# Evaluation of 25-hydroxyvitamin D quantification using a commercial HPLC kit method

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Vitamin D and its metabolites exist in two forms, cholecalciferol (vitamin $D_3$), which is produced in the skin via a photochemical reaction with 7-dehydrocholesterol or consumed via the diet or most supplements, and ergocalciferol (vitamin $D_2$), which is derived from ergosterol in plants and is found in a few dietary supplements or fortified foods.[1–3] Increased risk for numerous diseases, such as osteoporosis, several types of cancer, diabetes, hypertension and cardiovascular disease, has been associated with lower vitamin D intake or circulating concentrations of vitamin D metabolites.[4–6] 25(OH)D concentration in the circulation is the accepted measure to assess vitamin D status in humans.[4,7] Several analytical methods have been used to determine the serum concentration of 25(OH).[4,7] Advantages and disadvantages exist for each method, based on methodological characteristics.

In 1971, the competitive protein binding assay (CPB) method was introduced by Haddad and Chyu[8] and Belsey *et al*.[9] This assay involves the use of the vitamin D binding protein and radiolabelled tritiated 25(OH)$D_3$. With these CPB methods, individual sample loss and recovery had to be estimated, due to the extensive extraction process.[3] The first useful direct ultraviolet (UV) detection assay for 25(OH)D was reported in 1977.[4,10] The main advantage of this high-performance liquid chromatographic (HPLC) method is the ability to measure both vitamin $D_2$ and $D_3$ metabolites separately.[4,10] In 1985, Hollis and Napoli introduced a radioimmunoassay (RIA) for 25(OH)D quantification.[11] Although very sensitive, this RIA method had the disadvantage of being very complex and requiring a radioactive safety standard for the laboratory analysis. Components of the method include antiserum produced in an animal source, such as sheep, donkey or goat, and other radioiodinated or tritiated components.[12] A fluorometric assay for 25(OH)$D_3$ was reported in 1991 by Shimizu *et al.*[13] This method, although reported to be comparable to the HPLC-UV method, nonetheless required a fluorescence-labelled step and use of a radioactive standard.

In 1993, Hollis *et al,* working with the DiaSorin Corporation (Stillwater, MN), introduced an improvement to their earlier RIA method.[3,14] This improved RIA method became the default standard method for the measurement of total circulating 25(OH)D.[3,14] In 2004, the DiaSorin Corporation further developed 25(OH)D quantification using a chemiluminescence method. The method was a fully automated procedure on the LIAISON analyser.[3,4,15] Antibodies specific for 25(OH)$D_2$ and 25(OH)$D_3$ were the defining component of the method, a very specific, accurate and sensitive automated technique that replaced the improved RIA standard approach as the most widely accepted reference method for the quantitative assay of total 25(OH)D in serum or plasma.[3] Results from the chemiluminescence method are reported as total 25(OH)D, and some comparative assays with HPLC-UV techniques have shown good correlation and confirmed the ability to detect the vitamin $D_2$ and $D_3$ metabolites.[3,15]

In 2005, an LC-mass spectroscopy (MS) method was introduced by Maunsell *et al*.[16] This technique has the ability to separate and potentially more accurately quantify both 25(OH)$D_2$ and 25(OH)$D_3$, as compared to other methods.[4] Additional LC-MS methods that are demonstrated to be accurate and sensitive have been introduced over time, but they have the major disadvantage of being far too expensive for most research and clinical laboratories.

The primary aim of this study is to compare total serum or plasma 25(OH)D concentration from human samples quantified by a commercial laboratory (Arup Laboratories, Salt Lake City, UT), using the DiaSorin-developed chemiluminescence method, and the combined total of 25(OH)$D_2$ plus 25(OH)$D_3$ measured with a commercially available HPLC-UV method (25[OH]Vitamin $D_2$/$D_3$ Kit, Iris Technologies, Olathe, KS). We also examine the correlates of circulating concentrations of total 25(OH)D using these two laboratory methods.

Human serum or plasma samples were collected from adult female volunteers from two multicentre clinical research projects, neither of which targeted a change in vitamin D intake or status. For both of these trials, the institutional review boards at all involved institutions approved the protocol prior to study initiation, and all participants provided written informed consent. The first set of 956 plasma samples was collected at enrollment (between 1995 and 2000) in a phase III clinical trial of diet intervention and breast cancer recurrence. The complete cohort for this clinical trial was 3088 women, but funding was available to measure vitamin D on only a subset of the women ($n$=956) oversampled for breast cancer recurrence.[17] The samples were separated and frozen at –80°C until analysed (for an average of 11 years). The second set of 1659 serum samples was collected as part of another clinical trial testing a weight loss intervention in overweight and obese women. These samples were collected from 442 volunteers at four time points between 2007 and 2010. After blood collection and processing, these samples were also stored at –80°C until analysed (for an average of one year). Of note, sample storage at –80°C has not been observed to affect adversely the stability or accuracy of measurements of vitamin D and its metabolites. Notably, 25(OH)D in pooled human samples stored at –20°C for >10 years has been reported to show no detectable degradation.[3]

Study participants in both trials provided self-reported data on demographic and other characteristics, including age, race/ethnicity, and use of dietary supplements containing vitamin D, including multivitamin/mineral supplements, calcium supplements with vitamin D, and vitamin D alone. Height and weight were measured using standardised procedures in both studies, and body mass index (BMI, weight[kg]/height[$m^2$]) was calculated.

Total serum or plasma 25(OH)D concentration was measured using a chemiluminescence immunoassay

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**Table 1.** Description of 1395 female study participants in whom serum or plasma 25-hydroxyvitamin D (25[OH]D) concentrations were measured by two methods.

| | | Breast cancer cohort ($n$=953) | Weight loss cohort ($n$=442) |
|---|---|---|---|
| Race/ethnicity (%)* | Non-Hispanic White | 811 (85.1) | 326 (73.8) |
| | African-American | 35 (3.7) | 38 (8.6) |
| | Hispanic | 52 (5.5) | 60 (13.6) |
| | Asian | 22 (2.3) | 2 (0.5) |
| | Pacific Islander | 9 (0.9) | 2 (0.5) |
| | Native American | 1 (0.1) | 5 (1.1) |
| | Mixed | 15 (1.6) | 5 (1.1) |
| | Other | 8 (0.8) | 4 (0.9) |
| Age, years, mean (SD)* | | 51.3 (9.1) | 44.6 (10.2) |
| Body mass index (BMI, weight[kg]/height[m$^2$]), mean (SD)* | | 27.6 (6.2) | 33.9 (3.4) |
| Vitamin D dietary supplements (IU/d, mean[SD])* | | 246 (261) | 129(425) |

Age, BMI, and vitamin D supplement data shown on this table are those reported at study enrollment.

*$P$<0.01 between cohorts, $\chi^2$ test (categorical) or independent sample $t$-test (continuous).

principle with the DiaSorin LIAISON chemiluminescence procedure by a commercial laboratory (Arup Laboratories, Salt Lake City, UT). During the first incubation, 25(OH)D is dissociated from its binding protein and binds to the specific antibody on the solid phase. After 10 min the tracer (25[OH]D linked to an isoluminol derivative) is added. After a 10 min incubation, the unbound material is removed with a wash cycle. Subsequently, the starter reagents are added to initiate a flash chemiluminescence reaction. The light signal is measured by a photomultiplier as relative light units (RLU) and is inversely proportional to the concentration of 25(OH)D present in calibrators, controls or specimens. The assay range is 7.0–150 ng/mL and the lowest reportable value is 7.0 ng/mL.

We used HPLC equipment from Varian (Walnut Creek, CA), a model 410 autosampler, 325 UV/VIS dual wavelength detector, and Prostar 230 reagent pump. The ClinRep Complete 25(OH)Vitamin $D_2$/$D_3$ Kit (Iris Technologies) was used for in-house laboratory determinations. All required components for this assay were supplied or purchased separately as part of the kit.

In the HPLC method, 25(OH)$D_2$ and 25(OH)$D_3$ were separated and quantified. The method principle involves a two-step procedure. The first step is the rapid extraction of 25(OH)$D_2$ and 25(OH)$D_3$ from serum with an organic-based precipitant. Following extraction, the treated sample is centrifuged to remove protein precipitants. An aliquot from the organic phase of the treated sample is then assayed on a Varian HPLC system with Starworks software by direct injection onto a reverse phase column heated at 40°C. Separation and detection is at 264 nm, and calculation is based on peak height. One calibrator and five quality control samples are analysed with each batch of samples. Recovery is 99–104% and assay linearity is between 3.0–500 ng/mL for 25(OH)$D_2$ and 2.6–500 ng/mL for 25(OH)$D_3$. The lower limit of detection is 1.1 ng/mL and 2.6 ng/mL, respectively. To monitor the HPLC method performance, we participate in the International Vitamin D External Quality Assessment Scheme (DEQAS) proficiency survey,[1,3,18] and, more recently, the newly inaugurated National Institute of Standards and Technology (NIST) vitamin D quality assurance exercise. We routinely used one in-house serum pool and four additional purchased quality control (QC) samples covering the analytical range from 6.0 ng/mL to 150 ng/mL. The batched sample results were accepted only if these internal QC results were within two standard deviations (SD) of the assigned values.

After excluding one outlier whose blood 25(OH)D concentration exceeded 100 ng/mL (greater than 5SD from the mean) and two subjects whose concentrations of 25(OH)$D_2$ and 25(OH)$D_3$ were not measured due to unavailability of sufficient material, we examined associations between circulating 25(OH)D concentration obtained from the two laboratory methods in the combined sample of 1395 individual study participants at the time of their enrollment into their respective studies and in all available 25(OH)D measures (including follow-up measures in the second cohort), for the purpose of comparing the results from the two laboratory methods.

We characterised the two cohorts by presenting distributions (at enrollment) of race/ethnicity and supplemental intake of vitamin D, as well as mean age and BMI, all of which are possible covariates of 25(OH)D concentration. Differences between cohorts in these predictors were tested using $\chi^2$ tests or two-sample $t$-tests as appropriate.

We show Pearson correlations between the two laboratory measures of 25(OH)D using individual subjects, and also using complete data, including repeated measures. Difference scores between the two methods of measurement were computed for each of the samples, and compared using $t$-tests. Bland-Altman plots (data not shown) and scatterplots were created. Associations between covariates and 25(OH)D levels measured by each laboratory method were analysed by repeated measures mixed models stratified by laboratory method within the cohort in which subjects were measured at four time points. Finally, 25(OH)D measured by each method was modelled in a multivariate analysis of variance among all baseline samples, including as predictors, cohort, ethnicity, geographical region, season of blood collection, age at blood collection, BMI categories, supplemental intake of vitamin D, and all

**Table 2.** Comparison of two methods of measuring serum or plasma 25-hydroxyvitamin D (25[OH]D) ($n$=2612).

| | | | Coefficient of variation | | | |
|---|---|---|---|---|---|---|
| | | | Intra-assay | | Inter-assay | |
| | *n* | Mean (SD) 25(OH)D (ng/mL) | High | Low | High | Low |
| Baseline samples (correlation coefficient ρ=0.91, $P$<0.0001) | | | | | | |
| Chemiluminescence (DiaSorin) | 1395 | 23.3 (10.3) | 2.7% | 8.0% | 5.4% | 6.1% |
| HPLC | 1395 | 24.2 (9.7) | 6.9% | 7.4% | 4.7% | 6.9% |
| All samples (correlation coefficient ρ=0.93, $P$<0.0001) | | | | | | |
| Chemiluminescence (DiaSorin) | 2612 | 23.5 (10.4) | 2.7% | 8.0% | 5.4% | 6.1% |
| HPLC | 2612 | 24.5 (10.1) | 6.9% | 7.4% | 4.7% | 6.9% |

first-order interactions between cohort and other predictors. Statistical analysis was performed using SAS version 9.2 (Cary, NC).

Demographic and other characteristics at study entry for the two cohorts are shown in Table 1. In the breast cancer cohort, BMI ranged from 17.1 to 64.1 kg/m$^2$ and age ranged from 27 to 74 years. In the weight loss cohort, BMI ranged from 27.5 to 41.5 kg/m$^2$, and age ranged from 18 to 69 years. As mentioned above, one subject from the breast cancer cohort with extremely high 25(OH)D concentration was excluded from analysis, and data from that subject are not presented. Cohorts differed significantly by racial/ethnic composition, age, BMI and intake of supplemental vitamin D (all $P$<0.01).

Table 2 presents a comparison of results obtained from the two laboratory analysis methods. Mean total 25(OH)D by the chemiluminescence DiaSorin method was 23.3 ng/mL compared with 24.2 ng/mL by the HPLC method in 1395 unique individual subjects, and it was 23.5 ng/mL for DiaSorin and 24.5 ng/mL by HPLC in all available samples. The mean (SD) per sample difference between methods was 1.0 (3.9) ng/mL ($P$<0.001), but 96.8% of readings differed between the two laboratory methods by 8.0 ng/mL or less. In baseline samples the correlation coefficient between the two methods was 0.91, and in all samples the correlation coefficient between the two methods was 0.93. Among samples with detectable 25(OH)$D_2$ (9.2% of all samples), 25(OH)$D_2$ comprised an average of 35% of the total 25(OH)D. A scatterplot showing concordance of both methods is provided in Figure 1. Figure 2 shows a typical chromatogram from the HPLC method.

Regardless of the assay used, we found that race/ethnicity, obesity, use of dietary supplements containing an average of at least 200 IU per day of vitamin D, and season of blood collection were associated with 25(OH)D concentration. Table 3 shows the associations identified using the two different laboratory methods. We found that African-Americans and Hispanics had lower 25(OH)D concentrations than non-Hispanic whites. Age did not show a consistent trend for association with 25(OH)D concentration in the mixed models, and obese, but not overweight, individuals had lower levels than those of normal weight. There was a strong trend observed between intake of dietary vitamin D supplements and serum or plasma 25(OH)D concentration. Circulating 25(OH)D was lower in samples collected during the winter than in those collected in the spring, summer or autumn ($P$<0.01), and subjects at a clinical site in Oregon had lower measured circulating 25(OH)D concentrations than those in the reference category in California using the DiaSorin, but not the HPLC, method. In the multivariate regression model with interaction terms for cohort with each of age, ethnicity,

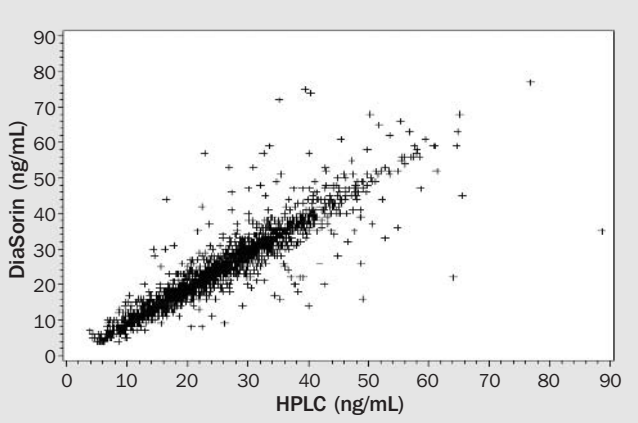


**Fig. 1.** Total serum or plasma 25-hydroxyvitamin D (ng/mL) measured by the DiaSorin method compared with concentrations computed by summing 25-hydroxyvitamin $D_2$ plus 25-hydroxyvitamin $D_3$ measured by high-performance liquid chromatography ($n$=2612).

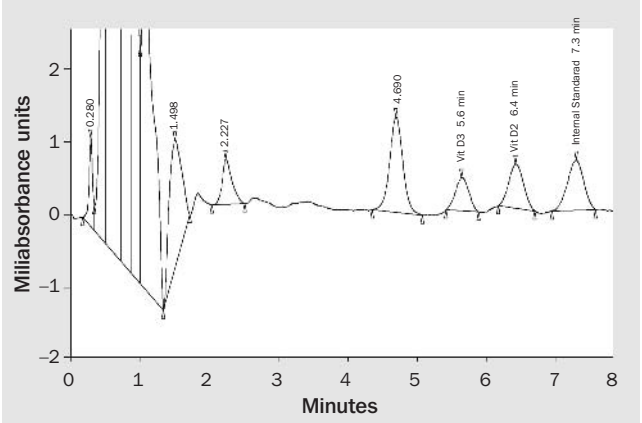


**Fig. 2.** Typical serum chromatogram obtained using the recommended high-performance liquid chromatography column and conditions at 264 nm and flow rate of 1.3 mL/min.

**Table 3.** Serum or plasma 25-hydroxyvitamin D (25[OH]D) concentrations measured by two methods ($n$=2612).

| | | *n* | Mean (SEM) measured by DiaSorin | Mean (SEM) measured by HPLC |
|---|---|---|---|---|
| Cohort | Breast cancer trial | 953 | 24.3 (0.3) | 25.3 (0.3) |
| | Weight loss trial | 1659 | 23.0 (0.3) | 24.0 (0.3) |
| Ethnicity | Non-Hispanic White | 2054 | 24.5 (0.2) | 25.6 (0.2) |
| | African-American | 175 | 16.5 (0.7)* | 16.8 (0.6)* |
| | Hispanic | 259 | 20.4 (0.6)* | 21.2 (0.6)* |
| | Asian | 30 | 18.2 (1.7) | 19.3 (1.5)* |
| | Pacific Islander | 17 | 21.9 (2.0) | 22.3 (1.9)* |
| | Native American | 21 | 29.5 (2.2) | 29.5 (2.2) |
| | Mixed | 32 | 24.7 (1.6) | 25.7 (1.6) |
| | Other | 24 | 19.6 (2.0) | 19.1 (1.6) |
| Age (years) | <40 | 572 | 23.8 (0.4) | 24.4 (0.4) |
| | 40–49 | 835 | 23.0 (0.4)* | 23.9 (0.3)* |
| | 50–59 | 902 | 23.3 (0.3) | 24.3 (0.3) |
| | 60–74 | 303 | 25.0 (0.6) | 26.7 (0.6) |
| Body mass index (kg/m$^2$) | Not overweight (<25) | 464 | 26.9 (0.5) | 27.5 (0.4) |
| | Overweight (25.0–29.9) | 788 | 25.4 (0.4) | 26.6 (0.4) |
| | Obese (>30) | 1359 | 21.2 (0.3)* | 22.2 (0.3)* |
| Vitamin D dietary supplements | None | 1392 | 21.0 (0.3) | 21.8 (0.3) |
| | 1–200 IU/d | 324 | 23.0 (0.5) | 24.2 (0.5) |
| | 20–400 IU/d | 440 | 26.0 (0.5)* | 27.0 (0.4)* |
| | IU/d | 317 | 26.9 (0.5)* | 28.5 (0.5)* |
| | >1000 IU/d | 139 | 33.7 (1.0)* | 35.2 (1.0)* |
| Geographical location | Northern California | 466 | 24.4 (0.5) | 25.1 (0.4) |
| | Southern California | 628 | 23.5 (0.4) | 24.2 (0.4) |
| | Oregon | 435 | 21.7 (0.5)* | 22.7 (0.5) |
| | Arizona | 574 | 23.6 (0.4) | 25.0 (0.4) |
| | Texas | 83 | 21.7 (1.0) | 22.7 (1.1) |
| | Minnesota | 426 | 24.6 (0.5) | 25.7 (0.5) |
| Season | Winter | 934 | 21.2 (0.3) | 22.1 (0.3) |
| | Spring | 421 | 23.4 (0.5)* | 24.5 (0.5)* |
| | Summer | 519 | 25.3 (0.4)* | 26.7 (0.4)* |
| | Autumn | 738 | 25.2 (0.4)* | 25.9 (0.4)* |

*$P$<0.05 within the weight loss cohort in a multivariate repeated measures mixed model stratified by laboratory method.
For each covariate the reference category is listed first.

BMI, supplemental intake, and season, cohort alone was not a significant predictor of 25(OH)D concentration (data not shown).

Mean total 25(OH)D concentrations obtained using the reference chemiluminescence and HPLC methods showed very high concordance, with no clinically meaningful differences. Some concern has been expressed about the ability of methods currently available to measure accurately $25(OH)D_2$ and $25(OH)D_3$ metabolites as a result of the observed variability in results across laboratories that has been observed in external quality control samples.[18–20] It has been suggested that results obtained by one method may not be assumed to be directly comparable to another method.

Also, questions about the comparability of methods and variability in results have been considered in setting and interpreting the reference ranges for 25(OH)D, and attempts have been made to develop reference intervals based on the analytical method used for quantification.[21] Lack of agreement about reference ranges may be due in part to inter-method variability.[20] The quoted reference ranges of 30–80 ng/mL and 20–70 ng/mL for 25(OH)D by the chemiluminescence and HPLC methods, respectively, differ only slightly, and it is important to recognise that factors such as geographical location, climate, seasonal and racial/ethnic factors are important components in establishing the reference range for either method.

We found that $25(OH)D_3$ was the major form of total 25(OH)D in the circulation. The $25(OH)D_2$ metabolite was detected in fewer than 10% of our samples, indicating that the majority of 25(OH)D is obtained from endogenous synthesis or vitamin $D_3$ consumed as a food fortificant or

dietary supplement. Ergocalciferol is reported to be less potent a supplement or fortificant than cholecalciferol, and cholecalciferol appears to maintain 25(OH)D concentrations up to 10-fold higher than ergocalciferol.[22] Our results showing measurable $25(OH)D_2$ in few samples are similar to the results reported by Roth *et al*.,[22] in which 277 of 291 had undetectable $25(OH)D_2$. However, as ergocalciferol is reportedly used as a dietary supplement in some countries (e.g., Australia), assay recognition and measurement of $25(OH)D_2$ is important.[20] High-performance liquid chromatography methods could also have an advantage when evaluating the effect of supplementation or fortification with ergocalciferol versus cholecalciferol.[23] The major advantage of the HPLC method is the ability to identify and quantify the vitamin $D_2$ and $D_3$ metabolites, although results from our study and from previous reports show that the vast majority of serum or plasma 25(OH)D currently is the vitamin $D_3$ metabolite.[3,4]

Consistent with current literature,[24–26] our results also demonstrate significant associations with race/ethnicity, BMI, season of sample collection, and use of dietary supplements containing vitamin D, regardless of assay used. The higher concentration of 25(OH)D in association with non-Hispanic white race/ethnicity has been suggested to relate possibly to limitations of the methodology. It has been reported that black populations have a high frequency of a genetic allele responsible for a strong affinity of the vitamin D binding protein.[27] This characteristic may prevent 25(OH)D from being released completely from its protein binding during the analytical extraction process,[1,22,28] resulting in lower measures of blood level in this population group. Lower blood levels of 25(OH)D in non-white subpopulations has been attributed most commonly to the effect of greater skin pigment, which affects endogenous synthesis of vitamin D.[29] Owing to the role of sun exposure in enabling endogenous synthesis,[29] season of measurement is another factor that would be expected to affect the concentration of 25(OH)D in the circulation, as we observed in the present study.

Lower levels of 25(OH)D in association with excess adiposity has been suggested to be due to sequestration in fat,[30] although confounding by less sun exposure in obese individuals is another possible explanation.[31] In the present study, subjects of normal body weight and BMI were excluded from the second cohort; thus the effects of cohort and BMI were likely confounded. When we tested this interaction in a multivariate model, cohort alone was not a significant predictor of circulating level of 25(OH)D. Associations with geographical location and 25(OH)D concentration are confounded by differential racial/ethnic and other characteristics, which underscores the importance of multivariate modelling in disentangling these influencing factors.

Notably, our results suggest that there is no effect of serum versus plasma aliquots as the blood compartment in which 25(OH)D is quantified. We were able to examine this issue because the two cohorts involved samples from these two compartments, and there was no cohort difference in mean concentration after controlling for age, race/ethnicity, BMI, and intake of supplemental vitamin D. This is important to future study design wherein an aliquot from one compartment may be available but an aliquot from another compartment is not. Also, the differential and relatively long storage time resulted in similar mean concentrations, which suggests that long-term storage at –80°C does not alter the analytical measurement results.

This study confirms that the HPLC method offers a useful, accurate and comparable alternative for the quantification of total 25(OH)D. Indeed, it has been argued[20] that current commercial assays should be able to recognise $25(OH)D_2$ and $25(OH)D_3$ specifically and sensitively. In a recent study, the HPLC type of analytical method was chosen as the reference method because it fulfils many criteria that define an appropriate reference method.[20] Additionally, systematic bias was detected in other evaluated methods except HPLC.[22]

It has been argued that there is a demand for clinically feasible validated commercial assays for 25(OH)D.[1] The DiaSorin chemiluminescence method, like LC-MS, is accurate and reliable. However, these other methods do require more expensive and specialised equipment and a higher level of technical expertise that ordinarily would be considered too expensive for routine analytical and most research laboratories. Although the HPLC-UV methods also require instrumentation, the equipment and software are not specialised and can be used for laboratory analysis and quantification of many other compounds.

The major criticism of the HPLC-UV method under study is that the system uses only one single point calibrator. However, our results demonstrate that the method is accurate and comparable to the reference (chemiluminescence) method. Since completion of these assays, the kit now offers multipoint calibrators. This HPLC method extraction and analytical process is simple, it does not require additional equipment or solid-phase extraction columns, and, as $25(OH)D_3$ and $25(OH)D_2$ and the internal standard are eluted from the HPLC column within 10 min, the method is suitable for large batched analytical runs, particularly in an overnight setting. The single most critical item of great importance is the addition of the internal standard. The recommendation is that the internal standard must be added cold, and our experience is that it must be kept at –80°C until immediately before use. The frozen internal standard is removed from the freezer and the container is gently inverted to mix the contents while facilitating a more rapid thaw. A repeater pipette is used to dispense the required volume in a minimum amount of time.

In conclusion, the HPLC-UV method under study, which is available and distributed as a commercial kit, offers advantages for use in research and clinical settings and produces results that are comparable to a reference chemiluminescence method. □

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# Effect of swab type on the analytical sensitivity of five point-of-care tests for group A streptococci


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The majority of point-of-care rapid antigen detection tests (RADTs) for group A β-haemolytic streptococci (GABHS) are sold by manufacturers with kit swabs provided. This is convenient for the purchaser but may have unexpected effects on kit performance.

Most clinical validation studies compare the performance of RADTs against culture and in these studies the clinical throat samples are often collected using various swab types; usually swabs provided with bacteriology transport media. It is widely assumed that swab type has no impact on RADT performance, and despite the fact that manufacturers often provide swabs with their kit that have been specifically validated for use with a RADT,[1] many clinical validation studies routinely disregard these recommendations by using a variety of swab types.[2–7]

This study presents a brief report on the impact of swab type on the analytical sensitivity of five point-of-care RADTs used for GABHS throat infection. The full study protocol is


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