Clin. Lab. 2025;71:XXX-XXX
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LETTER TO THE EDITOR

Comparison of Homoarginine Serum Levels with HPLC Method and Commercial ELISA Assay

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(Clin. Lab. 2025;71:xx-xx. DOI: 10.7754/Clin.Lab.2024.241115)

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Letter to the Editor accepted November 15, 2024

KEYWORDS

homoarginine, high pressure liquid chromatography, enzyme linked immunosorbent assay, method comparison

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Serum levels of homoarginine (hArg) are inversely associated with increased cardiovascular mortality. This observation was published for the first time by März et al. 2010 [1]. Subsequent studies demonstrated an association between low hArg levels and cardiovascular events, congestive heart failure, diastolic heart failure, and peripheral artery disease and several other diseases (e.g., stroke and chronic kidney disease). Low hArg was also associated with increased cardiovascular mortality in population-based studies of elderly individuals and in the general population [2]. There is an inverse association between hArg levels and the risk of adverse cardiovascular outcomes (e.g., high cardiovascular risk is predicted by low rather than high hArg levels), which makes it plausible to normalize systemic hArg levels via oral supplementation. Clinical data suggest that hArg supplementation in healthy volunteers is safe and well tolerated [3]. A sensitive and accurate method for hArg measurement is necessary to investigate blood levels of this biomarker. Many techniques have been developed for hArg determination. High pressure liquid chromatography (HPLC) is a simple, rapid, precise, and accurate method for hArg in various biological samples [4]. However not all laboratories have the adequate technical equipment and trained staff. Therefore, the enzyme linked immunosorbent assay (ELISA) technique, which is much easier to establish, could be an alternative

Table 1. Characteristics of patients.

Patients	n = 124
Age (mean ± SD) range (years)	58 ± 11.3, 31 - 84
Gender (female/male)	20/104
Homoarginine (µmol/L), ELISA Median (25th - 75th percentile)	1.76, 1.37 - 2.09
Homoarginine (µmol/L), HPLC Median (25th - 75th percentile)	1.89, 1.47 - 2.13

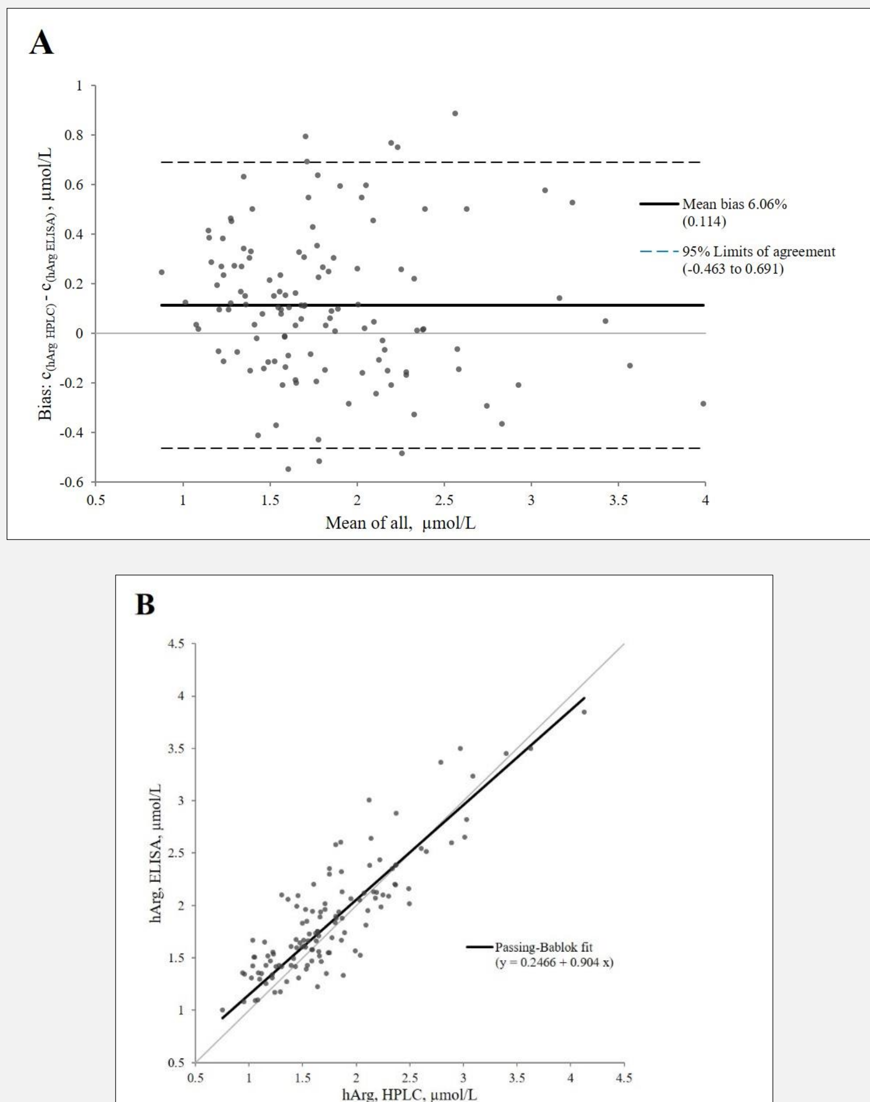

Figure 1. A) Bland-Altman plot of homoarginine (hArg) assay comparison. The mean relative bias in percent for the immuno-assay compared to the HPLC method is represented as a plain line (6.06%). The limits of agreement are illustrated by dashed lines. B) Passing Bablok fit of hArg assay comparison. Bold lines represent Passing-Bablok fit. The regression equation with the included 95% confidence intervals for the slope and intercept is presented next to the regression lines.

method in routine laboratories.
The aim of the study was to compare a recently launched ELISA (Immundiagnostik, Bensheim, Germany) with HPLC, which was well established in our laboratory [5].
The overall sample consisted of 124 study participants (104 males, 83.2%; 20 females, 16.8%). Blood samples were drawn from patients hospitalized at the Department of Cardiology at the Medical University of Graz, Austria, immediately after acute myocardial infarction. This study is in accordance with the ethical standards of the Declaration of Helsinki. The ethical approval was provided by the Ethical Committee of the Medical University of Graz, Austria. The new assay is a competitive ELISA, in which the well plate is coated with a polyclonal antibody against hArg derivate. Diluted serum samples were mixed with a derivatization reagent and then transferred to wells together with the assay reagent, which contains a defined amount of hArg derivative (tracer). The target antigen in the sample competes with the tracer for binding to the polyclonal antibody. After a washing step to remove unbound components, the peroxidase substrate tetramethylbenzidine (TMB) is added. The enzyme reaction is stopped by the addition of acid, resulting in a color change from blue to yellow. The resulting chromogenic compound is measured photometrically at 450 nm. The intensity of the color is inversely proportional to the concentration of the analyte measured. Within-run and between-run imprecisions of ≤ 15% were found within the whole working range (0.31 - 3.79 µmol/L). The limit of quantification (LOQ) was the lowest working range level. In the HPLC method hArg was purified with solid phase extraction and measured after precolumn derivatization and separation on a special reversed phase column with fluorescence detection. Within-day coefficients of variants (CVs) were 4.7% (1.21 µmol/L) and 2.2% (3.53 µmol/L), and between-day CVs were 7.9% (1.25 µmol/L) and 6.8% (3.66 µmol/L).
Patients' characteristics are given in Table 1. Bland-Altman plot is illustrated in Figure 1A. Compared to the HPLC method the new ELISA assay demonstrated a mean relative bias of 6.06%. Passing-Bablok plot is shown in Figure 1B ($y = 0.2466 + 0.904x$).
In conclusion, the new ELISA assay evaluated in this study is a useful method for measuring hArg serum levels in clinical laboratories. The performance characteristics are suitable for routine diagnostics.

Declaration of Interest:
The authors declare that they have no conflicts of interest.

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