Letter to the Editor

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Analytical performance and method comparison study of the total homocysteine immunoassay on the AIA 600II analyser

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To the Editor,

Plasma total Hcy (tHcy) is the biochemical marker routinely evaluated and considered clinically useful.

Hyperhomocysteinaemia (HHcy) is a well-established independent risk factor for cardiovascular diseases; the role of homocysteinaemia in risk stratification was largely supported by epidemiological studies [1].

Hyperhomocysteinaemia has recently also been associated with other pathological conditions such as peripheral neuropathy, renal failure, hypotiroidism, Alzheimer disease and other dementias, pregnancy complications and birth defects [1, 2].

Since homocysteine (Hcy) metabolism is catalysed by enzymes requiring B vitamins as cofactors, tHcy can also be considered a marker for folate and cobalamin deficiency [3, 4].

Thanks to the introduction of automated platform, tHcy concentration is more and more widely routinely measured as independent risk factor for cardiovascular diseases, or along with other metabolically-related markers.

We report the results of a study evaluating the analytical performance of the new immunoenzymatic assay ST AIA-Pack HomoCYS for measuring tHcy in human blood on AIA 600II Analyser (Tosoh Bioscience, Tokyo, Japan); the method was also compared with other commercial methods, namely Fluorescence Polarization ImmunoAssay (FPIA) Homocysteine assay on AxSYM (Abbott Diagnostics, Abbott Park, IL, USA), Chemiluminescent Microparticle ImmunoAssay (CMIA) Homocysteine assay on Architect i2000SR (Abbott Diagnostics), and Homocysteine liquid enzymatic assay (Sentinel Diagnostics, Milan, Italy) on Modular P analyser (Roche Diagnostics, Indianapolis, IN, USA). The aim was to evaluate whether this new method reaches the established analytical goals and can be considered suitable for routine testing.

The ST AIA-PACK HomoCYS is a competitive immunoenzymatic assay which after sample pre-treatment is performed entirely in the ST AIA-PACK HomoCYS cups. Sample Hcy competes with enzyme-labelled Hcy for a limited number of binding sites on homocysteine-specific antibody immobilised on magnetic beads. After incubation, the beads are washed to remove the unbound enzyme-labelled homocysteine and then incubated with a fluorogenic substrate, 4-methylumbelliferyl phosphate. The amount of enzyme-labelled homocysteine that binds to the beads is inversely proportional to the homocysteine concentration in the test sample. A standard curve using a range of known standard concentrations (from 0 to 55 μmol/L) is constructed and unknown sample homocysteine concentrations are calculated using this curve.

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Imprecision was determined by using the two levels' controls provided by the manufacturer and three different patient-pools measured 20 times in the same run (for intra-assay imprecision) and in duplicate in every single run during 20 days (for inter-assay imprecision).

Intra- and inter-assay imprecision were expressed as coefficient of variation (CV) %.

The limit of detection (LoD) was determined according to CLSI EP 17A2 document [5].

Recovery was assessed by using a plasma sample with known high tHcy concentration (37.7 µmol/L) spiked at a ratio of 1:10 with five plasma samples with known low tHcy concentrations (range 6.7–8.2 µmol/L); all samples were tested before and after spiking, and the recovery was calculated as the percentage of obtained/expected.

Linearity test was performed by diluting five patients' samples (concentration near 40 µmol/L) with AIA-PACK HomoCYS sample diluent in order to obtain serial dilutions (up to 1:32); all samples were processed in duplicate and the resulting percent recovery was calculated.

Method comparison was assessed on EDTA plasma samples obtained from 243 subjects coming to our laboratory for tHcy routine determination with Homocysteine liquid enzymatic assay (Sentinel Diagnostics) on Modular P analyser (Roche Diagnostics). Plasma aliquots were stored at −80°C unless analysed the day of collection. In particular, out of the 243 samples, 143 were also used for method comparison with AxSYM (Abbott), and 85 for method comparison with Architect (Abbott).

All statistical analysis were performed on software MedCalc [6].

As shown in Table 1 total analytical imprecision for the AIA-PACK HomoCYS was 6.2% for controls and 6.05% for plasma pools. Mean recovery and mean dilution recovery were 103.9% (range 100.4%–107.2%) and 102.5% (range 96.6%–110%), respectively; linearity test showed optimum results at dilutions up to 1:16 (mean r=0.94) and good results up to 1:32 (mean r=0.91). The LoD for AIA-PACK HomoCYS was 0.15 µmol/L.

tHcy concentrations measured in 243 plasma samples in parallel on AIA 600II and Modular P ranged from 4.6 to 46.8 µmol/L and from 3.8 to 50.0 µmol/L, respectively; tHcy values obtained in 143 samples on AxSYM and in 85 samples on Architect were 4.0–45.3 µmol/L and 4.0–37.6 µmol/L, respectively.

AIA-PACK HomoCyS compared well with all the three methods evaluated as shown in Table 2; results of the Passing and Bablok and Deming regression analyses were nearly equivalent.

The agreement of tHcy measurements, obtained with AIA-PACK HomoCYS Tosoh and with each of the other methods, was analysed with the Bland and Altman plots showing mean difference of −0.8 µmol/L (95% CI −3.5 and +2.0 µmol/L), −0.3 µmol/L (95% CI −3.0 and +2.4 µmol/L) and −0.5 µmol/L (95% CI −3.1 and +2.0 µmol/L) with Modular-P, AxSYM and Architect, respectively.

The reference method for determining tHcy is liquid chromatography [2, 7], but it is generally unsuitable for large routine. During recent years several methods have been developed and proposed on automated analysers, due to the increasing need of Hcy evaluation in a variety of physiopathologic conditions [2].

The physiopathological interval (0.5th–99.5th percentiles of the general population) for tHcy concentration is approximately 3–40 µmol/L [2] and it fits in the analytical range of the immunoenzymatic method evaluated.

Total analytical imprecision was <7%; in particular total CV% was <6% for tHcy concentrations near 15 µmol/L, the critical level corresponding to the upper reference limit.

Table 1 Imprecision of AIA-PACK HomoCyS from controls and plasma pools.

		Total homocysteine concentration		Imprecision, CV%
		Target value, µmol/L	Mean±SD, µmol/L	
Intra-assay	Control 1 (n=20)	15.8	16.83±0.83	4.9
	Control 2 (n=20)	27.3	29.54±2.20	7.5
	Pool 1 (n=20)	NA	19.18±1.00	5.2
	Pool 2 (n=20)	NA	23.35±1.24	5.3
	Pool 3 (n=20)	NA	20.12±0.76	3.8
Inter-assay	Control 1 (n=20)	15.8	16.47±0.97	5.9
	Control 2 (n=20)	27.3	28.23±1.85	6.5
	Pool 1 (n=20)	NA	18.63±1.04	5.6
	Pool 2 (n=20)	NA	23.12±1.88	8.1
	Pool 3 (n=20)	NA	19.51±1.60	8.2

CV, coefficient of variation; NA, not applicable; SD, standard deviation.

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Table 2 Correlation of AIA-PACK HomoCyS (Tosoh) with FPIA Homocysteine assay on AxSYM (Abbott), CMIA Homocysteine assay on Architect i2000SR (Abbott), and Homocysteine liquid enzymatic assay Sentinel on Modular P (Roche).

Assay comparison	Tosoh/Sentinel-Roche n=243	Tosoh/AxSYM n=143	Tosoh/Architect n=85
Deming regression			
Slope (95% CI)	0.9238 (0.8953 0.9522)	0.9701 (0.9321 1.0081)	0.9865 (0.9329 1.0402)
Intercept (95% CI)	1.9069 (1.5413 2.2725)	0.7376 (0.2071 1.2681)	0.7469 (0.0229 1.4709)
Passing and Bablok			
Slope (95% CI)	0.9767 (0.9492 1.0000)	0.9924 (0.9630 1.0185)	1.0028 (0.9639 1.0396)
Intercept (95% CI)	1.1953 (0.9000 1.4881)	0.2950 (–0.0598 0.69637)	0.3681 (–0.1579 0.8880)
Spearman's rank correlation	0.983 (p<0.0001)	0.979 (p<0.0001)	0.979 (p<0.0001)

Refsum et al. recommended a total imprecision for tHcy assays <5%, nevertheless imprecision determined for the ST AIA-Pack HomoCYS should be considered acceptable.

The ST AIA-Pack HomoCYS on Tosoh AIA 600II Analyser demonstrated good overall comparability with the other three immunoassays considered and routinely used in clinical biochemistry laboratories. Both Deming regression and Passing and Bablok test showed slope values near 1.00 and positive y-intercepts ranging from 0.2950 (Tosoh vs. AxSYM) to 1.9069 (Tosoh vs. Sentinel-Roche). The Bland-Altman plots highlighted a good agreement of ST AIA-Pack HomoCYS and the other immunoassays with <7% of values differing by 2 SD, and on average a little overestimate in comparison to all the other methods, with no remarkable difference in wideness of bias depending on tHcy concentration.

In conclusion, considering the acceptable performance and the good agreement with other routine methods, together with the analytical characteristics of AIA 600II Analyser (low reagent/consumable cost, number results/hour, user friendly, fully automated), ST AIA-Pack HomoCYS can be considered suitable for routine use, evaluated alone or as a part of the metabolic panel together with the related vitamin.

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Conflict of interest statement

Authors' conflict of interest disclosure: The authors stated that there are no conflicts of interest regarding the publication of this article. Research support played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.
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References

1. Lippi G, Plebani M. Hyperhomocysteinemia in health and disease: where we are now, and where do we go from here? Clin Chem Lab Med 2012;50:2075–80.
2. Refsum H, Smith AD, Ueland PM, Nexo E, Clarke R, McPartlin J, et al. Facts and recommendations about total homocysteine determinations: an expert opinion. Clin Chem 2004;50:3–32.
3. Stabler SP. Clinical practice. Vitamin B12 deficiency. N Engl J Med 2013;368:149–60.
4. Obersby D, Chappell DC, Dunnett A, Tsiami AA. Plasma total homocysteine status of vegetarians compared with omnivores: a systematic review and meta-analysis. Br J Nutr 2013;109:785–94.
5. CLSI. EP17A2 Evaluation of detection capability for clinical laboratory measurement procedures; Approved guideline, volume 32, 2nd ed. Wayne, PA: CLSI, 2012.
6. Stephan C, Wesseling S, Schink T, Jung K. Comparison of eight computer programs for Receiver-Operatic Characteristic Analysis. Clin Chem 2003;49:433–9.
7. Pfeiffer CM, Huff DL, Gunter EW. Rapid and accurate HPLC assay for plasma total homocysteine and cysteine in a clinical laboratory setting. Clin Chem 1999;45:290–2.

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