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Evaluation of Enzymatic Method using an Automated Chemistry Analyzers for Homocysteine Measurement

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In clinical practice, homocysteine has gained popularity because its elevated values are strongly associated with an increased risk of cardiovascular disease. More recently, a new enzymatic colorimetric assay for homocysteine in biological sample, suitable for automated clinical analyzers, has been proposed. To evaluate one of these enzymatic methods and compare the results obtained with this method with those of an immunoenzymatic method, thirty-two samples were analyzed for total homocysteine by HiSens® homocysteine reagent on the automated chemistry analyzers TBA 200FR and compared to the widely used immunoenzymatic method ADVIA Centaur. In TBA 200FR, the within-run CVs of two control materials were 3.23% and 0.92%, respectively; the between run CVs were 4.58% and 2.55%, respectively. And in ADVIA 1650, the within-run CVs were 6.81% and 0.99%, respectively; the between run CVs were 9.0% and 3.9%, respectively. The recovery for homocysteine was 100% (60.8 μmol/L), 99.1% (48.64 μmol/L), 96.3% (36.48 μmol/L), 96.1% (24.32 μmol/L), and 92.1% (12.16 μmol/L). The regression equation of TBA 200FR vs. ADVIA Centaur was y=0.9095x – 2.5086 (*r*=0.9632). And the regression equation for the ADVIA 1650 chemistry vs. Immulite 2000 was y=0.8418x + 0.3207 (r=0.9625). In conclusion, this enzymatic method using automated chemistry analyzer for homocysteine assay shows acceptable analytical performance. I suggest that this assay will be suitable for routine analysis.

Key Words : Homocysteine, Method comparison, Enzymatic colorimetric assay, TBA 200FR

I. INTRODUCTION

Clinical utilization of homocysteine measurements include the detection of homocystinuria, folate and vitamine B12 deficiency, and cardiovascular disease risk assessment (Rasmussen and Moller, 2000; Refsum *et al*, 2004). Additionally, its use has been suggested in management of renal failure, cognitive impairment, and psychiatric disorders. Homocysteine is an essential amino acid, derived from methionine.

Early methods for homocysteine measurement were based on the amino acid analysis, radioenzymatic assay, gas chromatography-mass spectrometry, HPLC with fluorescence or electrochemical detector and most of these methodologies, however, are time consuming and require sophisticated equipment not commonly present in clinical chemistry routine laboratories (Ueland *et al*, 1993; Powers and Moat, 2000; Ubbink, 2000). And it required manual pretreatment of samples. The increasing clinical interest for measuring homocysteine in the 1990s called for rapid,

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automated methods with high sample throughput more suitable for routine use. Measurement of homocysteine by an immunoassay appeared to have become the preferred analytical approach. But a limit to use of the immunoassay, at least for some clinical laboratories, is often represented by the rather high costs of both reagents and instrumentation needed for immunoenzymatic analyses. To avoid this inconvenience, an automated method was desired.

More recently, a new enzymatic assay for homocysteine in biological sample, suitable for automated clinical analyzers, have been proposed (Roberts and Roberts, 2004; Kellogg *et al*, 2005).

In this paper, I evaluated one of these enzymatic methods and compared the results obtained by this method with those of immunoassay method.

II. MATERIALS AND METHODS

The serum samples were obtained by a standardized procedure. Blood was collected by venipuncture into SST tubes. The serum was separated by centrifugation at 2000×g for 15 min at 4°C within 30 min of collection, and samples were immediately stored at −80℃ until analysis. To evaluate HiSens® homocysteine reagent (HBI, Anyang, Gyeonggi-Do, Korea), thirty-two samples were analyzed for total homocysteine by the HiSens® homocysteine reagent on automated chemistry analyzers Toshiba 200FR (Toshiba, Tokyo, Japan) compared to the widely used immunoenzymatic method ADVIA Centaur (Siemens, Tarrytown, NY, USA). And twenty-two samples were analyzed by HiSens® homocysteine reagent on automated chemistry analyzers ADVIA 1650 (Siemens, Tarrytown, NY, USA) compared to the widely used immunoenzymatic method Immulite 2000 (Siemens, Pacific Concourse Drive, LA, USA).

The principle of the method is as follows. In the first step, the sample is reduced by dithiothreitol to generate free homocysteine. Simultaneously, homocysteine methyl-transferase transfers the methyl group of D-methionine methylsulfonium to homocysteine, leading to the generation of D-methionine and L-methionine. In the second step, D-amino acid oxidase oxidized D-methionine with the production of hydrogen peroxide, followed by oxidation of redox indicator. The reagent contains N-ethylmaleimide to capture the remaining dithiothreitol, enabling the oxidation of the redox. Sample blank assay is performed at the same time to avoid the influence of D-amino acids in the sample.

To assess linearity, a serum sample with elevated total homocysteine concentration was serially diluted and assayed in triplicate. The within-run CV of the method was established by analysis of control materials, Low and high homocysteine levels (BIO-RAD, Irvine, CA, USA). The between-run CV was established by analyses of the same control on 30 separate batches over the course of 6 weeks.

Data were expressed as means ±SD All data were analyzed using the statistical program SAS 9.1 (SAS Institute, Cary, NC, USA).

III. RESULTS

1. Precision and recovery studies

Precision studies were obtained with two control materials. In TBA 200FR, the within-run CVs were 3.23% and 0.92%, respectively; the between run CVs were 4.58% and 2.55%, respectively. And in ADVIA 1650, the within-run CVs were 6.81% and 0.99%, respectively; the between run CVs were 9.0% and 3.9%, respectively (Table 1). The recovery for homocysteine was 100% (60.8 μmol/L), 99.1% (48.64 μmol/L), 96.3% (36.48 μmol/L), 96.1% (24.32 μmol/L), and 92.1% (12.16 μmol/L). As clearly shown, a recovery higher than 92% was obtained with the five samples analyzed. And Fig. 1 indicated that the enzymatic method shows a good linearity.

Table 1. The precision of within-run and between-run on the plasma homocysteine

	TBA 200FR			
	Within-run		Between-run	
	Mean SD	CVa (%)	Mean SD	CV (%)
Low level	14.0 ± 0.5	3.23	10.9 ± 0.5	4.58
High level	32.3 ± 0.3	0.92	36.4 ± 0.9	2.6
	ADVIA 1650			
	Within-run		Between-run	
Low level	15.0 ± 1.0	6.81	15.3 ± 1.4	9.0
High level	43.4 ± 0.4	0.99	43.8 ± 1.7	3.9

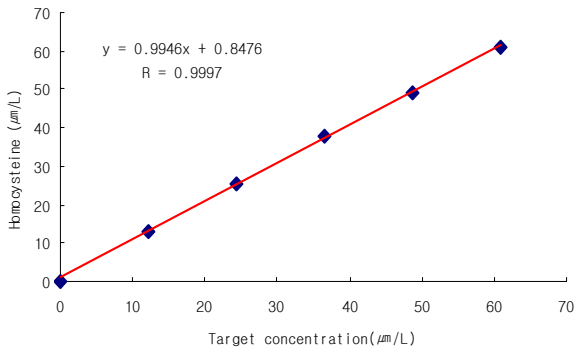
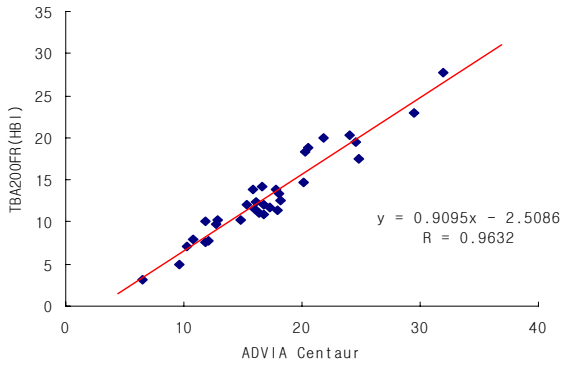


Fig. 1. Linearity of dilution with homocysteine diluent.

2. Comparison of methods

The assay was compared to a enzymatic method using ADVIA 1650 and a immunoassay on the Immulite 2000, and enzymatic method on the TBA 200FR and a immunoassay by ADVIA Centaur. Fig. 2A and B show the correlation between the enzymatic method and a immunoassay method. The regression equation of TBA 200FR vs. ADVIA Centaur was y=0.9095x−2.5086 (*r*=0.9632) (Fig. 2-A). The regression equation for the ADVIA 1650 chemistry vs. Immulite 2000 was y=0.8418x +0.3207 (r=0.9625) (Fig. 2-B). In both cases, the regression analysis clearly indicates a good correlation with both methods.

A.



B.

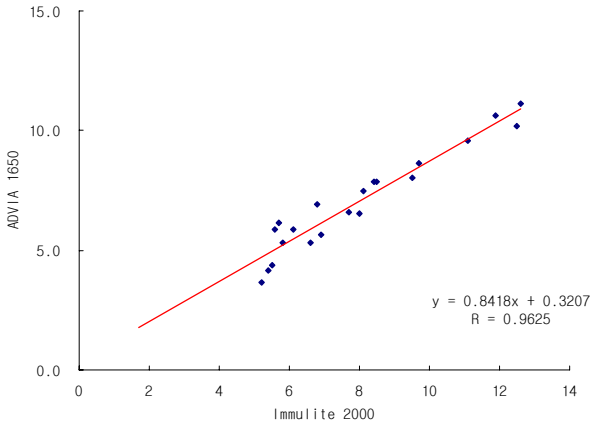


Fig. 2. Linear regression comparing the chemistry method using Chemistry method by TBA 200FR and a immunoassay method by ADVIA Centaur (A) and ADVIA 1650 and Immulite 2000 (μmol/L) (B). Forty-three samples were assayed on each platform. The regression equation of TBA 200FR vs. ADVIA Centaur was y=0.9095x − 2.5086 (*r*=0.9632). The regression equation for the ADVIA 1650 chemistry vs. Immulite 2000 was y=0.8418x + 0.3207 (*r*=0.9625).

IV. DISCUSSION

Homocysteine is an endogenous sulfhydryl amino acid, which is generated by the demethylation of methionine. In human, 15～20 mmol of homocysteine are synthesized each day, but most of them are converted to cysteine, under the enzymatic control of the cystathione β-synthase (CBS), or to methionine.

In clinical practice, homocysteine has gained popularity because its elevated values are strongly associated with an increased risk of cardiovascular disease (Malinow, 1994; D'Angelo and Selhub, 1997; Nygard *et al*, 1999).

The increasing interest in measuring total homocysteine in plasma has led to the development of several different methods (Ueland *et al*, 1993). In fact, starting from the original methods such as gas chromatography or HPLC with a fluorometric or electrochemical detector, nowadays, several reliable automated methods, mostly immunoenzymatic, are commercially available for homocysteine measurement. A wide variety of assays are available to measure homocysteine and are basically grouped into chromatographic and immunoassays.

The immunoassays include the fluorescent polarization immunoassay (FPIA), a chemiluminescent immunoassay (ICL) and enzyme immunoassay (EIA). Analytical performance of the ICL method developed on the ADVIA Centaur analyzer or Immulite 2000 was similar to those of other documented immunoassay-based commercial methods for total homocysteine determination.

While immunoassays provide clinical laboratories with rapid, simple and precise measures of homocysteine, these assays suffer from a lack of standardization and expensive reagents. More recently, two variations on enzymatic assays have become available (Tan *et al*, 2000; Huijgen *et al*, 2004). Recently, some new automated methods (Roberts and Roberts, 2004; Kellogg *et al*, 2005), based on a recombinant enzymatic cyclic assay for homocysteine, have been proposed.

Like immunoassay, enzymatic methods offer rapid and simple means of analysis. Additionally the enzymatic methods can be implemented on a much wider variety of automated platforms.

In this study, I have compared the homocysteine values, obtained by using the kit from HBI on an automated chemistry analyzer, with an immunoassay method (ADVIA Centaur, immulite 2000). These results show that this new enzymatic method is well correlated both to the immunoassay method. These results support a similar conclusion made by Zappacosta *et al* (2006) in their comparison of FPIA and new enzymatic method using automated chemistry analyzer. This method is applicable to various colorimetric-based automatic analyzers.

In conclusion, this method seems to be more suitable for routine analysis than immunoenzymatic methods and more feasible because it needs only common laboratory instrumentation unlike the fluorescent immunoenzymatic method. I suggest that this assay will be suitable for routine use,

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국문요약

호모시스테인은 관상동맥질환, 뇌혈관 질환의 위험인자로 알려지고 있다. 초기의 호모시스테인 측정은 HPLC를 이용하여 일반적인 검사실에서 이용하기에는 어려움이 많았으며 현재 여러 가지 면역분석기가 도입되어 시행되고 있다. 하지만 최근 자동화학분석기를 이용한 새로운 효소법의 시약이 개발되어 이 시약의 유용성 평가를 하기위해 본 연구에서는 기존의 면역분석기와 비교분석하였다. 그 결과 정밀도 평가에서 변이계수는 0.98 - 3.23%, 2.55~4.58%를 나타내었고, 자동화학분석기(TBA 200FR와 Advia 1650)를 이용한 새로운 효소법(HBI)과 기존의 면역분석기(Advia Centaur와 Immulite 2000)간의 상관관계 평가에서 상관계수는 0.9632와 0.9625로 우수한 상관관계를 보였다. 이 결과를 토대로 호모시스테인 측정에 있어 새로운 효소법의 자동화학분석기를 이용한 방법이 제시되어 보다 간편하고 쉬운 routine analysis에 적합하리라 사료된다.