Performance verification and sigma metrics of creatinine assays

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Abstract

Introduction: Creatinine enzymatic method is more accurate but its own higher cost is the main reason why the use of Jaffe assays is still in practice. The verification step is performed because assay procedure confirms to the manufacturer's claims in the end users' setup. Desirable specification based on biological variation gives Allowable Total Error (ATE) and Sigma metrics provides summative evaluation of method performance.

Objectives: Verification of manufacturer's precision claims. Calculation of bias, Total Analytical Error (TAE) and sigma metrics.

Materials and Methods: Modified Jaffe's (No deproteinization) and enzymatic creatinine Erba XL liquid pack on Erba EM360 fully auto analyser were used. Intra assay and inter assay imprecision done on 20 replicates each. The estimates of bias uses data from precision experiment.

Results & Discussion: The performance of jaffe's and enzymatic serum creatinine is comparable to manufacturer's stated claimed value and are within desirable specification based on biological variation. The bias calculated in both assays is less than allowable bias. Total Analytical Error (TAE) is less than Allowable Total Error (ATE). Both the methods overall showed ATE ≥ bias + 4 SD. Within-run showed ATE ≥ bias + 5 SD complying Six Sigma concepts.

Conclusion: Both Jaffe's and enzymatic creatinine assays gives good performance and better sigma metrics. This makes enzymatic method a better choice due to inherent advantages such as increased accuracy and less interference if cost is neglected.

Keywords: Allowable Total Error, Bias, Creatinine, Imprecision, Sigma metrics, Total Analytical Error.

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Introduction

Creatinine is commonly measured by enzymatic and Jaffe's method in serum and urine. Laboratories are under pressure to maintain quality at the lowest possible price. Enzymatic method is more accurate but its own higher cost is the main reason why the use of Jaffe or compensated Jaffe assays is still in practice.[1] More accurate staging of Chronic Kidney Disease (CKD) had resulted from enzymatic methods with generally fewer interferences than the Jaffe methods.[2]

The verification step is to be performed by end users so that the assay procedure confirms to the manufacturer's claims in the end users' setup. This is because there may be new challenges in the environment in which the working measurement procedure will be implemented, such as different storage conditions of reagents or other factors that could change the performance of the test method.[3] For the clear determination of the major source of error, the individual interpretation of imprecision (I) and bias (B) by using Fitness for purpose criteria was used. Desirable specification based on biological variation gives Allowable Total Error (ATE). Whereas, summative evaluation of method performance was provided by sigma metrics.

Objectives

The study was done to;

1. Verify manufacturer's precision claims for jaffe's and enzymatic creatinine assays,
2. Calculation of bias for comparison against allowable bias and calculation of Total Analytical Error (TAE) for comparison against Allowable Total Error (ATE)
3. Calculation of sigma metrics

Materials and Methods

The reference materials with stability, assigned values and of human origin were recommended[4] and hence used. To measure the performance of Modified Jaffe's (No deproteinization)[5] and enzymatic creatinine by creatininase method, Erba XL liquid system assay packs on Erba EM360 fully auto analyser were used. Informed written consent was not

required as study based on anonymised samples. Within run (intra assay) and between run (inter assay) imprecision estimate were calculated based on 20 replicates each.

Precision claim were verified and bias estimated in one experiment. The estimates of trueness (by calculating bias) uses data from precision experiment, providing samples have assigned values.[4] After experiment was completed data were reviewed for consistency, correctness and outliers due to known causes such as transcription errors. Calculation of Total Analytical Error (TAE) for comparison against Allowable Total Error (ATE) was done. Six Sigma tolerance limits were applied and corresponds to the laboratory limits for ATE which facilitates calculation of a sigma metrics. For data compilation and calculations Microsoft^R Office Excel worksheet was used. The following formulae were used.

1. Calculation of imprecision (I) in verifying sample and comparing with manufacturer's claim samples.
   Imprecision expressed in percentage is equal to percentage of coefficient of variation (CV) which were determined as follows-
   CV% = (SD/Mean) x 100
   where SD is the standard deviation
2. Calculation of bias for comparison against allowable bias and calculation of Total Analytical Error (TAE) for comparison against Allowable Total Error (ATE)
   Bias (B)% = (Average absolute deviation from the target value/
   Target) x 100
   Total Analytical Error (TAE) = % Impression (I) x 1.65 + %Bias(B)
3. Sigma metrics
   Sigma metric = (ATE – Bias)/SD or
   Sigma metric = (% ATE – %Bias)/% CV

Results and Discussion

The estimated test results of verifying sample parameter for jaffe's and enzymatic serum creatinine are tabulated.

The results of precision experiment on verifying sample along with manufacturer's claim value were tabulated (Table 1and 2). They are comparable or near to manufacturer's stated claimed value. The bias and total analytical error calculated from values of same precision experiment along with allowable total error based on biological specifications are tabulated (Table 3).

The total analytical error were within desirable specification and near to optimum specification based on biological variation. Slight deviation in performance could be due to enzymatic reagents requiring narrow optimum conditions than Jaffe's non enzymatic reagents. Improving conditions for reagent stability and handling of enzymatic method improves the performance.

Other study[6] but on two different analyzers showed Creatinine assay's (method not specified) TAE of 7.9 and 9.6 respectively. Another study[7] with pooled frozen serum of higher than normal creatinine levels showed error within minimal specifications.

The bias calculated in both assays is less than allowable bias. Total Analytical Error (TAE) is less compared against Allowable Total Error (ATE) (Table 3). ATE is taken from Ricos[8] developed database of biologic goals based on published studies of biologic variation and recommendations for allowable SDs, biases, and biologic total errors. This is in accordance with Fraser's guidelines for combining allowable SDs and biases.[9] "Ricos goals," are evidence based and our instruments should strive to reach those goals. Ricos goals were used for Sigma metrics.

To characterize test quality sigma metric was used. The better the quality of the testing process, the higher is the sigma metric. Earlier the original recommendation for a total error criterion was ATE ≥ bias + 2 SD, and recent papers recommended ATE ≥ bias + 4 SD.[10] ATE ≥ bias + 5 SD and ATE ≥ bias + 6 SD in Six Sigma concept.[11]Both the methods overall showed ATE ≥ bias + 4 SD (Table No. 4). Within-run showed ATE ≥ bias + 5 SD (not shown in table) complying Six Sigma concepts. Only slight difference shown is between-run performance which may be due to reagent stability and handling.

The tabulation for intra-assay and inter-assay precision results of Modified Jaffe's are in Table 1.

Table 1: Precision results of Modified Jaffe's creatinine assay

precision for intra-assay(N=20) & inter-assay (N=20)	Mean (mg/dL)		SD (mg/dL)		CV (%) = I%	
	intra-assay	inter-assay	intra-assay	inter-assay	intra-assay	inter-assay
Claim Sample 1	2.97	1.25	0.043	0.021	1.45	1.71

Claim Sample 2	4.49	3.51	0.052	0.031	1.16	0.95
Verifying Sample	3.84	3.86	0.057	0.068	1.50	1.74

N= number of samples

The tabulation for intra-assay and inter-assay precision results of enzymatic creatinine assay are in table no. 2.

Table 2: Precision results of Enzymatic creatinine assay

precision for intra-assay(N=20) & inter-assay (N=20)	Mean (mg/dL)		SD (mg/dL)		CV (%) = I%	
	intra-assay	inter-assay	intra-assay	inter-assay	intra-assay	inter-assay
Claim Sample 1	1.02	1.02	0.012	0.023	1.19	2.18
Claim Sample 2	3.7	3.55	0.035	0.061	0.94	1.72
Verifying Sample	3.92	3.96	0.059	0.072	1.49	1.81

The tabulation of %I, %B, TAE & ATE results of Modified Jaffe's and enzymatic assay are in table 3.

Table 3: %I, %B, TAE & ATE

Error calculation (including both intra & inter assay) N=20+20	%I	%B	Total Error
Allowable Error (Specifications)	2.98	3.96	Minimal <13.3 Desirable <8.9 Optimal <4.5
Analytical Error (Verifying Sample by jaffe)	1.62	1.26	3.94 (TAE)
Analytical Error (Verifying Sample by enzymatic)	1.72	1.74	4.58 (TAE)

The tabulation of sigma metrics were done in table 4.

Table 4: Sigma Metrics

Sigma metrics (including both intra & inter assay) for N=20+20	Sigma by total error criteria	Sigma by Six Sigma concept
Recommended Sigma metrics	>4	>5 or >6
Analytical Sigma metrics (Verifying Sample by jaffe)	>4 (4.14)	<5 (4.14)
Analytical Sigma metric (Verifying Sample by enzymatic)	>4 (4.68)	<5 (4.68)

Conclusion

Verification of measurement procedure is important to check manufacturer's claims in the end users' setup. Both Jaffe's and enzymatic creatinine assays gave comparable precision. Both methods TAE were near optimum specifications. And both methods were comparable to sigma by total error criteria and near to six sigma concept on sigma metrics. The inherent advantages of enzymatic method such as less interference from bilirubin, etc, were not tested in this process. Many laboratory adopting enzymatic creatinine assay worldwide may also bring down the cost. Overall enzymatic method is a better choice if cost neglected for quality. Larger studies on different instruments with reagents from different manufacturers and at different setup are required to come for any conclusion.

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