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Total Errors of TSH results by fluorescent immunoassay technique from dried blood spots in a newborn screening program for congenital hypothyroidism - variations in time

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Abstract

***Objective**:the aim of this study was to evaluate the imprecision of the method used to determine TSH (Thyroid Stimulating Hormone) levels from dried blood spots in a newborn screening program and how the value of the coefficient of variation influences the total error of the method. **Methods**:A short-term evaluation of imprecision was compared with the imprecision of the method assessed over five years. The coefficient of variation from the best quality control period and the worst quality control period were used. For Bias assessment mean results from the external quality program were used. Total Error was calculated with Bias and Coefficient of Variation values. A freely available software was used for standard deviation and coefficient of variation profiling. **Results**:The values of the coefficient of variation for the short-term were lower than values obtained in worst quality control period but higher than in the best quality control period. Total error was higher than the accepted value for low-level control in the worst quality control period. Images obtained with the software showed that for high-level control coefficient of variation is concentration-dependent but this finding is not similar for low-level control. **Conclusions**:Total Error of the Method may be subject to change in time. Initial evaluation of imprecision must be performed on a short term analysis but a continuous evaluation should be performed as the performance of a method may change in time. The evaluation should be performed on clinically significant levels for each parameter.*

***Keywords:** Six Sigma, TSH, Imprecision, dried blood spot (DBS)*

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Introduction

The performance of laboratory tests may be evaluated using models based on the concept of Total Allowable Error (TEa). Irrespectively of the model of TEa used (analytical or clinical model) by the laboratory to evaluate the performance of a certain method, a first step is to evaluate the imprecision of the method. For the TE model based on analytical criteria, the formula (1):

$$TE\% = Bias\% + 1.65 \times CV\%$$

is applied, where CV% (coefficient of variation) is obtained from internal statistical quality con-

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trol (SQC) data and Bias% value from an external quality assessment (EQA) program.

It is recommended that imprecision studies be performed for an initial evaluation by performing at least 5 determinations/day for five days, not necessarily consecutive days. These results can be used to verify the manufacturer's precision claims. For this initial evaluation, at least 25 values must be obtained for each level that is clinically significant (2). Standard Deviation (SD) of a method can vary in the long term even by 30% compared with the initial evaluations, if 100 values are used. In a study performed by Sadler concerning the imprecision of Thyroid-stimulating hormone (TSH) measurement, the performance of the method varied in time and staff changes were one of the factors that influenced the performance of the method (3). Our study aimed to evaluate the imprecision of the method used for TSH determination in a newborn screening program for congenital hypothyroidism and how the value of the CV% used influenced the value of TEa. TSH was measured with a fluorescent enzyme immunoassay (FEIA) technique from dried blood spots (DBS) and a cut-off value of 10 mUI/L was used.

Material and methods

To evaluate the imprecision of the FEIA method for TSH, two models were applied: one that follows Clinical Laboratory Standard Institute (CLSI) EP15 A3 User verification of Precision and Estimation of Bias (2) recommendations and one that uses the results of routine SQC.

Model 1: following CLSI EP 15 A3 recommendations, two levels of control samples were tested 5 times/run for five days. The two control samples used in this experiment were internal QC samples included in the TSH kit (Oy LabDiagnostics, Finland).

Model 2: All SQC results from January 2014 to December 2018 were included: 314 values (each lot from at least 10 to 39 values) for each control level. For each lot, a Levey-Jennings chart was created using Excel spreadsheets. SD, mean and CV% were computed for each control lot and level using formulas from Excel. During the five year evaluation, some staff changes occured: from 2014 to 2015, staff member A performed all the samples; from 2016 to 2017, staff members A, B and C performed the tests; and from 2017 to 2018, staff member D performed all the tests.

For Bias% estimation, values from the EQA program from January 2019 were used. Five samples were assayed five times each and a mean value was obtained. Mean values for our laboratory and mean values for all methods reported were used to compute the Bias% value using the formula available at www.westgard.com. All participants in EQA schemes used the same sampling method (DBS) and results were assessed by the provider, by own group, and by all methods.

TE% was calculated using the formula stated above and a comparison was performed between the short-term estimated TE (5 days) and the long-term estimated TE (for the best/worst QC periods). Using a variance software program (VFP) freely available online at http://www.aacb.asn.au/resources/useful-tools/variance-function-program-v14 (5), imprecision profiling was evaluated. All SD and mean values from the internal QC data were tabulated in the software. Graph curves for low-level and high-level QC were obtained.

Results

Model 1:

After performing the precision study according to CLSI EP15 A3 guidlines, using an internal control with two different levels, values for mean, SD and CV% were calculated. The results are presented in table I.

Table I. Calculated TSH mean values, SD and CV% for Model 1 (CLSI EP 15-A3 guidelines).

Sample	Mean mUI/L blood	SD mUI/L blood	CV%
QC level 1	7.44	0.80	10.71
QC level 2	23.41	3.32	14.20

Model 2:

The evolution of CV% in all lots of control material is shown in figure 1

Based on the CV% values for the two models and the Bias% values as calculated from samples with known values, the values of TE% were determined and presented in table II. After using the VFP software, the following images were obtained: figure 2.

Discussions

The CV% obtained after performing the experiment according to Model 1 was higher than the value stated by the manufacturer in the precision claims. The CV% value calculated for a mean concentration of 8.4 mUI/L was 10.71%, higher than 6.5% which is the value stated by the manufacturer. Also, the CV% value of 14.20%, corresponding to a concentration of 43.9 mUI/L,

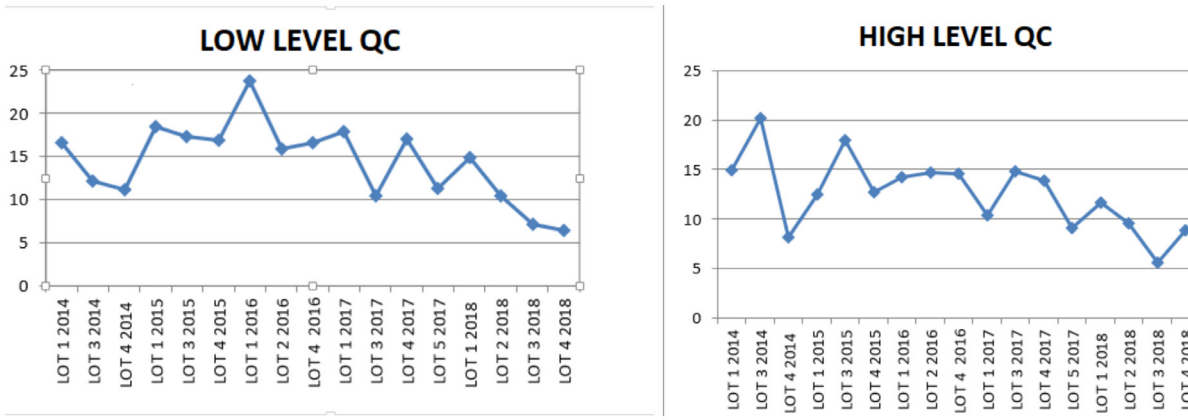

Fig. 1. The evolution of CV% across the evaluated 5-year period (left side: low-level; right side: high-level). The value of CV% for each lot of control material is represented as a unique dot. X-axis: reagent lot; and Y-axis: corresponding CV% value.

Table II. TE% value obtained using different values for CV%. Accepted values for each column are values stated in Desirable Variations tables from EFLM database, available at https://biologicalvariation.eu/.

	CV%			BIAS%			TE%		
	Low Level	High Level	Accepted value	Low Level	High Level	Accepted value	Low Level	High Level	Accepted value
5 days	10.71	14.20	9.7	5.00	10.00	6.9	13.87	21.70	22.80
Best QC	6.45	3.14	9.7	0.45	0.45	6.9	10.32	4.62	22.80
Worst QC	20.17	5.60	9.7	11.81	11.81	6.9	24.59	16.64	22.80

Note: First line of table show results of CV% after model 1 was used and TE% value obtained. With these data TE% obtained value was bellow TEa% value. Line 2 shows results obtained in Best QC period: CV%, Bias% and TE% had lower value than the accepted value. Line 3 shows results for Worst QC period when both CV% and TE% values for Low Level control were higher than the accepted values.

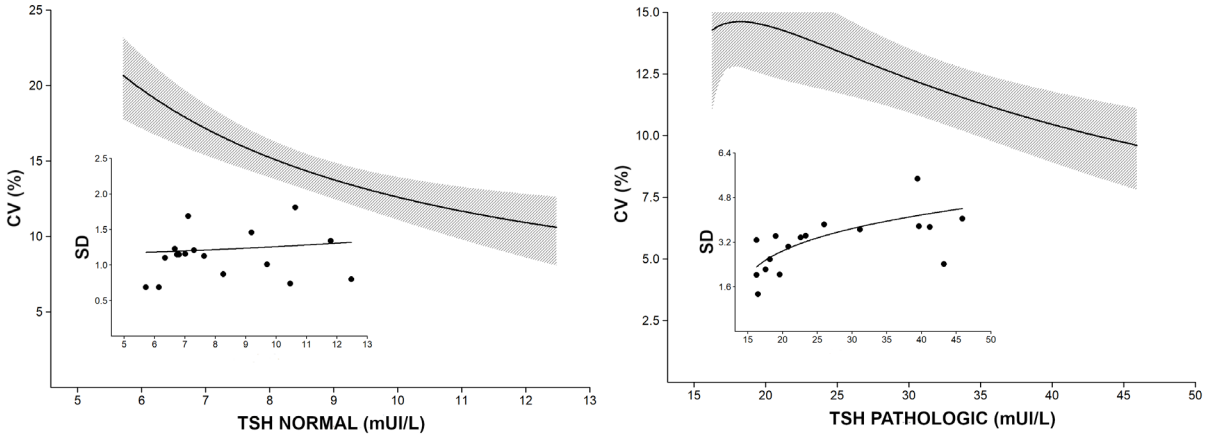

Fig. 2. Imprecision profiling as rendered by the VFP software. Y-axis: CV% value; X-axis: mean value of each control lot (mUI/L blood). The curve represents the evolution of CV% and the lined contour represents the limits of CV%. In the cassette of each figure the relationship between SD and mean value is represented. Each dot represents the SD value of each lot that was used.

was higher than 8.8% which is the value stated by the manufacturer for high-level concentrations.These values were obtained by three staff members using three different reagent lots. The CV% values obtained after performing the same experiment with three patient samples were also above the values stated in the manufacturer's precision claims. The CV% results for patients' samples had higher value than the accepted value for imprecision of 9.7% from Desirable Biological Variation (4). These results showed that the fluorescent method for TSH does not always meet the same performance characteristics as serum TSH methods.

The results obtained for CV% by using the data from the 5 years of SQC show that the test performance changed over time. The lowest values for CV% were obtained for lots 3/2018 and 4/2018. For lot 3/2018, the CV% value was below the value of 6% stated by the manufacturer in the TSH insert. Of the 5-year interval that was evaluated, certain periods showed a CV% value even higher than the value of TEa of 22.6%. These changes may be partially explained by staff changes: a higher CV% was obtained in the period in which lot 1/2016 was used and three different staff members (out of whom two less experienced) performed the tests while a lower CV% was obtained when lot 3/2018 was used and only one experienced staff member performed the tests. Calibration effect was evaluated in a different study (data not published yet) and the obtained value (8.6 %) was lower than the intraindividual biological variation of 9.6%.

In a one-year study performed by Karmisholt et al. on samples collected from patients with overt hypothyroidism, a CV of 6.9% was reported for serum TSH (6). Rawlins and Roberts evaluated six third-generation TSH assays and the values reported for CV% were between 2.8% and 6.4%. In all evaluated assays, values were below the accepted value of 9.6%. All methods evaluated in this study used plasma or serum for TSH measurement and chemiluminescent (CLIA) or electrochemiluminescent (ECLIA) immunoassays (7).

In a study concerning performances of four analytical platforms that use CLIA or ECLIA technology in an external quality assessment program over five years, only 50% of the evaluated

laboratories met the desired quality performances. In all cases, CV% values were below the desired value of 9.6% (8).
Another study published by Usha et al., in which Six Sigma performance of TSH tests were evaluated, a CV% value of 9.4% was obtained for a CLIA measurement (9).
In a study regarding the imprecision stability of TSH methods according to the CLSI guidelines, Sadler showed that results do not reflect the long term method performance, nor calibration effect or lot-to-lot variability being evaluated (3).
In our study, the CV% obtained in some periods was as high as double the CV% value obtained with model 1 for low-level control while in other periods the CV% value was half of the value obtained with the same model. As for high-level control, the CV% obtained in the model 1 experiment showed lower variation, the obtained values were closer to the ones obtained during the five-year analysis.
In two out of the five QC samples that were evaluated, the calculated Bias% was higher than 6.9% which is the accepted value. One of these samples had values that are close to 10 mUI/L which is the cut-off value used by our laboratory.
The relationship between SD value and mean value has different representations for low- and high-level values: for low-level values, the relationship between the two is described by an almost straight line while SD value is not concentration-dependent. For high-level control, the relationship is best described by a curve and SD value is concentration-dependent.

Conclusions

CV% and SD are important tools used to evaluate the performance of a method and have an important contribution in quality assessment of laboratory methods. Our study on the imprecision of a FEIA method for the measurement of TSH from DBS, showed that the manufacturer's precision claims are rarely met, irrespectively of the model used to verify the precision claims. Additionally, values for the CV% are above the accepted values from Desired Values for Biological Variation. However, these values refer to serum TSH measurements while we performed the tests on whole blood. Therefore, different CV% values may be acceptable. Quality assessment should be a continuous and dynamic process in clinical laboratories and TE% of the method should be re-evaluated after the initial imprecision study.

Abbreviations

TEa - Total allowable error
CV% - Coefficient of Variation
SQC - Statistical Quality Control
FEIA - flourescent immunoassay
DBS - dried blood spots
CLSI - Clinical Laboratory Standard Institute
SD - Standard Deviation
TE - Total Error
CLIA - chemiluminescent immunoassay
ECLIA - electrochemiluminescent immunoassay
EFLM - European Federation of Clinical Chemistry and Laboratory Medicine
TSH - Thyroid Stimulating Hormone

Authors' contribution

ORO: Conceptualization; Methodology; Investigation; Formal Analysis; Writing (original draft).
MZ: Data curation; Formal Analysis.
MD: Supervision; Writing (review and editing).

Conflict of interest

The authors declare no conflicts of interest.

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