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Letter to the Editor

Performance evaluation of the Vitros®3600 immunodiagnostic system for the determination of free thyroid hormones

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In serum, most thyroxine (T4) and triiodothyronine (T3) is bound to binding proteins, and only 0.02% of T4 and 0.3% of T3 are free (FT4 and FT3) (1–4). It is generally accepted that only free hormones in circulation are metabolically active. Chemiluminescence enzyme immunoassays are among the most frequently used in routine clinical laboratories for the determination of FT4 and FT3.

We undertook this study to compare the performance of two analyzers for FT3 and FT4 determination, the former ''Vitros Eci/Eciq'' and the new ''Vitros®3600'', both from Ortho-Clinical Diagnostics (Rochester, NY, USA). In both methods a direct, competitive immunoassay technique that employs a horseradish peroxidase (HRP)-labeled antibody conjugate and a ligand on the well surface is used. The bound HRP conjugate is measured by a luminescent reaction, and the signal is inversely related to the concentrations of FT4 or FT3 in serum. Both methods use the same reagent pack and the same set of calibrators. They both feature Microwell™ with enhanced chemiluminescence properties and Intellicheck® Technology that verifies analytical performance and reduces potential errors by providing real-time operator notification and tracking. In addition, Vitros®3600 features Microsensor™ Technology that automatically performs sample quality indice checks: hemolysis, icterus and turbidity can be quantified without additional reagents or sample volume, with no impact on turn around time (TAT) and costs. This capability reduces the time, labor and error potential of manual interpretation. With the Vitros®3600, the use of a disposable tip, in addition to dispensing the sample, has been extended to the dispensing of reagents: this eliminates any possible contamination of reagent/reagent, and also drastically decreases the use of wash solution and the production of waste liquid, because it is no longer required a washing protocol of fixed probes before and after contact with a reagent. The opportunity for loading and unloading of reagents with the routine in progress has also been introduced, and wash and waste liquids can be changed 'on the fly'. Up to 80 samples (+10 in emergency) can be loaded in the Vitros®3600 as compared to 60 samples in the Vitros Eci/Eciq. The high productivity of the Vitros®3600 (about twice that of Vitros Eci/Eciq) is obtained also by an increase in the size of the incubator, by doubling the number of microwell washing probes (two instead of one), by increasing the capacity of reagent supplies (31 positions instead of 20) and by integrated packages of luminol+enhancer (three instead of two).

Comparison studies were performed using samples obtained from 329 subjects (Total) grouped as follows: 118 euthyroid blood donors (Eu), 57 obese patients without thyroid diseases and free of medications affecting thyroid function (Ob), 54 hypothyroid (thyroidectomized) patients free of levo-thyroxine therapy before radioiodine treatment for thyroid cancer (Hypo), 57 hyperthyroid patients free of antithyroid medications, the day before administration of 131-iodine (Hyper), 21 patients with cardiac disease (CD), 22 uremic patients before hemodialysis (Ur 1). Samples from uremic patients were also obtained 4 h after hemodialysis (Ur 2). Assay imprecision was determined by analyzing a serum pool with 10 replicates in each run (intrassay) and in 10 different runs (interassay). The within-run imprecision values obtained with the Vitros®3600 were 1.9% (FT3) and 1.0% (FT4), while they were 3.7% (FT3) and 2.1% (FT4) with the former Eci/Eciq. The between-run imprecision values were 5.3% (FT3) and 4.0% (FT4) with the Vitros®3600, 4.1% (FT3) and 4.0% (FT4) with Eci/Eciq.

The values of FT3 and FT4 and the results of statistical analysis in various groups are reported in Tables 1 and 2. Close agreement between the two methods for measurement of both hormones was demonstrated. The slight differences observed in some patient's subgroups, mainly in the case of FT3 measurements, may not be attributed to a better performance of one assay over the other, and from a clinical point of view, they do not affect interpretation of the results. Furthermore, both methods have a similar ability to detect changes in thyroid hormone balance. Low FT3 concentra-

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Table 1 Results of FT4 and FT3 assays by Vitros Eci/Eciq and Vitros®3600.

	FT4 Vitros Eci/Eciq	FT4 Vitros®3600	FT3 Vitros Eci/Eciq	FT3 Vitros®3600
Eu				
Mean ± SD	10.0 ± 1.6	10.4 ± 1.6	3.8 ± 0.4	3.8 ± 0.5[a] (1)
Median and IQR	10.0 (7.9 to 11.9)[b]	10.2 (7.9 to 12.6)[b]	3.8 (3.3 to 4.3)[b]	3.8 (3.1 to 4.4)[b]
Ob				
Mean ± SD	10.0 ± 1.8	10.5 ± 1.9	3.9 ± 0.4	3.4 ± 0.5[c] (1)
Hypo				
Mean ± SD	0.3 ± 0.7	0.3 ± 0.6	1.1 ± 0.5	0.9 ± 0.4[d] (1)
Hyper				
Mean ± SD	14.1 ± 7.1	13.8 ± 6.4	5.2 ± 2.9	4.8 ± 2.6
CD				
Mean ± SD	12.9 ± 3.0	12.2 ± 2.7[a] (2)	2.9 ± 0.4	2.9 ± 0.4
Ur 1				
Mean ± SD	12.5 ± 3.0	12.3 ± 3.1	3.0 ± 0.7	2.8 ± 0.4[a] (3)
Median and IQR	11.5 (9.2 to 15.6)	11.4 (9.9 to 15.2)	2.9 (2.8 to 3.3)	2.8 (2.5 to 3.0)[e]
Ur 2				
Mean ± SD	21.0 ± 9.5	17.3 ± 5.7	3.6 ± 1.3	3.1 ± 0.8
Median and IQR	17.7 (14.1 to 27.2)[f]	16.2 (13.4 to 21.5)[e,f]	3.2 (2.6 to 4.2)[f]	3.1 (2.6 to 3.5)[e,f]
Total				
Mean ± SD	9.5 ± 5.6	9.7 ± 5.4	3.5 ± 1.8	3.3 ± 1.7[c] (2)

Data are presented as mean ± SD (pg/mL). For uremic patients and euthyroid controls, data are also presented as median and IQR. The Passing-Bablok non-parametric test (5) vs. Vitros Eci/Eciq (reference method) for FT4 and FT3 was used. A significant proportional bias (slope β) indicates that the disagreement between the two assays is concentration-dependent, while a significant constant bias (intercept α) means a systematic difference along the entire range of concentrations: [c](α) different from 0; [a](α) different from 0 and (β) different from 1; [d](β) different from 1. Wilcoxon test for paired samples, [e]$p < 0.01$ vs. Vitros Eci/Eciq. Kruskal-Wallis Test, [f]$p < 0.05$ vs. pre-hemodialysis; [b]$p < 0.05$ vs. Ur 1 and Ur 2 (post-hoc comparisons using the Bonferroni correction of significance were conducted when necessary). [a](1): $\alpha = -0.62$, 95% CI: −1.26 to −0.12; $\beta = 1.16$, 95% CI: 1.04 to 1.33; [a](2): $\alpha = 1.63$, 95% CI: 0.4 to 2.9; $\beta = 0.91$, 95% CI: 0.8 to 0.9; [a](3): $\alpha = 0.57$, 95% CI: 0.2 to 1.1; $\beta = 0.74$, 95% CI: 0.56 to 0.87; [c](1): $\alpha = -0.99$, 95% CI: −2.21 to −0.17; [c](2): $\alpha = -0.20$, 95% CI: −0.28 to −0.12; [d](1): $\beta = 0.81$, 95% CI: 0.71 to 0.90.

tions were found in uremic patients with no relevant differences between the two methods. Chronic kidney disease may cause an alteration in serum thyroid hormone concentrations in the absence of underlying intrinsic thyroid disorders. This non-thyroidal illness syndrome is mainly characterized by a decrease in total and unbound T3 concentrations due to impaired conversion of T4 to T3 via peripheral deiodination (6–8). The results of our study, indicating low FT3 concentrations in uremic patients, are in agreement with most reports in the literature, with no relevant differences between the two methods. After hemodialysis, an increase in FT4 values above the range of euthyroid subjects was observed. This artifactual increase was the same regardless of the equipment used, and could be explained by changes in serum proteins or fatty acids, the appearance of circulating inhibitors of plasma T4 binding activity, heparin administration and/or reduction of extracellular fluid volume after haemodialysis (9, 10). This increase was smaller for the Vitros®3600 than for the Vitros Eci/Eciq. Yet, we conclude that FT4 measurement by both methods is affected too severely to be useful for routine work in this specific clinical setting.

Table 2 The relationship between FT4 and FT3 concentrations measured with the Vitros Eci/Eciq and Vitros®3600 was tested using Pearson's correlation or Spearman's rank correlation in case of non-Gaussian distribution of measurements.

	Vitros Eci/Eciq	Vitros®3600
Eu	0.547[a]	0.541[a]
Ob	0.06	0.098
Hypo	0.630[a]	0.696[a]
Hyper	0.897[a]	0.882[a]
CD	0.222	0.207
Ur 1	0.301	–0.123
Total	0.835[a]	0.834[a]

A significant correlation was found in the total population and in the subgroups of unselected patients with normal thyroid profile, hypothyroid and hyperthyroid patients ([a]$p < 0.01$). There were no significant differences between the correlation coefficient values obtained by Vitros Eci/Eciq or Vitros®3600 in these groups. Isolated T3 thyrotoxicosis was not observed in the hyper group.

In conclusion, the results of our study indicate that: 1) from a clinical point of view, FT3 and FT4 measurements by the Vitros®3600 are comparable to those obtained by the former Vitros Eci/Eciq, with an increase in productivity and no impact on turnaround time or costs; 2) a manufacturer, using the same immunochemical reagents and the same technical components, may provide the same results for a given assay using two different analyzers; 3) the similarity in the results is strengthened by the observation that the same artifacts are observed in both cases.

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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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