ORIGINAL ARTICLE

Estimation of uncertainty measurement — A prerequisite of ISO1589 accreditation for clinical laboratories

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Abstract

Objective: To estimate relative expanded uncertainty measurement of routine clinical chemistry analytes for international organisation for standardisation 15189 accreditation.

Methods: This cross-sectional study was conducted at Dow International Medical College, Karachi, from September 2013 to May 2014. During the process of international organisation for standardisation 15189 accreditation, measurement uncertainty was estimated for 13 clinical chemistry analytes using top-down approach. Relative combined uncertainty of each analyte was calculated by combining uncertainties of imprecision, bias and calibrators. Results of estimated imprecision, bias and expanded uncertainties were observed for allowable imprecision, bias and total analytical error for the respective analyte.

Results: Uncertainties of imprecision were found within acceptable limits for all analytes except total protein (2.4% vs. 1.3%). Uncertainties of bias of all analytes were found within allowable limits. Relative expended uncertainties of all analytes were found acceptable except total protein (4.7%vs 3.63%).

Conclusion: The approach used to estimate the measurement uncertainty may be found simple and feasible by clinical laboratories interested in getting the relevant accreditation.

Keywords: Uncertainty, Clinical chemistry, Quality control, Accreditation. (JPMA 67: 701; 2017)

Introduction

Laboratory-generated results are critical for diagnosis, treatment and prognosis of disease. Quality performance of clinical laboratory has been an issue of primary interest for clinicians and laboratory management as well. An extensive evaluation and surveillance of clinical laboratory performance is a part of laboratory accreditation.[1] For laboratories generating quantitative results, uncertainty measurement of analytes is mandatory for international organisation for standardisation (ISO) 15189 accreditation.[2] Measurement uncertainty (MU) is defined in ISO Guide to Expression of Uncertainty in Measurement (GUM) as a parameter associated with the result of a measurement, which characterises the dispersion of the values that could reasonably be attributed to the measurand.[3] Uncertainty describes the dispersion from the reference value and provides information about quality and effectiveness of results. Different sources can contribute to the dispersion around the reference value; including matrix effect, reagent and calibrator concentrations and environmental conditions during test analysis. The fundamental principles for estimating MU are described in the international vocabulary of metrology (VIM).[4] To estimate the MU, "bottom up" and "top down" approaches are used in reference calibration and medical laboratories respectively.[5] The top down methodology for MU is suitable for medical laboratories where the main focus of interest is to identify the dispersion from the reference value.[6] The smaller the dispersion the greater the reliability of result is considered. Imprecision and bias are the major contributors of MU for quantitative assays. An easy approach to calculate the imprecision and bias is to use internal quality control (QC) and proficiency testing for inter-laboratory QC data, respectively.[7] Besides imprecision and bias, possible potential sources of uncertainty are observed, including different concentrations of calibrators and reagent in different lots, multiple technologists' involvement and environmental conditions such as humidity and temperature fluctuations. These factors could also be anticipated with QC data over a period of time.[8]

There is no study carried out in Pakistan to elaborate how to calculate the MU of laboratory analytes by using simple laboratory data. Hence, the current study was planned to estimate the MU of routine clinical chemistry analytes in our laboratory for accreditation.

Material and Methods

This cross-sectional study was carried out at the Chemical Pathology section of the Department of Pathology, Dow International Medical College, Karachi, from September 2013 to May 2014.

During the process of laboratory accreditation with ISO

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15189, MU was estimated for selected analytes proposed for accreditation. A top down approach was used to estimate the MU. Internal and external quality control data and vendor-provided calibrator uncertainty were used to estimate relative combined and expanded uncertainties. Relative expanded uncertainty was taken as final MU to evaluate the reliability of the reported result. The study was approved by the institutional ethical research review board.

MU was estimated for 13 routine clinical chemistry analytes, including serum cholesterol, triglyceride, high-density lipoprotein (HDL) cholesterol, creatinine, total protein, albumin, total bilirubin, direct bilirubin, magnesium, iron, uric acid, alanine transaminase (ALT) and aspartate transaminase (AST). All analytes were analysed on Architect c8000 (Abbott Laboratories, IL, USA)), by using calibrators and reagents of same manufacturer (Table-1).

Table-1: Specification of analytes considered for uncertainty estimation.

Analyte	Measurand	Normal Reference value/interval (Adult)	Measurement unit	Methodology	Interfering Substances
Cholesterol	Concentration of cholesterol in the serum	<200	mg/dl	Enzymatic	Bilirubin Hemoglobin Ascorbate Lipids
Triglyceride	Concentration of triglyceride in the serum	<150	mg/dl	Glycerol Phosphoxidase	Bilirubin Hemoglobin Ascorbate
HDL-Cholesterol	Concentration of HDL cholesterol in the serum	40-60	mg/dl	Accelerator Selective Detergent	Bilirubin Hemoglobin Ascorbate
Creatinine	Concentration of Creatinine in the serum	M*: 0.7-1.2 F**:0.5-0.9	mg/dl	Kinetic Alkaline Picrate	Bilirubin Hemoglobin Lipds Ascorbate Glucose Protein
Total Protein	Concentration of total protein in the serum	6.4-8.3	g/dl	Biuret	Bilirubin Hemoglobin Triglyceride
Albumin	Concentration of Albumin in the serum	3.5-5.2	g/dl	Bromocresol Green	Bilirubin Hemoglobin Lipids
Total Bilirubin	Concentration of total Bilirubin in the serum	<1.2	mg/dl	Diazonium salt	Hemoglobin Lipids
Direct Bilirubin	Concentration of direct Bilirubin in the serum	0.0-0.30	mg/dl	Diazo reaction	Hemoglobin Lipids Triglycerides
Magnesium	Concentration of Magnesium in the serum	1.6-2.6	mg/dl	Enzymatic	Bilirubin Hemoglobin Glucose
Iron	Concentration of Iron in the serum	M*: 50-160 F**: 40-160	µg/dl	Ferene	Bilirubin Hemoglobin Triglyceride
Uric Acid	Concentration of uric acid in the serum	M*:3.5-7.2 F**: 2.6-6.0	mg/dl	Uricase	Bilirubin Hemoglobin Ascorbate Lipids
ALT***	Activity of ALT by measuring NADH	M*:<45 F**:<34	U/l	NADH(without P-5′-P)	Bilirubin Hemoglobin Lipids
AST****	Activity of AST by measuring NADH	M*:<35 F**:<31	U/l	NADH(without P-5′-P)	Bilirubin Hemoglobin Lipids

A statistical approach was used to estimate the relative combined uncertainty (U_C) by combining relative uncertainties of imprecision (U_{imp}), bias (U_b) and calibrators (U_{ref}). To calculate the values all equations were adopted from published guidelines of quality management programme of laboratory services of Ontario Medical Association.[9] U_{imp} and U_b were calculated from internal and external QC data respectively, over the period of six months (Table-2). According to laboratory internal QC programme, 2 levels of control material (Randox Laboratories, United Kingdom) are singly analysed in each run of total three per day. Therefore 540 values of each level of control were available to calculate the imprecision from intra-laboratory QC data for each analyte. Similarly, from inter-laboratory QC data total 12 values were available for each analyte from 12 samples over a period of six months, as laboratory is participating in external QC programme (Randox international quality assessment scheme, Randox Laboratories, United Kingdom), fortnightly. Information about uref was obtained from calibration certificates provided by manufacturer.

Uimp was calculated with the following equation:

$$U_{imp} = (CV_1^2 + CV_2^2/2)^{0.5} \quad \text{equation 1}$$

Where, CV_1 and CV_2 are the co efficient of variations of normal and pathological control materials respectively.

U_b was calculated by using following equation:

$$U_b = \text{Mean } (U_m / P_g) \quad \text{equation 2}$$

Where, U_m and P_g are lab and peer group results of samples received from external QC programme. Mean of total 12 samples results were taken for each analyte.

U_{ref} was calculated with the mean of uncertainties of calibrators, provided by manufacturer.

Relative Combined uncertainty (U_c) was calculated as following:

$$U_c = (U_{imp}^2 + U_b^2 + U_{ref}^2)^{0.5} \quad \text{equation 3}$$

A coverage factor (*k*) of 2 was used to calculate relative expanded uncertainty (*U*), to achieve 95% confidence level, as following:

$$U = U_C K \quad \text{equation 4}$$

Table-2: Data of calibrators' uncertainties, internal and external quality controls to calculate relative uncertainties of analytes.

Analytes	Units	Calibrator		Internal Quality Control		External Quality Control	
		Value	Respective uncertainty	Mean Value	CV%	Reference Value*	*Ub*
Cholesterol	mg/dl	98	1.31	190	2.50**	211.06	0.1
		384	5.12	275	3.20***		
Triglyceride	mg/dl	93	2.08	87	3.60**	171.00	1.3
		470	10.50	259	3.90***		
HDL	mg/dl	60	0.53	59	4.90**	75.10	0.5
				103	5.00***		
Creatinine	mg/dl	0.85	0.02	1.50	2.00**	2.89	0.3
		5.25	0.14	4.30	2.50***		
Total Protein	g/dl	3.95	0.05	6.00	2.40**	5.43	0.1
		7	0.09	4.70	2.60***		
Albumin	g/dl	1.75	0.02	4.00	1.50**	3.58	0.3
		5.2	0.07	2.90	2.00***		
Total Bilirubin	mg/dl	1.40	0.03	1.70	4.40**	3.20	0.5
		17	0.38	4.80	6.70***		
Direct Bilirubin	mg/dl	0.80	0.01	1.40	8.40**	1.40	0.9
		8.20	0.18	1.90	7.20***		
Magnesium	mg/dl	0.85	0.01	2.20	2.00**	3.00	0.6
		3.40	0.06	4.30	2.20***		
Iron	ug/dl	100	2.00	108	5.10**	137.70	0.3
				212	5.70***		
Uric Acid	mg/dl	4.05	0.11	5.90	4.00**	7.50	0.2
		9	0.24	9.80	5.20***		
ALT	U/l	50	3.00	32	6.40**	76.23	0.4
				123	4.10***		
AST	U/l	70	6.00	32	4.00**	86.90	0.1
				121	4.00***		

*Mean value from peer group data. **At normal level. ***At pathological evel.

A range was defined for U of each analyte . Goals of acceptable uncertainties were set by taking the desirable imprecision, bias and total analytical error values from Westgard biological variation database specifications for U_{imp}, U_b and *U* respectively.[10]

Results

Uncertainties of imprecision were found within acceptable limits for all analytes except total protein (2.4% vs. 1.3%) (Table-3). Uncertainties of bias of all analytes were found within allowable limits. Relative expended uncertainties of all analytes were found acceptable except total protein (4.7%vs 3.63%) (Table-4).

On applying Westgard rules on the internal quality control data of protein all runs were acceptable at both control levels. However, opportunities of random errors (measurement errors that in replicate measurements vary in unpredictable manner) were closely observed and an increased frequency of QC measurement was adopted for protein assay for further analysis.

Discussion

To demonstrate the laboratory proficiency and performance, accreditation plays an important role. Generating effective and accurate result is the reflection of laboratory performance.[5] MU is an important tool to

Table-3: Relative (%) uncertainties of bias (U_b), imprecision (U_{imp}) and calibrators (U_{ref}), combined uncertainty (U_c), expanded uncertainty (U) and range applied for expanded uncertainty (U) of analytes.

Analytes	U_b	U_{imp}	U_{ref}	U_c	U	Range of U(units)
Cholesterol	0.1	2.8	3.20	4.2	8.4	98 - 384 (mg/dl)
Triglyceride	1.3	3.7	6.20	7.4	14.8	93 - 470 (mg/dl)
HDL	0.5	4.9	0.50	4.9	9.8	-
Creatinine	0.3	2.2	0.08	2.2	4.4	0.85 - 5.25 (mg/dl)
Total Protein	0.1	2.4	0.07	2.3	4.7	3.95 - 7.0 (g/l)
Albumin	0.3	1.7	0.04	1.7	3.4	1.75 - 5.2 (g/l)
Total Bilirubin	0.5	5.6	0.20	5.5	11.0	1.4 -17 (mg/dl)
Direct Bilirubin	0.9	7.8	0.09	7.8	15.6	0.8 - 8.2 (mg/dl)
Magnesium	0.6	2.0	0.03	2.0	4.0	0.85 - 3.4 (mg/dl)
Iron	0.3	5.4	2.00	5.7	11.4	-
Uric Acid	0.2	4.6	0.15	4.6	21.1	4.05 - 9.0 (mg/dl)
ALT	0.4	5.3	3.00	8.0	16.0	-
AST	0.1	4.0	6.00	7.2	14.4	-

HDL: High-density lipoprotein.
ALT: Alanine transaminase.
AST: Aspartate transaminase.

Table-4: Allowable imprecision, bias and total analytical errors of analytes.

Analyte	Desirable Goal Specifications from Westgard database		
	Allowable Imprecision (%)	Allowable Bias (%)	Allowable total analytical error (%)
Cholesterol	2.9	4.1	9.0
Triglyceride	9.9	9.5	25.9
HDL	3.6	5.6	11.6
Creatinine	2.9	3.9	8.8
Total Protein	1.3	1.3	3.6
Albumin	1.6	1.4	4.0
Total Bilirubin	10.9	8.9	26.9
Direct Bilirubin	18.4	14.2	44.5
Magnesium	1.8	1.8	4.8
Iron	13.3	8.8	30.7
Uric Acid	4.3	4.8	11.9
ALT	9.7	11.4	27.4
AST	6.1	6.5	16.6

HDL: High-density lipoprotein.
ALT: Alanine transaminase.
AST: Aspartate transaminase.

observe the variability of within laboratory results. MU provides quantitative estimate of the level of confidence about the laboratory-generated results and defines the accuracy of the test report. It also provides a measure of the expected variability in laboratory quantitative result when the same test is performed on more than one occasion.[11]

According to clinical laboratory standard improvement guideline (CLSI C51A), "top down" approach is suitable to measure uncertainty in medical laboratories.[2] We applied the same approach to estimate uncertainties of imprecision and bias with intra and inter-laboratory QC data respectively, by using the methodology adopted by Crawford et al.[9] U was derived with the QC data over a period of six months to include all possible sources that could contribute to uncertainty, including different lots of reagent and calibrators, multiple users, variation in environmental conditions (temperature and humidity) and effect of analyser preventive and corrective maintenance.

In a study by Linko et al, the combined uncertainties of serum calcium and glucose were calculated with the intra and inter-laboratory QC data. Their methodology was different from this study.[12] Disagreement of methodologies and diversities in uncertainty budgets have been observed in literature.[12-15] However, the general equation outlined in the GUM can be applied for various functional relations for the calculation of measurement uncertainty.[7,16,17]

This work accentuates that the laboratory QC performance can provide much of the information required for uncertainty measurement.[18] The current evaluation for 13 clinical chemistry analytes can easily be applied to other available clinical chemistry analytes. In this study the inherent analytical uncertainties of analytes were calculated with imprecision, bias and calibrators, but pre and post-analytical sources of uncertainties were not excluded. The pre and post-analytical sources of uncertainties could easily be controlled if all processes are carried out strictly according to standard operating procedures of international standards such as ISO.

Conclusion

The approach used to estimate the MU is simple and feasible by utilising the data available within laboratory.

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Conflict of Interest: None.

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References

1. Aoyagi T. ISO 15189 medical laboratory accreditation. Rinsho Byori. 2004; 52: 860-5.
2. Clinical and Laboratory Standards Institute. Expression of Measurement Uncertainty in Laboratory Medicine. Wayne, PA: CLSI, 2012.
3. Joint Committee for Guides in Metrology. Evaluation of measurement data-guide for the expression of uncertainty in measurement. Paris: JCGM. [Online] 2008 [Cited 2015 Dec 15]. Available from URL: http://www.bipm.org/en/committees/jc/jcgm.
4. Joint Committee for Guides in Metrology.International Vocabulary of Metrology-Basic and General Concepts and Associated Terms. Paris: JCGM. [Online] 2012 [Cited 2015 Dec 15]. Available from URL: http://www.bipm.org/en/publications/guides/vim.html.
5. Burtis CA, Edward R. Ashwood, David EB. Selection and analytical evaluation of Methods-With statistical Techniques. In: BurtisCA, Edward R. Ashwood, David EB, eds. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics. 5th edition. Philadelphia: Saunders, 2012; pp 38-42.
6. Carling R. A practical guide to ISO 15189 in laboratory medicine. Ann Clin Biochem. 2014; 51: 720.
7. White G, Farrance I. Uncertainty of measurement in quantitative medical testing. Clin Biochem Rev. 2004; 25: S1-S24.
8. Eurachem. Cooperation on International Traceability in Analytical Chemistry. Quantifying Uncertainty in Analytical Measurement. Third edition 2012. EURACHEM/CITACGuideCG4. [Online][cited2015Dec20]. Available from URL: http://www.citac.cc/QUAM2012_P1pdf.
9. Crawford L, Moses G. Traceability and uncertainty of measurement for medical laboratories-OLA's expectations. [Online] 2007 [Cited 2015 Dec 16]. Available from URL: http://www.qmpls.org/pub_resources/publications/qmpls_news/pdf/qmplsnews118.pdf.
10. Westgard J. Desirable Specifications for Total Error, Imprecision and Bias, Derived from Biologic Variation. [Online] [Cited 2015 Dec 16]. Available from URL: http://www.westgard.com/biodatabase1.htm.
11. Theodorsson E, Magnusson B, Leitol. Bias in clinical chemistry. Bioanalysis. 2014; 6: 2855-75.
12. Linko S, Ornemark U, Kessel R, Taylor PD. Evaluation of uncertainty of measurement in routine clinical chemistry-applications to determination of the substance concentration of calcium and glucose in serum. Clin Chem Lab Med. 2002; 40: 391-8.
13. Chen H, Zhang L,B iX, Deng X. Two evaluation budgets for the measurement uncertainty of glucose in clinical chemistry. Korean J Lab Med. 2011; 31: 167-71.
14. Paulson WD, Roberts WL, Lurie AA, Koch DD, Butch AW, Aguanno JJ. Wide variation in serum anion gap measurements by chemistry analyzers. Am J Clin Pathol.1998; 110 :735-42.
15. Stanczyk FZ, Cho MM, Endres DB, Morrison JL, Patel S, Paulson RJ. Limitations of direct estradiol and testosterone immunoassay kits. Steroids. 2003; 68: 1173-8.
16. Lee JH, Choi JH, Youn JS, Cha YJ, Song W, Park AJ. Comparison between bottom-up and top-down approaches in the estimation of measurement uncertainty. Clin Chem Lab Med. 2015; 53: 1025-32.
17. Farrancel, Frenkel R. Uncertainty of Measurement: A Review of the Rules for Calculating Uncertainty Components through Functional Relationships. Clin Biochem Rev. 2012; 33: 49-75.
18. Kristiansen J. Description of a generally applicable model for the evaluation of uncertainty of measurement in clinical chemistry. Clin Chem Lab Med. 2001; 39: 920-31.