Appendix 1
Clause 5.5: Examination processes

Performance characteristics in verification of examination procedures

No.	Discipline	Measurand*	Accuracy		Linearity	Biological reference intervals	Remarks
			Trueness	Precision			
Example	**Clinical Biochemistry**	Glucose in Serum by Glucose oxidase method (mg/dL)	√	√	√	√**	
Example	**Clinical Biochemistry**	Urea in Serum by Urease method (mg/dL)	√	√	√	√	
Example	**Hormone**	TSH in Serum by ECL method (mIU/L)	√	√	√	√	
Example	**Hematology**	Hemoglobin(Hb) in Whole Blood by Cell Counter … (g/dL)	√	√	√	√	
1							
2							
3							
4							
5							

* **The definition of the measurand according to the national standard of Iran- 4723**: The quantity intended for measurement.
Note: The information that the laboratory shall enter in the measurement column for the requested accreditation scope is the name of the analyte such as glucose, the type of sample such as serum, the principles of measurement such as the glucose oxidase method, unit of measurement such as mg/dL.

**There is no need to verify the reference range if the determination of the reference range or clinical decision-making values is considered from valid scientific references in the laboratory.

5.5.1 Selection, verification and validation of examination procedures

Method of execution: 5.5.1.2 Verification of examination procedures

Which method has been used to verify/estimate the precision of the examination method in the laboratory?

Verification of the precision claim of the examination method (kit) according to CLSI Document Ep15-A3 " User Verification of Precision and Estimation of Bias:"

- Conducting the examination with at least two or more patient samples or any other control sample similar to the human sample matrix or a sample compatible with the examination method that covers the entire concentration range claimed by the manufacturer in 5 working days each, 5 repetitions per day and statistical review for repeatability and Within Lab Precision comparison with the claimed values of the kit to verify the precision of the examination method.

Note 1: This method is simple and can be used for laboratories, and in the case of using Certified Reference Material (CRM) with a specific concentration, it is possible to simultaneously verify the precision and estimate bias.

Note 2: Repeatability is equivalent to within run precision and Within Lab Precision is equivalent to total precision including repeatability, between run precision (run to run precision) and in case of performing more than one run in a working day, in addition to they include between working days precision (day to day precision).

Estimating of the Within Lab Precision; including the repeatability and total precision of the examination method (kit) based on the third edition of Dr. Westgard's book; Basic Method Validation.

- Performing the examination at least 20 repetitions in 1 working day to evaluate repeatability (intra-run precision) and at least 20 repetitions in 20 working days on 20 aliquots of two or more patient samples or any other control sample similar to the human sample matrix or a sample compatible with the examination method that all cover the manufacturer's claimed concentration range and evaluate the within lab precision (total precision) and compare the coefficient of variation (CV%) with the laboratory criterion to estimate the precision of the examination method.

- Conducting the examination within 10 working days every day twice (10x2) on 10 aliquots from two or more patient's samples or any other control sample similar to the human sample matrix or a sample compatible with the examination method that covers the entire concentration range claimed by the manufacturer and comparison coefficient of variation (CV%) with the laboratory criterion to estimate the precision of the examination method.

- Conducting the examination within 5 working days every day 4 (5x4) on two or more patient's samples or any other control sample similar to the human sample matrix or a sample compatible with the examination method that covers the entire concentration range claimed by the manufacturer and comparison coefficient of variation (CV%) with the laboratory criterion to estimate the precision of the examination method.

Note 1: Laboratory criterion: To determine the qualitative characteristics of imprecision and bias, the laboratory can use the model of biological variation (BV) in which the contribution of the allowable imprecision and the allowable bias is well defined, or use the total allowable error (TEa) model in which the coefficient of variation (CV%) of the examination method under the condition of zero bias should be less than half of the total allowable error.

Note 2: According to standard 15189 in clause 5-5-1-2 and the text of the Health Reference Laboratory (Legal document in Iran) in the same clause, confirming the manufacturer's claim about functional characteristics of the examination method should be one of the goals of the laboratory.

Which method has been used to verify/estimate the trueness of the examination method in the laboratory?	○ **Using the method comparison test to launch a new method**: Method comparison test (regression method with analysis at decision-making levels) by examination of at least 20 patient samples in the reportable range of kit; so as to cover different points of the claimed measurement interval of the method, to compare the new method with a method that has the capability of metrological traceability.
	○ **Using paired t-test to launch a new method on the condition of having a narrow analytical interval** : **Keeping in mind that the use of the paired t-test is only applicable for tests that have a narrow analytical range, such as sodium, calcium, and potassium, and it does not have enough sensitivity for the rest of the tests**. Conducting the examination with at least 20 patient samples in the reportable range of the kit; so as to cover different points of the claimed measurement interval of the method, and then, evaluate the significance of the difference between the new method and the method that has the capability of metrological traceability.
	○ **Using Certified Reference Material with specific concentration (CRM) and estimation of Bias**
	○ **Using Certified Reference Material with specific concentration (CRM) and Verification of the precision claim of the examination method (kit) according to CLSI Document Ep15-A3 " User Verification of Precision and Estimation of Bias:"**
	○ **Using the recovery method to determine the relative systematic error.**
	○ **Other: (Specify)**

Which method has been used to evaluate the trueness of the examination method in the laboratory, When does the lot number of kit changes?	○ **Assessment of Between-Reagent Lot Variation according to CLSI Document Ep26-A " User Evaluation of Between-Reagent Lot Variation:"** Comparison of two manufacturing identifiers with the number of samples determined based on EP26 tables; with using of Critical Difference and S_R (Repeatability) and S_{WL} (Within Lab Precision). • The list of measurands, that have been used in the laboratory using this method to evaluate the trueness of the examination method at the time of changing the manufacturing number should be mentioned: ○ **"Regression method" according to the published guidelines of Health Reference Laboratory OF Ministry of Health of Iran** Conducting the examination of at least 5 patient samples in non-hospital laboratories and 10 patient samples in hospital laboratories and referral laboratories and reference laboratories so that these samples are all points of the measurement interval (reportable range of the kit) cover, and performing Deming regression to evaluate the variability between different manufacturing identifiers from an operational method of measuring and comparing the components of slope (Slope) and width from the origin (Y-intercept) with the Empirical table of Henry book, edition 21. • The list of measurands, that have been used in the laboratory using this method to evaluate the trueness of the examination method at the time of changing the manufacturing number should be mentioned: ○ **Other: (Specify)**
Which method has been used to verify the linearity of the examination method in the laboratory?	○ **"Evaluation of the Linearity of Performance Quantitative Measurement Procedures" according to CLSI Document Ep06-A and the third edition of Dr. Westgard's Basic method validation book** Conducting the examination on at least 4 and preferably 5 dilutions of a sample 3 times from each dilution and drawing a curve and comparing the results with the total allowable error values. ○ **Other: (Specify)**

5.5.1 Selection, verification and validation of examination procedures

Method of execution: 5.5.1.3 Validation of examination procedures

Which options of functional characteristics is done for validation of examination procedures in the laboratory?

NOTE:

Mentioning the source is mandatory.

for example

Precision evaluation based on CLSI EP05-A3

The laboratory shall validate examination procedures derived from the following sources:

a) Non-standard methods;
b) Laboratory designed or developed methods;
c) Standard methods used outside their intended scope;
d) Validated methods subsequently modified.

Performance characteristics of an examination procedure should include consideration of:

- Measurement trueness,
- Measurement accuracy,
- Measurement precision including measurement Repeatability and measurement intermediate precision (Within Lab Precision),
- Measurement uncertainty,
- Analytical specificity, including interfering substances,
- Analytical sensitivity,
- Measurement linearity
- Detection limit
- Quantitation limit,
- Measuring interval,
- Diagnostic specificity (for qualitative tests)
- Diagnostic sensitivity (for qualitative tests)

5.5.1 Selection, verification and validation of examination procedures

Method of execution: 5.5.1.4 Measurement uncertainty of measured quantity values

Which approach has been used to measurement uncertainty estimation in the laboratory?	○ **Top-Down approach (according to ISO-TS 20914)**
	○ **Other: (Specify)**

Method of execution: 5.5.2 Biological reference intervals or clinical decision values

Regarding the biological reference range of the test method, which steps have been performed in the laboratory?	○ **Definition of biological reference ranges or clinical decision values.**
	○ **Verification of the reference range, claimed by the kit manufacturer in the brochure of the kit.** • The list of measurands, which verification of claimed reference range by the manufacturer is done for them should be mentioned:
	○ **Determination of clinical decision-making values according to valid scientific references regarding some tests such as blood sugar and lipid profiles, etc., in which case there is no need to verify claimed reference range by the manufacturer.** • The list of measurands, which clinical decision-making values have been determined in the laboratory according to valid scientific references, in which case there is no need to verify the claimed reference range by the manufacturer should be mentioned:
	○ **changing biological reference ranges or clinical decision-making values when method or pre-examination procedure is changed.**

Method of execution: 5.5.3 Documentation of examination procedures	
How is the documentation of examination procedures done in the laboratory?	○ Using the kit brochure, the important parts of which are well defined for recognition and attention in a suitable way, such as highlighting.
	○ Using a summary table including items A to T of Clause 5-5-3 of ISO 15189:2012 standard, which is attached to the kit brochure.
	○ Using work instruction related to codified tests in the laboratory with the document control ID; including items A to T of Clause 5-5-3 of ISO 15189:2012 standard; If it is applicable in the examination procedure.

Records keeping for a suitable period of time for review in evaluations is the laboratory's responsibility.

The laboratory's approach to record keeping:

- **Electronic**
- **Paper**
- **movie**
- **Other: (Specify)**

References:

- *Basic Method Validation. Publisher: Westgard QC; 3rd Edition; January 2008. ISBN: 1-886958-25-4.*
- *CLSI. User Verification of Precision and Estimation of Bias; Approved Guideline—Third Edition. CLSI document EP15-A3. Wayne, PA: Clinical and Laboratory Standards Institute;2014.*
- *CLSI. Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline—Third Edition. CLSI document EP05-A3. Wayne, PA: Clinical and Laboratory Standards Institute;2014.*
- *CLSI. User Evaluation of Between-Reagent Lot Variation; Approved Guideline. CLSI document EP26-A.Wayne, PA: Clinical and Laboratory Standards Institute; 2013.*
- *CLSI. Evaluation of the Linearity of Performance Quantitative Measurement Procedures; a statistical approach; Approved Guideline—Second Edition. CLSI document EP06-A. Daniel, W: Clinical and Laboratory Standards Institute; 2003.*
- *McPherson R.A. Pincus M.R. Henry's Clinical Diagnosis and Management by Laboratory Methods. 21st Edition, St. Louis, Missouri : Elsevier, 2007.*
- *ISO/TS 20914- Medical laboratories - Practical guidance for the estimation of measurement uncertainty, 2019.*
- Medical laboratories — Requirements for quality and competence; ISO 15189:2012 = INSO-ISO 15189
- Technical guide for methods of obtaining assurance of validity of laboratory results (the examination processes and the quality control of results) - notified by the Health Reference Laboratory of Ministry of Health of Iran (The letter number is 948/307D dated 16 October 2019).