Validation of some analytical methods in Department of Biochemistry - 108 Military Central Hospital

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Summary

Objective: Validation method and homogeneity assessment of 6 biochemistry tests. Which were measured with AU 5,800 with these tests were performed on 5 other biochemistry instruments and 2 immuno biochemistry tests were performed on two Cobas e601 that were analyzed at Department of Biochemistry, 108 Military Central Hospital. *Subject and method:* Evaluating within-run precision, day-to-day precision, accuracy, LOQ, linear range, uncertainty of the assigned value of 8 biochemistry tests in 8 bio-chemistry equipments. *Result:* 8 tests which have within-run precision, day-to-day precision, accuracy, liner range are absolute by the manufacturer's announcement, promulgate uncertainty and realistic LOQ of each bio-chemistry test, 6 devices ensure the similarity. *Conclusion:* 6 tests - glucose, cholesterol, triglyceride, AST, ALT, ure on 6 AU and 2 test - TSH, FT4 in 2 Cobas e601, which are currently analyzed at Department of Biochemistry in 108 Military Central Hospital, are accepted by within-run precision, day-to-day precision, accuracy, limit of determinative, linear range, and equipment similarities according to manufacturer's claims and 5.5.3 and 5.6.4 clauses of ISO standard 15189:2012.

Keywords: AU5800, Cobas e601, validation method, 108 Military Central Hospital.

1. Background

ISO 15189 is an international standard based on ISO/IEC 17025 and ISO 9001 that provides quality and capacity requirement for medical laboratories. Validation method, homogeneity assessment and uncertainty measurement are mandatory requirements for the laboratory system, especially for the bio-chemistry laboratories that can be accepted by ISO 15189 or other organizations which are CAP, JC, Nata and other obligations [1], [7]. Analytical methods need to be validated for the following reasons: Firstly, a result is affected by many factors such as: The object was changed into routine use, change of the laboratory location, implemented by different staffs, the change of laboratory environment, edit or update of the equipment. Therefore, the methods are applied in the real labor need to be validated to satisfy manufacturer's claims. Secondly, the validation method support to evaluate the method being used at different moments as a regular rule. Thirdly, before using medical equipment, we should demonstrate that the results manufacturer's announcement is reliable for the patients. In addition, validation method is an important task of

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each laboratory, is professional responsibility in practicing.

Similarity: Section 5.6.4. ISO 15189: 2012 requires that the laboratory should have a technique to compare the processes, equipments and methods and establish a comparability of the results for the specimen in the duration of the treatment. This technique can apply for similar or different processes, equipment, locations, or all of them. As a result, we need to evaluate the similarity of the test results in different equipments.

Uncertainty of the assigned value is a specialized parameter for the dispersion of an analytical substance. All information, which is provided from the uncertainty of the assigned value that is associated with within-subject biological variation, is useful in analyzing and interpreting of the test results [1], [7].

All the above reasons implement the management of quality that can be accepted by ISO 15189:2012 in the Department of Biochemistry - 108 Military Central Hospital and constantly improve the quality of testing, we conduct the topic: Validation of some Analytical Methods in Biochemistry Department - 108 Military Central Hospital with the following purposes:

Validating the analytical method of 6 tests (glucose, AST, ALT, cholesterol, triglyceride, urea), Which were measured with AU 5800 and 2 tests TSH, FT4 were performed in Cobas e601 in Department of Biochemistry - 108 Military Central Hospital.

Evaluating the similarity among 6 equipments (AU 5811, AU 5821, AU 2700, AU 640, AU 400-1, AU 400-2); and 2 equipments Cobas e601-1 and Cobas e601-2 being used in Department of Biochemistry - 108 Military Central Hospital.

2. Subject and method

2.1. Subject

6 biochemistry equipments: AU 5811, AU 5821, AU 2700, AU 640, AU 400-1, AU 400-2 and 2 immune biochemistry equipments: Cobas e601-1 and Cobas e601-2 being used in Department of Biochemistry - 108 Military Central Hospital.

8 tests: Glucose, AST, ALT, cholesterol, triglyceride, urea, TSH, FT_4.

2.2. Testing sample

Accuracy (Bias): Using QC sample (quantity control) with 2 different levels (normal and high) of Randox brand.

Within - run precision: Using 3 different levels of patient serum sample (low - middle - high). We collected 7 samples for each level.

Day-to-day precision: Same as within - run precision.

Linear range: Using QC sample/ real sample divide into 5 different concentrations that is diluted in 1, 2, 3, 4, 5 levels respectively. The diluent level should be noted for each test to make five dilute concentrations cover the analytical measurement range - the reference range and the linear range according to manufacturer's claims.

Quantitative limit (LOQ: Limit of quantitation): Use QC sample.

2.3. Time and place of study

The study was conducted at the Department of Biochemistry - 108 Military Central Hospital from November 2017 to February 2018.

2.4. Research Method

2.4.1. Trueness assessment

Measure 2 to 3 QC samples with different concentrations.

Experimental layout: Each QC sample is repeatly measured 10 times in a short period of time by a laboratory staffs in a device.

Calculation of some parameters: Mean, standard deviation (SD), dispersion (CV). Then calculate the bias-D% by the formula: D% = (Mean-X)/X*100.

Where: D% is the deviation, mean is the mean value when running on the equipment, X is the average value of the QC sample.

Bias compare with biological variation (westgard.com/biodatabase). If it is greater than the biological variation according to reagent's claims: Unacceptable results, have to run again or contact the manufacturer.

2.4.2. Repeatability evaluation

Select patient samples at 3 concentrations (low, medium and high) with 7 samples/concentration (scattered data within ≥ 20 days to ensure assessment of the influence of the factors on the test).

Experimental layout: Run each sample 5 times/day for 7 days. Each run is performed by a different employee. Each specimen was run at least 2 days apart and ran 7 samples within ≥ 20 days.

Calculate the parameters: Mean, SD, CV.

Compared with the manufacturer's published data:

If less than SD/CV value of the reagen's announcement, the method meets the requirements.

If larger than the manufacturer's SD/CV compared to 1/4 TEa (TEa: Total error acceptable, westgard.com).

2.4.3. Day-to-day precision assessment

Use the results of running the patterns in repeatability. Each concentration of ≥ 7 samples, repeat 3 - 5 times. Therefore, we will have 21 to 35 values.

Enter data into the calculations of repetition, repeatability (excel, BM.04 / QTQL.30) to calculate parameters: Mean, SD, CV.

Compared with the manufacturer's published data:

If less than the declared SD/CV value of the reagent's claims, the method meets the requirements.

If larger than SD/CV of the reagent's claims compare to 1/3 TEa - Permitted error (website: westgard.com).

2.4.4. Linear range

Divide the linear range into 4 intervals, taking the QC samples / real samples mixed into 5 different dilluted concentrations (1 : 1, 1 : 2, 1 : 3, 1 : 4, 1 : 5). Sample dilution concentrations should be noted for each test so that five concentrations of dilute concentrations cover the measurement range - the reference range and within the linear range of the machine.

Analysis based on R^2 (Correlation coefficient) calculated:

Linearity of good when $R^2 \geq 0.99$.

If you do not meet the need to do experiment again and can contact the manufacturer to be supported.

2.4.5. Define quantitative limits

LOQ is usually a multiple of LOD (detection limit): LOQ = 3x actual LOD. However, it is necessary to investigate the actual situation by: Preparing the diluted samples from low control to have low concentration around theoretical LOQ. Runs in parallel 20 times. At what concentrations found 19/20 results with good pinching, that is the actual LOD of the lab.

Compared with the manufacturer's claims:

If less than the LOQ value of the reagen's annocuncement, the method meets the requirement.

If it is greater than LOQ value of the reagent's declared: The result is not acceptable, must run again or contact the manufacturer.

For some test procedures with a linear principle, the LOQ is understood to be the bottom boundary of the linear range. However, the lab still has to survey its actual LOQ.

2.4.6. Measurement the uncertainty (U)

Estimated total uncertainty of test procedure:

$$U^2c = U^2_{LL} + U^2_{TL} + U^2_{Bias}$$

In which: $U_{LL} = SD_{LL}$, $U_{TL} = SD_{TL}$, $U_{Bias} = SD_{Bias}$ (SD_{LL}: Standard deviation of within-run precision, SD_{TL}: Standard deviation of day-to-day precision, SD_B: Standard deviation of accuracy).

Calculate the expanded uncertainty of the test procedure:

UMR = k × Uc (k: Coefficient, k = 2). Reliability: 95%. Uc: Uncertainty measure of synthesis. UMR: Expanded uncertainty of the assign value).

2.4.7. Equivalence assessment

Use internal quality control sample at levels of low, medium and high levels. Each level runs 10 times at the same time on all machine systems. Calculate the standard deviation of each machine.

In case of 2 machines (2 cobras e601): Use F-test.

The calculated value is calculated as: Ftt = $(SDmax / SDmin)^2$.

Where: Ftt is the calculated F value. SDmax is the larger SD value. SDmin is a smaller SD value.

Look up table F-test with significance levels of 5% and 1% (Appendix 1 and 2) with the number of runs minus 1 to obtain the F value of the table (Fb).

- If there are more than 2 machines (6 AUs): Use Cochran test.

The calculated value is calculated by the formula: Ctt = (SDmax / ΣSD) 2.

Where: SDmax is the largest SD value in the machine. Look at the Cochran's test table (Appendix 3) with the number of runs minus 1 to get the C value of the table (Cb).

If Ctt < Cb then the similarity of the machines is accepted [1], [6], [7].

3. Result

3.1. Result of within-run precision, day-to-day precision and accuracy

Table 1. Evaluation results of the with-run precision in AU5800 and Cobas e601

No	Test	SD_{max} of AU5800 compare to $SD_{manufacturer's\ claims}$ (SD_{max}: The daily maximum level of SD)		
		Low	Middle	High
1	Glucose	0.02 ≤ 0.02	0.03 ≤ 0.03	0.05 ≤ 0.08
2	AST	0.45 ≤ 0.53	0.55 ≤ 0.59	1.79 ≤ 2.42
3	ALT	0.45 ≤ 0.53	0.8 ≤ 0.8	1.92 ≤ 2.88
4	Choletesrol	0.03 ≤ 0.03	0.04 ≤ 0.04	0.08 ≤ 0.08
5	Triglycerid	0.01 ≤ 0.01	0.02 ≤ 0.03	0.04 ≤ 0.08
6	Ure	0.04 ≤ 0.05	0.04 ≤ 0.1	0.26 ≤ 0.34
No	Test	SD, CV_{max} of Cobas compare to CV, SD_{NSX}		
7	TSH	0.002 ≤ 0.002	1.89% ≤ 5%	0.064 ≤ 0.102
8	FT4	0.08 ≤ 0.08	0.147 ≤ 0.213	0.353 ≤ 0.381

Comment: SD analysis valuation of 6 tests (glucose, AST, ALT, cholesterol, triglyceride, urea) of AU 5800 and two tests (TSH, FT4) of the Cobas e601 are passed (they are smaller than or equal to SD announced by the manufacturer).

Table 2. Results of the day-to-day precision of the AU 5800 and Cobas e601

No	Test	SD of AU5800 compare to $SD_{manufacturer's\ claims}$		
		Low	Middle	High
1	Glucose	0.03 ≤ 0.04	0.05 ≤ 0.06	0.16 ≤ 0.18
2	AST	1.01 ≤ 1.16	0.770 ≤ 1.16	0.90 ≤ 0.95
3	ALT	0.54 ≤ 0.57	0.69 ≤ 1.0	1.0 ≤ 3.67
4	Cholesterol	0.03 ≤ 0.03	0.09 ≤ 0.09	0.11 ≤ 0.13
5	Triglycerid	0.01 ≤ 0.01	0.03 ≤ 0.04	0.12 ≤ 0.15
6	Ure	0.04 ≤ 0.06	0.06 ≤ 0.23	0.13 ≤ 0.91
No	Test	SD, CV of Cobas e 601 compare to CV, $SD_{manufacurer's\ claims}$		
7	TSH	0.005 ≤ 0.005	4.98% ≤ 7%	0.093 ≤ 0.12
8	FT4	0.1 ≤ 0.1	0.141 ≤ 0.217	0.475 ≤ 0.516

Comment: SD analysis valuation of 6 tests (glucose, AST, ALT, cholesterol, triglyceride, urea) of AU 5800 and two tests (TSH, FT4) of the Cobas e601 are passed (they are less than or equal to SD announced by the manufacturer).

Table 3. The accuracy testing result of AU5800 and Cobas e601

No	Test	Compare Bias of lab with Bio-Variation		
		QC level	Bias	Bio-variation
1	Glucose	Level 1	-1.38	2.34
		Level 2	0.22	2.34
2	AST	Level 1	-1.79	6.54
		Level 2	1.03	6.54
3	ALT	Level 1	0.56	11.48
		Level 2	-3.97	11.48
4	Cholesterol	Level 1	3.13	4.1
		Level 2	2.9	4.1
5	Triglyceride	Level 1	2.34	9.57
		Level 2	3.06	9.57
6	Ure	Level 1	2.74	5.57
		Level 2	1.32	5.57
7	TSH	Level 1	3.43	7.8
		Level 2	1.25	7.8
8	FT_4	Level 1	-3.5	3.3
		Level 2	-4.97	3.3

Comment: The absolute value of the trueness (Bias of lab) obtained when analyzing the test in AU 5800 and Cobas e601 are acceptable (≤ bio-variation value).

3.2. Results of LOQ evaluation, linear

Table 4. LOQ results, linear range of AU5800 and Cobas e601

No	Test	LOQ	Linear Range

		Mean	SD_{lab}	Requirement	R^2	Requirement
1	Glucose	0.6	0.02	≤ 0.02	0.999	≥ 0.995
2	AST	0.8	0.03	≤ 0.05	0.999	≥ 0.995
3	ALT	0.1	0.01	≤ 0.01	0.999	≥ 0.995
4	Choletesrol	0.5	0.01	≤ 0.03	0.999	≥ 0.995
5	Triglycerid	3	0.49	≤ 0.53	0.999	≥ 0.995
6	Ure	3	0.44	≤ 0.53	0.999	≥ 0.995
7	TSH	0.012	0.001	≤ 0.001	0.999	≥ 0.995
8	FT4	3.18	0.056	≤ 0.08	0.999	≥ 0.995

Comment: The SD_{lab} value of the test results (glucose, AST, ALT, cholesterol, triglyceride, urea) in the AU 5800 and two tests (TSH, FT_4) in Cobas e601 are passed (less than or equal to the SD published by the manufacturer). Correlation coefficient R2 when the linear range of the indicators are guaranteed.

3.3. Confirmation uncertainty results

Table 5. The results of the uncertainty of the assigned value in AU5800 and Cobas e601

Test	Uncertainty measurement of U has a coefficient of k = 2 for a realiable level of 95% ($\bar{X}$: Average value of the analysis)		
	Low	Middle	High
Glucose	$\bar{X}$ ± 0.41 (mmol/L)	$\bar{X}$ ± 0.43 (mmol/L)	$\bar{X}$ ± 0.86 (mmol/l)
Ure	$\bar{X}$ ± 0.59 (mmol/L)	$\bar{X}$ ± 0.6 (mmol/L)	$\bar{X}$ ± 1.78 (mmol/l)
Triglyceride	$\bar{X}$ ± 0.12 (mmol/L)	$\bar{X}$ ± 0.21 (mmol/L)	$\bar{X}$ ± 0.36 (mmol/l)
Cholesterol	$\bar{X}$ ± 0.37 (mmol/L)	$\bar{X}$ ± 0.56 (mmol/L)	$\bar{X}$ ± 0.64 (mmol/l)
AST	$\bar{X}$ ± 5.16 (U/L)	$\bar{X}$ ± 5.16 (U/L)	$\bar{X}$ ± 14.78 (U/L)
ALT	$\bar{X}$ ± 4.85 (U/L)	$\bar{X}$ ± 5.6 (U/L)	$\bar{X}$ ± 18.23 (U/L)
TSH	$\bar{X}$ ± 0.17 (µIU/mL)	$\bar{X}$ ± 0.24 (µIU/mL)	$\bar{X}$ ± 0.70 (µIU/mL)
FT_4	$\bar{X}$ ± 1.23 (pmol/L)	$\bar{X}$ ± 1.38 (pmol/L)	$\bar{X}$ ± 3.4 (pmol/L)

3.4. Result of similarity assessment

Table 6. The result of similarity measurement between six machines: AU400-1, AU 400-2, AU640, AU 2700, AU5811, AU 5821

Test	QC levels	T_{tn} of QC running in 6 AU machines	Tb with α = 0,05	Requirement
Glucose	Middle	0.27	0.33	Ttn ≤ Tb
	High	0.28	0.33	
AST	Middle	0.31	0.33	
	High	0.25	0.33	
ALT	Middle	0.31	0.33	
	High	0.33	0.33	
Cholesterol	Middle	0.23	0.33	

	High	0.25	0.33
Triglycerid	Middle	0.29	0.33
	High	0.29	0.33
Ure	Middle	0.31	0.33
	High	0.32	0.33

Table 7. The result of similarity measurement between Cobas e601_1 and Cobas e601_2

Test	QC levels	Ftn of QC running in 2 Cobas machines	Fc with a = 0,05	Requirement
TSH	Middle	1.93	4.03	Ftn ≤ Fc
	High	2.25	4.03	
FT_4	Middle	1.49	4.03	
	High	1.80	4.03	

4. Discussion

4.1. Result of within-run precision, day-to-day precision and accuracy

Precision is the approximation of the results which are independently conducted at the same sample and the same conditions. Precision is as to the distance between singular test results and mean values. The dispersion of the test results has an opposite trend with the precision. There are two types of precision that should be evaluated: Short-term precision or within-run precision or between-day precision, day-to-day precision, long-term precision or between-day precision, day-to-day precision. For the assessment of precision, it is possible to use CMR samples, QCs or patient's sample, with its own strengths and weaknesses, it is important to use the sample as substrate as possible. Precision is affected by random errors. Experimental evaluation of random error estimation results from different factors in the course of the method. This is considered as the first content in evaluating a new method. An unfocused method is often unreliable, so if the method does not pinch, then no further experiments are required or the causes of random errors need to be identified and removed in advance when testing is continued [1], [3], [4].

Acuracy is the approximation between the measurement result and the real value of the measurement. A test result is obtained when the result is approximately equal to its actual value. Actual value is an ideal concept that is difficult to implement in practice but only in real terms. One must repeat many times on the same pattern and the result must be repeated many times as real numbers. The purpose of the technical's acuracy is to detect and eliminate system errors that may occur during testing.

Calculating the bias of the test results using the method of validation with a reference method is also a way of assessing the accuracy. Compare the calculated deviation with the maximum deviation allowed for each standardized test proposed by the organization or association to assess the degree of accuracy. The deviation is less than the desired deviation, the test method looks as valid [1], [5].

4.2. Results of LOQ evaluation, linear

The limit of quantification (LOQ) is the lowest concentration that can be quantified by the survey method and result in consistent accuracy and precision [4], [5].

The linearity range of an analytical method is the concentration range where there is linear dependence between the measured quantity and

the analyte concentration, in simple words they are the smallest and the highest of test result may be reliable enough to report. The linear range is also known as the analytical measurement range (AMR), which is the measurement range of the device that can be used for reportable range, which does not require dilution or concentrated sample [2].

For the most quantitative methods, a linear range is required. A regression analysis can be used to assess whether the evaluation range is linear. Long or short linear ranges depend on many factors, the most important of which is the nature of the analytes and the analytical method used [6], [8].

4.3. Confirmation uncertainty results

The clinical value of uncertainty of the assigned value is important for the cumulative data and laboratory information systems allow comparison of new test results with previous test results. To assess whether or not the two test results of the same patient have significant differences, biologically speaking, to consider both the uncertainty of the measurement and the biological variability of the analyse. Two results of an analyte on the same patient were statistically and biologically different when they differed by at least 2.77 $\sqrt{U_C^2 + CV_{intra}^2}$ (95% confidence intervals) where U_c: Coordinated uncertainty, CV_{intra} within subject biological variation, the effects of combinations of uncertainty and biotransformation in an individual. The uncertainty not only provides information about the quality of the measurement, but also helps to compare the quality of laboratories that are recognized, but is also useful for analyzing and interpreting test results [9].

4.4. Result of similarity assessment

Section 5.6.4. comparison of laboratory results according to ISO 15189: 2012 required that the laboratory must build up a defined technique to compare all testing processes, equipments and methods and establish the comparability for all the results during appropriate treatment period. This technique can be applied for different processes and equipment, and different positions. As a result, we conduct to estimate the similarity among 6 AUs in 6 biochemical tests and 2 Cobas in 2 test TSH, FT4. The results of Table 7 show that the comparison between the comparative equipment is consistent result of the test [7].

5. Conclusion

6 indicators of glucose, cholesterol, triglyceride, AST, ALT, ure in 6 AU and 2 test TSH, FT4 in 2 e601 Cobas which are currently being analyzed at 108 Millitary Central Hospital are suitable. They have within-run precision, day-to-day precision, accuracy, quantitative limit, linear range and similarity among machines appropriate for the manufacturer's claims and in accordance with 5.5.3 and 5.6.4 of ISO 15189:2012.

References

1. Trần Thị Chi Mai (2018) *Thẩm định phương pháp trong phòng xét nghiệm y khoa*. Tạp chí y học Việt Nam 470, tr. 18-27.
2. Clinical Laboratory Standard Institute (2003) *Evaluation of the linearity of quantitative measurement procedures: A statistical approache, approved guideline.* 3rd edition. CLSI document EP06AE.
3. Clinical Laboratory Standard Institute (2012) *Evaluation of detection capability for clinical laboratory measurement procedures. Approved guideline*. 2nd edition. CLSI document EP17-A.
4. Clinical and Laboratory standards institute (2010) *Evalution of precision performance of quantitative measurement methods; Approved Guideline*. 2nd edition. CLSI document EP5-A2.
5. Clinical Laboratory Standard Institute (2010) *Measurement procedure comparison and bias estimation using patient samples. Approved guideline.* 3rd edition. CLSI document EP09.
6. Linnet K, Boyd JC (2006) *Selection and analytical evaluation of methods - with*

statistical techniques. In: Burtis CA, Ashwood ER, Bruns DE, editors. Tietz textbook of clinical chemistry. 4thed. Philadelphia: Sauders.

7. International Organization for Standardization (2012) *Medical laboratories particular requirements for quality and competence. ISO 15189.* International Organization for Standardization (ISO), Geneva.
8. Jeffrey S, Jhang et al (2004*) Evaluation of linearity in clinical laboratory.* Arch Pathol Lab Med 128: 44-48.
9. Westgard JO et al (2008) *Basic method validation*. 3nd edition. Madison: Westgard QC Inc.