04)	7e-04(1e-04)
Cobas 8000 (c 502)	14	1e-04(4e-04)	0.0013(3e-04)
Cobas 8000 (c702)	10	4e-04(2e-04)	4e-04(1e-04)
Thermo Scientific	4	0.0013(0.0017)	0.001(8e-04)

Table 1: Overview of measurement procedures, number of laboratories and coefficients that describe the relation between bias (Intercept (a) and slope (b)) on the one hand and variability (d, e) on the other hand for the commutable samples.

Measurement procedure	Number of laboratories	Intercept (a)	Slope (b)	d	e	Standard error on measurement uncertainty
Alinity c (Abbott)	7	0.0138 (0.0066)	1.035 (0.0116)	7e-04 (4e-04)	0.0035 (6e-04)	0.0134
Architect c 16000 (Abbott)	7	0.0102 (0.0033)	1.001 (0.005)	2e-04 (1e-04)	6e-04 (1e-04)	0.0060
Architect c 8000 (Abbott)	6	0.0153 (0.0044)	0.9988 (0.0066)	4e-04 (2e-04)	8e-04 (2e-04)	0.0080
Cobas 6000 (c 501) (Roche)	44	0.015 (0.0017)	0.9914 (0.0025)	1e-04 (1e-04)	7e-04 (1e-04)	0.0030
Cobas 8000 (c 502) (Roche)	11	0.0119 (0.0043)	1.0004 (0.0043)	5e-04 (1e-04)	5e-04 (1e-04)	0.0061
Cobas 8000 (c702) (Roche)	11	0.0385 (0.0094)	0.9837 (0.0053)	7e-04 (2e-04)	0.001 (2e-04)	0.0108
Thermo Scientific (Thermo Scientific)	5	0.0067 (0.0051)	1.0074 (0.0104)	9e-04 (8e-04)	0.0036 (0.001)	0.0117
Vitros 5600 (OCD)	7	0.005 (0.0026)	0.9939 (0.0061)	0 (1e-04)	0.001 9(2e-04)	0.0066

The meaning of the coefficients can be derived from Eq. (4). Values shown are parameter estimates, their standard errors are between parentheses.

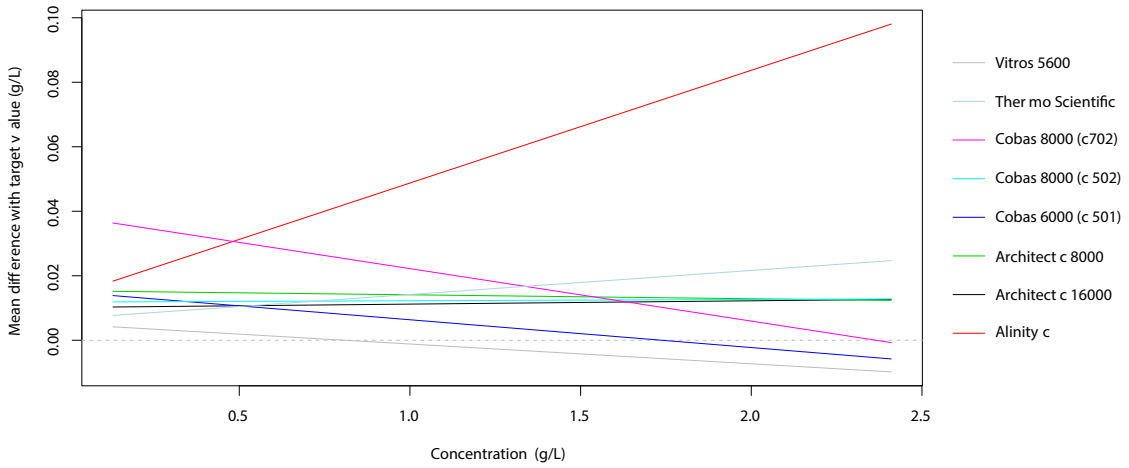

Figure 1: Differences between regression lines between target concentration and reported values for the different measurement procedures and 45°-line for the commutable samples.

from –0.00073 g/L for Cobas 8000 with the c702 module from Roche to 0.098 g/L for Alinity c from Abbott. At the lowest investigated concentration of 0.13 g/L, the bias ranges from 0.004 g/L for Vitros 5600 from Ortho-Clinical Diagnostics (OCD) to 0.018 g/L for Alinity c.

The largest slope, which reflects an increase in bias with increasing concentration, was recorded for Alinity c. Of note, all enzymatic methods have a small positive bias for the low concentrations, while three methods have a small negative bias for the higher concentrations: Cobas 6000 with the c501 module and Cobas 8000 with the c702 module, both from Roche, and Vitros 5600. All negative biases remained, in absolute value, smaller than 0.0098 g/L at a concentration of 2.41 g/L.

Figure 2 shows the relation between the variability and the target concentration, as calculated from the characteristic function coefficients *d* and *e*, collected for the commutable samples and displayed in Table 1. The Architect c8000 and c16000 from Abbott and Cobas 6000 and 8000 using the c501/c502 module show the lowest variability over the whole concentration range. There is no single method that exhibits the largest variability over the entire concentration range. For low concentrations (<0.4 g/L), Cobas 8000 with the c702 module shows the highest variability. For higher concentrations, Thermo Scientific and Alinity c show the highest variability. The Vitros 5600 system shows the lowest variability for concentrations up to 0.4 g/L, but this increases steadily, to result, in comparison with other methods, in an intermediate variability for the highest concentrations.

Figure 3 shows the same relation for data from 2021, collected for the samples with unknown commutability and for which the details are shown in Table 2. Three methods have an overall low relative variability: Cobas 8000 (c702), Cobas 6000 (c501) and Architect c8000.

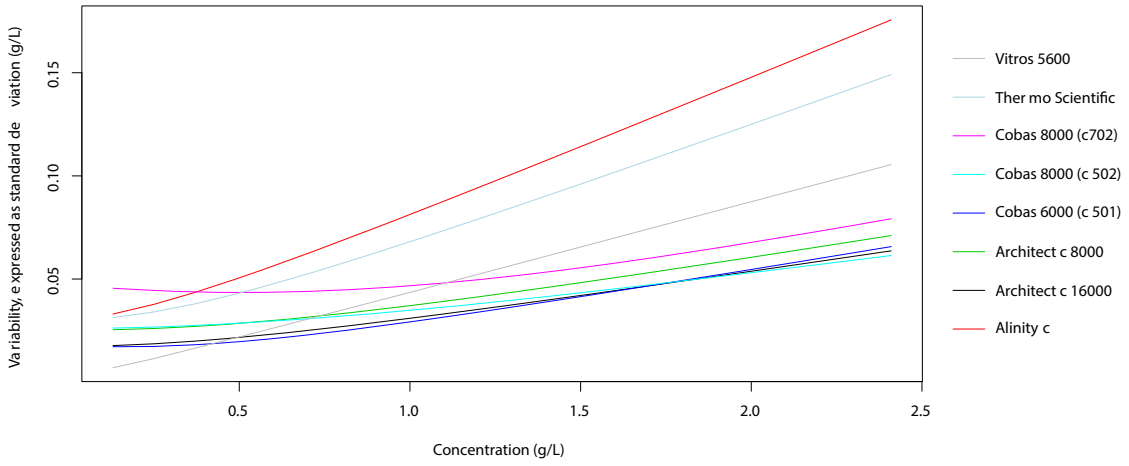

Figure 2: Variability of different measurement procedures for the commutable samples.

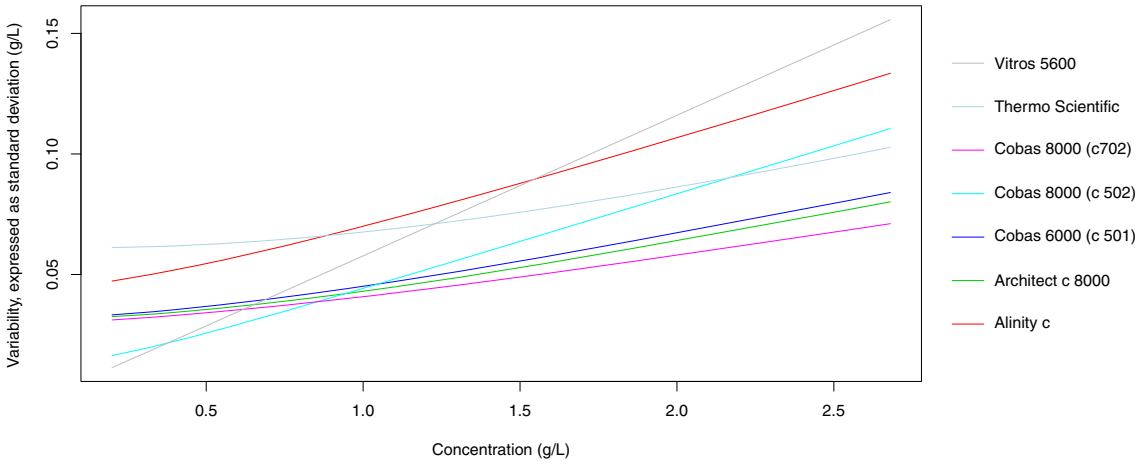

Figure 3: Variability of different measurement procedures for the samples with unknown commutability status.

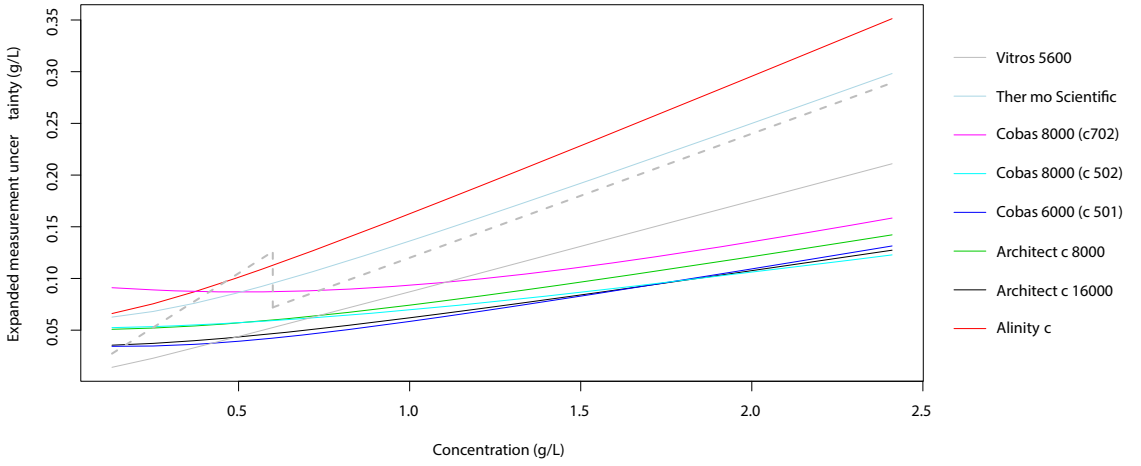

Figure 4: Expanded measurement uncertainty of the different measurement procedures. Dashed line is the 12% maximum deviation line as suggested by Rilibäk.

The variability of the Vitros 5600 ranges from the lowest variability for the lowest concentrations to the highest variability for the highest concentrations. Alinity c, Thermo Scientific and Cobas 8000 (c502) show an intermediate variability, with the Cobas 8000 (c502) having a relative low variability for concentrations below 1 g/L.

Figure 4 shows the measurement uncertainty as calculated via Eq. (4) for the commutable samples. For concentrations below 0.4 g/L, the Cobas 8000 (c702 module) shows the highest measurement uncertainty, exceeding the 21% for concentrations below 0.6 g/L suggested by Rilibäk [10]. For higher concentrations, i.e. 1 g/L and higher, on the contrary, this method is among the methods with the lowest measurement uncertainty. For these concentrations, Alinity c, Thermo Scientific and Vitros 5600 are the methods with the highest measurement uncertainty exceeding the 12% Rilibäk limits for concentrations above 0.6 g/L [10]. Their expanded measurement uncertainty exceeds the 12% limit suggested by Rilibäk over the whole concentration range.

The measurement performance of the different systems, as declared by the manufacturers, is shown in Table 3. The narrowest measuring range is reported for Vitros 5600 (0.1–3 g/L), while Alinity has the widest reported measuring range (0.025–6 g/L). Interpolated intermediate imprecision at 0.5 g/L ranges from 0.00793 for Architect c16000 + c8000 systems to 0.045 g/L for Thermo Scientific. The same order is kept for intermediate imprecision at 1.5 g/L, ranging from 0.0272 g/L for Architect systems to 0.0636 g/L for the c501 module on Cobas 6000 and 8000.

Data from Alinity c, Thermo Scientific and Vitros 5600 are obtained by interpolation, data from Roche systems by extrapolation.

Table 3: Measuring range and intermediate imprecision as declared by the manufacturers on the reagent inserts.

	Measuring range, g/L	Intermediate imprecision (target: imprecision), g/L	Interpolated intermediate imprecision at 0.5 g/L, g/L	Interpolated intermediate imprecision at 1.5 g/L, g/L
Alinity c	0.025–6	0.5: 0.0111 2.837: 0.0527	0.0111	0.0355
Architect c16000 + c8000	0.1–6	0.519: 0.0087 1: 0.02 2.929: 0.0668	0.00793	0.0272
Cobas 6000 (c501), Cobas 8000 (c501)	0.101–4.98	0.6: 0.03 0.8: 0.04	0.0235	0.0636
Cobas 8000 (c702)	0.1–4.98	1.24: 0.03 3.15: 0.04	0.0251	0.0315
Thermo Scientific (mixed)	0.1–6	0.5: 0.045 2.5: 0.067	0.045	0.0571
Vitros 5600	0.1–3.0	0.88: 0.019 1.98: 0.058	–	0.0453

Intermediate precision at 0.5 g/L for Vitros 5600 could not be calculated.

When the observed inter-laboratory variability based on EQA results is compared with the declared intermediate imprecision by the manufacturers, the relatively high intermediate imprecision that is declared by Thermo Scientific correlates with the relatively high inter-laboratory variability observed in EQA results. On the other hand, while the Alinity c system has one of the lowest declared intermediate imprecision values for both 0.5 and 1.5 g/L, it has the highest observed inter-laboratory variability observed in EQA results. The Architect systems both have a relatively low declared intermediate imprecision and a low observed inter-laboratory variability over the investigated measuring range.

No vigilance warnings have been issued for the investigated systems during or after the investigation period.

Discussion

EQA has already been reported to be capable of serving as an objective means to describe the measurement uncertainty associated with application of a certain method [5]. The approach that was applied in this study was to evaluate bias and variability in relation with concentration, and to combine both as a single measure of uncertainty.

The bias of the different enzymatic techniques with respect to the target concentration, obtained by chromatography, is nearly negligible for all methods except one. The Alinity c system has a bias of up to 0.12 g/L for concentrations of 3 g/L. In contrast, other Abbott systems are among the systems with the lowest bias.

Variability differs strongly between measurement procedures. Certainly for concentrations above 1 g/L, three methods have a variability that is clearly higher than the other ones: Alinity c, Vitros 5600 and Thermo Scientific. The variability of the Alinity c contrasts with that of the other Abbott systems, which exhibit amongst the lowest variabilities recorded, both for the declared intermediate imprecision as the observed inter-laboratory variability.

This study is the first to describe the performance of the Alinity c system for ethanol using data from an external quality assessment round. In contrast to previous findings [11, 12], this study shows that the Alinity c system from Abbott performs worse than other enzymatic measurement systems for concentrations higher than 0.5 g/L. Recent data for the samples with unknown commutability shows that the variability of the Alinity c is decreasing slightly with respect to Vitros 5600 and Thermo Scientific. A possible explanation may be found in the fact that laboratories using the Alinity c started calibrating more often than required by the manufacturer.

The expanded measurement uncertainty is, just as for the Thermo Fisher system, higher than the 12% maximum deviation from Rilibäk over the whole concentration range.

Overall, we found the differences in measurement uncertainty to be mainly influenced by the differences in variability, with the difference in performance of the different measurement systems paralleling the differences in variability.

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