Vitamin D immunoassay systems: a comparison

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Vitamin D deficiency has been reported in populations around the world.[1] This has attracted the attention of health professionals, especially given the mounting evidence linking vitamin D to overall health.[2–4] Consequently, there has been rising clinical demand for the assessment of vitamin D status.[5,6] The total 25(OH)D level (the sum of D2 and D3) is the best indicator of vitamin D body stores.[7,8] Currently, there is great variation and a lack of standardisation in the methods used for vitamin D measurement, resulting in considerable uncertainty. Hence, a simple and high-throughput method for reliably measuring vitamin D level has become an absolute requirement for clinical laboratories.[9]

In response to the need for vitamin D testing, different automated assays for measuring the 25(OH)D level have been developed and released by different companies. The most popular are LIAISON (DiaSorin), which is a direct competitive chemiluminescence immunoassay, the Elecsys system (Roche), which is based on electro-chemiluminescence immunoassay technology and is used on different platforms including the E170 module and cobas e602 modular analyser, and the ARCHITECT assay (Abbott), which is a chemiluminescence microparticle immunoassay. With the steadily growing list of 25(OH)D assays, the choice of an ideal method has become increasingly difficult.

The objective of this study is to compare the total 25(OH)D levels obtained by four automated immunoassay methods with those obtained by high-performance liquid chromatography (HPLC) to test the accuracy of these immunoassays and thus their suitability for routine use in high-volume laboratories.

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Table 1. Comparison of total 25(OH)D levels and percentage of deficient individuals obtained by the five different assay systems. Vitamin D deficiency was defined as 25(OH)D <50 nmol/L.

	Total 25(OH)D (nmol/L)	Deficient individuals
HPLC	68 (48–87)	24%
Elecsys cobas	67 (53–87)	24%
Elecsys E170	78 (57–93)	15%
Architect	68 (47–85)	27%
Liaison	61 (36–75)	36%

Results presented as median (interquartile range)

To convert 25(OH)D concentration to ng/mL, multiply by 0.4.

This multicentre study used 33 randomly selected blood samples from apparently healthy subjects sent for routine tests. All samples were checked for haemolysis and were left to clot for 30 minutes and then centrifuged at 3000 rpm for 10 minutes. The serum was split into five aliquots, and these were stored at –70°C until analysis. Samples were shipped frozen to different centres in the Riyadh area for analysis of total 25(OH)D levels. All samples were analysed within two weeks of collection using the same run on each analyser.

The total 25(OH)D level was measured using four immunoassay methods: the LIAISON chemiluminescence immunoassay (DiaSorin, Stillwater, MN, USA), the ARCHITECT i2000 chemiluminescence microparticle immunoassay (Abbott, IL, USA), and the Elecsys electrochemiluminescence immunoassay (Roche, Basel, Switzerland) run on the cobas e602 and E170 analysers. The results obtained using these four methods were compared with those obtained using HPLC (Waters Alliance, Milford, USA) using a commercial reagent kit (Chromsystems, Munich, Germany).

Vitamin D deficiency was defined as a 25(OH)D level <50 nmol/L.[10] Passing–Bablok regression analysis was performed. The results were also analysed using Bland-Altman plots. Statistical analyses were performed using SPSS 16.0 and MedCalc 12.5.0.0 (MedCalc, Belgium [www.medcalc.be]). $P<0.05$ was considered statistically significant.

The studied subjects comprised five males and 28 females (age range: 1–76 years). The median and interquartile range obtained with the different tests is show in Table 1. The percentage of deficient individuals ranged from 15% using the Elecsys E170 assay to 36% using the LIAISON assay (Table 1). The details of the Passing-Bablok regression analysis are summarised in Table 2 and illustrated in Figure 1. None of the four immunoassays showed deviation from linearity when compared with the HPLC assay ($P<0.05$). However, there was a small constant difference

Table 2. Passing-Bablok regression analysis of the evaluated methods using HPLC as a reference.

	Intercept	95% CI	Slope	95% CI	Deviation from linearity
Elecsys cobas e602	3.23	–3.4–13.6	0.96	0.8–1.1	$P<0.05$
Elecsys E170	13.02	6.69–23.39	0.93	0.79–1.04	$P<0.05$
Architect	3.85	–6.75–12.3	1.01	0.84–1.18	$P<0.05$
Liaison	–5.93	–14.11–4.00	0.96	0.79–1.11	$P<0.05$

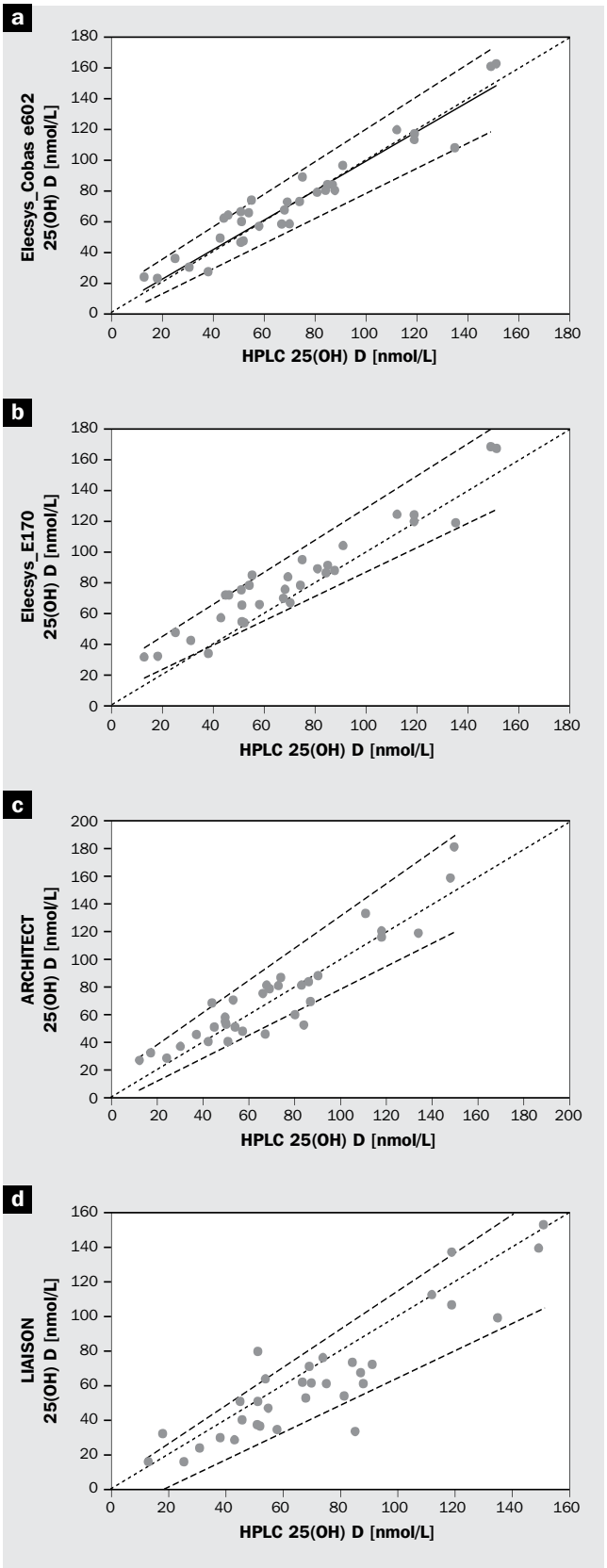

Fig. 1. Comparison of the evaluated methods with HPLC using Passing-Bablok regression analysis. Scatter diagrams showing regression line (solid), confidence interval (large dash) and identity line (small dash): **a)** y = 0.9 x + 3.23 (Elecsys cobas e602); **b)** y = 0.93 x + 13.02 (Elecsys E170); **c)** y = 1.00 x + 3.85 (Architect); **d)** y = 0.96 x – 5.93 (Liaison).

between the Elecsys E170 and HPLC assays, with an intercept of 13.02 (95% confidence interval [CI]: 6.69–23.39), but no proportional difference (slope: 0.93; 95% CI: 0.79–1.04).

The Bland-Altman plot analysis is illustrated in Figure 2. The mean bias with respect to the HPLC method was –2.5 nmol/L for the Elecsys cobas assay, –10.0 nmol/L for the Elecsys E170 assay, –1.9 nmol/L for the ARCHITECT assay, and +8.1 nmol/L for the LIAISON assay.

Determining the level of vitamin D has gained increased popularity among healthcare providers and researchers. This is of particular relevance in a country with year-round high prevalence of vitamin D deficiency, such as Saudi Arabia.[11] Furthermore, a recent meta-analysis by Reid,[12] undermining the role of vitamin D on bone mineral density, does not apply to a population with greater risk of D deficiency,[13] such as the Saudi population.

Many studies have compared different methodologies for vitamin D testing;[9,14–16] however, these studies had various objectives and findings. Some compared assays that measured D3 only,[14,17] while others compared automated assays with manual radioimmunoassay.[9] Interestingly, researchers have compared the LIAISON and ARCHITECT assays with the liquid chromatography-mass spectrometry (LC-MS) method and reported good performance.[15] Very recently, Abdel-Wareth *et al*.[16] compared HPLC with the Elecsys total 25(OH)D assay performed on the cobas e602 module and reported an acceptable bias.

The present study compared total 25(OH)D levels obtained by four different automated immunoassay methods with those obtained by an HPLC method. All centres participating in this study were part of the Vitamin D External Quality Assessment Scheme (DEQAS).[18]

Despite the fact that good agreement was found between the four evaluated methods and HPLC by the Passing-Bablok regression analysis, all four showed a bias when compared with HPLC using Bland-Altman plots. A bias not exceeding 15.8% has been described as acceptable in the literature.[15] Using this criterion, the Elecsys cobas e602, ARCHITECT and LIAISON assays had acceptable bias, whereas the Elecsys E170 assay exceeded this, with a negative bias of –23.2%.

A major limitation of the present study was the small sample size. In addition to the variable cut-off values used to define deficiency or insufficiency,[19–21] many important issues need to be considered when performing vitamin D testing. These issues include poor standardisation,[22] which has led to discrepancies between the results of different laboratories, and interference from vitamin D-binding protein.[23]

More recently, a standard reference material for vitamin D (SRM 972) has been developed by the National Institute of Standards and Technology.[24] The use of this standard should improve the confidence in 25(OH)D testing and the ability of these tests to identify individuals with suboptimal vitamin D status.

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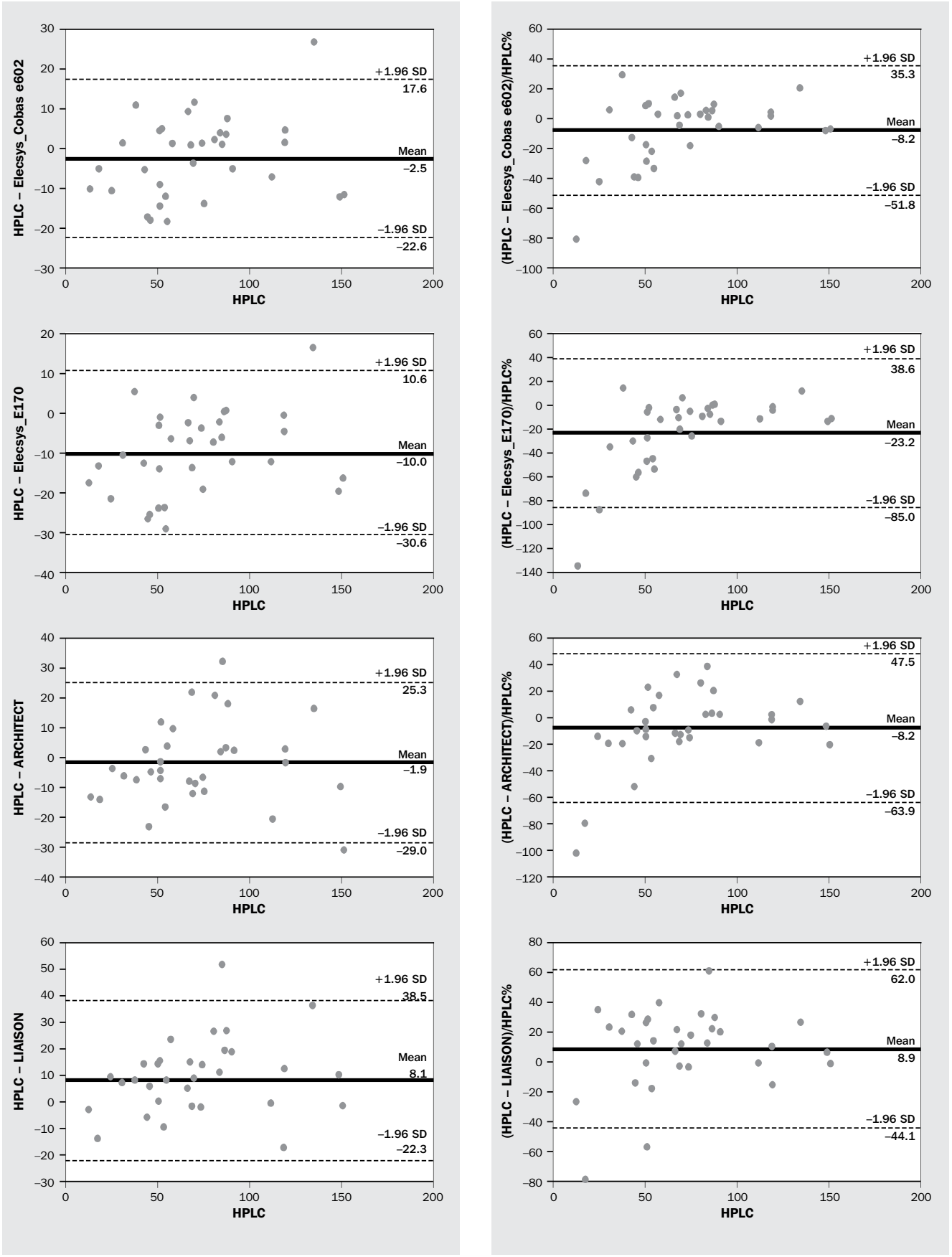

Fig. 2. Comparison of the evaluated methods with HPLC using Bland-Altman plots. The total 25(OH)D level in nmol/L obtained with the HPLC method was plotted against the difference between the values obtained with the HPLC method and the tested method. The Bland-Altman plots show bias in the level in nmol/L (left panel) and in the percentage (right panel).

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Observations on the variation in volumes of self-collected oral fluid samples submitted for HIV antibody detection

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Saliva is a safe, simple and abundant sample to collect for an ever-increasing number of assays.[1–3] The use of oral fluids for detecting antibodies to human immunodeficiency virus (HIV) has long been suggested as an alternative to the use of blood.[4–6] This could help to eliminate the occupational risks associated with needlestick accidents and injuries from phlebotomy. It could also decrease the patient discomfort and thus improve compliance with repeated testing.[7,8]

Although oral fluid from HIV-1-infected individuals contains antibodies to HIV-1, infectious virus in oral fluid is rare.[9,10] Early studies show that the volume and condition of oral fluid are important factors in successful antibody detection, therefore investigators developed specialised self-collection devices that would enhance the quality obtained and preserve the quality and concentration of antibodies by preventing microbial growth and proteolytic breakdown of the antibodies.[11,12]

Self-collection of samples, however, can lead to variability in the volume or quality of the sample submitted for analysis. Therefore, this study aims to determine the frequency of 'unacceptable' samples submitted by participants being screened for HIV-1 infection in three different settings: i) as part of an insurance application; ii) through an online healthcare company; and iii) at a local hospital under direct supervision of hospital staff.

Until March 2012, Quest Diagnostics provided the pathology services for a number of insurance companies that tested clients for HIV, and for an online medical company (which sent samples to the Quest walk-in clinic at Upper Wimpole Street). All these samples were self-collected

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