METHOD EVALUATION OF AN ENZYMATIC METHOD FOR SERUM CREATININE

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ABSTRACT

Selecting the correct method for routine analysis by 'method evaluation' is an important component of quality assurance. It is a step-wise procedure that evaluates various analytical parameters like accuracy, precision etc of the given method. Finally reference intervals are established for selected population. we evaluated an enzymatic method for serum creatinine. The results show that it is an acceptable method based on the above mentioned criteria.

KEY WORDS: Evaluation, Quality assurance, Allowable error, Recovery, Decision-Level.

INTRODUCTION

Clinical chemistry laboratories have to regularly introduce new analytical methods and change existing methods to improve accuracy or precision over existing methods, to allow automation, to reduce cost or to measure a new analyte. A method is selected after considering the equipment and personnel available, and the application characteristics like turnaround time, sample size, space required, cost per test, safety etc. After selecting a method it needs to be evaluated thoroughly before it is put into routine use. This 'method evaluation' is an important component of good laboratory practice and contributes to quality assurance.

Method evaluation is different from routine quality control of a method after it has been introduced into daily use. It is a systematic step-wise assessment of the inherent analytical errors like imprecision, inaccuracy etc. of the method. As a final step, newer method that is being introduced into the laboratory is compared with the method that is currently being followed or a reference method. All quality conscious laboratorians must rigorously evaluate a new method before it is put into routine use, failing which they land into quality assurance

problems. While reviewing the literature for an alternative to Jaffe's method for creatinine estimation, we came across an enzymatic method (1) for serum creatinine. We are presently communicating our method evaluation studies for serum creatinine using creatinine iminohydrolase.

MATERIALS AND METHODS

This study evaluated the creatinine iminohydrolase method for serum creatinine on Beckman CX4 autoanalyzer using a commercial kit. The standards for checking linearity were prepared in our laboratory. The quality control sera and calibrator for the analyzer were imported from Beckman Instruments Inc., U.S.A.

The method evaluation proceeded as described (2), in the following steps:

Defining quality goals: The medical decision levels for serum creatinine were taken as 1.0 mg/dL and 3.0 mg/dL. Precision goals were defined to be 0.08 mg/dL at 1.0 mg/dL, and 0.11 mg/dL at 3.0 mg/dL. Total error goals were 0.3 mg/dL at 1.00 mg/dL, and 0.45 mg/dL at 3.0 mg/dL.

Within-run precision: This was studied by analyzing 20 aliquots each of a normal control and an abnormal control.

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Linearity (analytical range): This was determined by duplicate analyses on a series of dilutions prepared from a stock creatinine solution of 50 mg/dL.

Recovery: Two pools (I and II) of sera were prepared initially. To 4.8 ml aliquot of pooled serum was added 0, 50, Or 200 μ L of a concentrated creatinine solution containing 100 mg/dL, plus 200, 150, or 0 μ l, respectively of normal saline to give a final volume of 5.0 ml each. Triplicate analyses were performed and the three values for each specimen were averaged. The amount recovered was determined by subtracting the amount originally present in the serum pools from the amount measured and the percent recovery was calculated from this. Average recovery was obtained from these individual recoveries.

Interference: This was not done for reasons explained in 'Results and Discussion'.

Day-to-day precision: This was evaluated by analyzing two quality control sera daily for 20 days.

Comparison of methods: Duplicate assays were performed on serum specimens from 50 patients using both the enzymatic method and the Jaffe Kinetic method that is routinely followed in our laboratory. After averaging the duplicates, regression analysis was applied.

RESULTS AND DISCUSSION

Within-run precision: The means and standard deviations (SD) of the controls were 0.80 mg/

dL and 0.03 mg/dL for the normal control, and 3.55 mg/dL and 0.80 mg/dL for the abnormal control. These SDs are acceptable when compared with the precision goals of 0.08 and 0.11 mg/dL.

Analytical range: Plotting of the results of this experiment showed that the enzymatic method is linear up to 10 mg/dL.

Recovery: The results are shown in table 1. The average recovery of 95% corresponds to a proportional error of 5%. In terms of actual error this will be 0.05 mg/dL at 1.0 mg/dL concentration, and 0.15 mg/dL at 3.0 mg/dl concentration. These are less than the allowable total error goals.

Interference: This is defined as "the effect of a substance present in the sample that alters the correct value of the result" (3). The ideal method for studying this involves adding the interfering substance (hemoglobin, bilirubin or lipid) directly and measuring its effects. Considering the limitations of this approach, the alternative suggested was to analyse a series of hemolyzed, icteric, and lipemic samples using the new method and an established reference method known to be free of such interferences. Since the Jaffe method that is currently followed is not free of the above problems, this interferences study was omitted.

Recently, the matrix properties of the samples were also found to be interfering with accuracy. We observed an analytical bias between serum and aqueous matrix calibrators (4).

Table 1. Results of recovery experiments.

Serum pool	Creatinine added (mg/dL)	Creatinine measured* (mg/dL)	Creatinine recovered (mg/dL)	Recovery (%)
I	0.0	0.80	-	-
I	1.0	1.75	0.95	95
I	4.0	4.64	3.84	96
II	0.0	3.70	-	-
II	1.0	4.64	0.94	94
II	4.0	7.50	3.80	95
				Average = 95

*Average of triplicate values.

Day-to-day precision: The means and SDs obtained were 0.84 mg/dL and 0.04 mg/dL for one control serum, and 3.43 mg/dL and 0.09 mg/dL for the other. These are again less than the allowable imprecision of 0.08 mg/dL and 0.11 mg/dL respectively.

Comparison of methods: Regression analysis (graph not shown) of the data gave these statistical data: correlation coefficient 0.996, slope 0.992, and Y-intercept - 0.02. The proportional analytical error, as obtained from the slope, is 0.8%; this corresponds to - 0.008 mg/dL at 1.0 mg/dL and - 0.024 mg/dL at 3.0 mg/dL. The Y-intercept shows a small constant systematic error of - 0.02 mg/dL. In order to decide the acceptability or otherwise of the test method based on these data, the systematic errors are to be examined at medically important concentration levels. Thus the systematic error (as calculated from the regression equation, $Y = 0.992X - 0.02$) at the level of 1.0 mg/dL is only 0.028 mg/dL, and the error at the level of 3.0 mg/dL is 0.044 mg/dL. These are less than the specified allowable total error.

Finally, the total error (TE) was calculated by adding systematic error (SE) to random error (RE = $1.96 \times \text{SD}$) (5). Thus the TE at 1.0 mg/dL level

was found to be 0.087 mg/dL, and at 3.0 mg/dL level was 0.20 mg/dL. These observed total errors are also less than the allowable total errors of 0.3 and 0.45 mg/dL.

From the above observations, the enzymatic method for serum creatinine estimation can be judged acceptable. After judging the analytical performance, further studies regarding the stability of reagents and the reference interval for selected population need to be undertaken by the individual laboratories.

Such evaluation procedures need to be undertaken by the clinical chemist to accept or reject a commercial method, a new "in-house" method, or a method published in literature. The decision to accept or reject an analytical method under consideration should be based on the ability of the method to meet the requirements of the final user, the physician. However these detailed method evaluation studies are feasible only for certain analytes like glucose, urea, creatinine etc. Recovery studies may not be possible for assaying enzymes. We have to limit the sample number in case of complex procedures like electrophoresis or expensive investigations such as immunoassays.

REFERENCES

1. Blijenberg, B.G., Brouwer, H.J., Kuller, T.J., Leeneman, R. and Van Leeuwen, C.J.M. (1994) Improvements in creatinine methodology: A critical assessment. *Eur. J. Clin. Chem. Clin. Biochem.* 32(7), 529-537.
2. Koch, D.D. and Peters, Jr., T. (1994) Selection and evaluation of methods. In: *Tietz text book of clinical chemistry*. Eds. Burtis, C.A. and Ashwood, E.R., W.B. Saunders Company, USA, 2nd ed, p. 508-525.
3. Kroll, M.H. and Elin, R.J. (1994) Interference with clinical laboratory analyses. *Clin. Chem.* 40(11), 1996-2005.
4. Saibaba, K.S.S., Srinivasa Rao, P.V.L.N., Ramana, G.V., Kumar, E.G.T.V. and Tripathi, R.L. (1995) Analytical bias due to calibrator matrix effects. *Ind. J. Clin. Biochem.* 10(2), 112-115.
5. Carey, R.N. and Garber, C.C. (1989) Evaluation of methods. In: *Clinical chemistry - theory, analysis and correlation*. Eds. Kaplan, LA and Pesce, A.J. The C.V. Mosby Company, U.S.A., 2nd ed, p. 290-310.