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Thyroid-stimulating hormone and free thyroxine on the ADVIA Centaur immunoassay system: A multicenter assessment of analytical performance

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Objectives: We assessed the analytical performance of the TSH and FT4 assays on ADVIA Centaur in a multicenter national evaluation.

Design and methods: A precision study and a method comparison were performed. Reference values stated by the manufacturer were checked from 379 normal subjects.

Results: For TSH and FT4, the intra-assay CVs were below 2.3 and 5.2%, respectively, and the inter-assay CVs below 4.4% and 7.2%, respectively. Therefore, the precision and reproducibility were acceptable. Bland-Altman bias plots revealed good correlation and agreement with Cobas assays. TSH and FT4 data yielded reference ranges of 0.64-3.24 mIU/L and 10.5-18.9 pmol/L, respectively.

Conclusion: These assays demonstrate reliable characteristics. The reference ranges obtained can be used for interpretation of thyroid function.

Keywords:

Thyroid-stimulating hormone; Free thyroxine; Evaluation; Reference values

1. Introduction

Thyroid disorders are an important health problem and have an estimated prevalence of 13% in France [1]. Serum thyroid-stimulating hormone (TSH) measurement is the most common test used for the diagnosis and monitoring of thyroid diseases. It requires highly sensitive TSH assays with third-generation functional sensitivity (National Academy of Clinical Biochemistry, NACB, guidelines [2]). Free thyroxine (FT4) should be measured in some cases to identify and manage thyroid dysfunctions. Thus, TSH and FT4 are the most frequently encountered immunoassays in routine clinical testing. These first-line tests are the subject of international collaborative investigations aimed at studying the key performances and comparability of results between the available tests [3,4]. The standardization of TSH and thyroid hormone measurements remain a concern for the application of clinical practice guidelines.

At the laboratory-scale, performance quality should be assessed to ensure agreement with the performance announced by the manufacturer. Indeed, studies have reported discordances between the manufacturers' claims and the laboratory assessment [5,6]. Consequently, when Siemens optimized the TSH3 and FT4 assays to produce the new and improved TSH3-ultra (TSH3-UL) and FT4 assays, we assessed the key analytical performance characteristics of the new assays on the ADVIA Centaur immunoassay system in a multicenter national evaluation. In a previous study, we had assessed the functional sensitivity of the TSH3-UL assay [7]. We report here all analytical performance characteristics of this TSH assay as well as those of the FT4 assay, and the checking of the reference values stated by the manufacturer.

2. Materials and methods

This collaborative study was conducted by the working group of "Biologie Spécialisée" (Société Française de Médecine Nucléaire). It was performed in three clinical laboratories in France (Strasbourg, Rennes and Lille). The precision study was performed and the reference ranges established in Strasbourg and Rennes. The method comparison was made in Strasbourg.

2.1. ADVIA Centaur assays

TSH3-UL and FT4 assays were performed on a fully automated ADVIA Centaur analyzer (Siemens Healthcare Diagnostics, Munich, Germany). TSH3-UL and FT4 are new assays based on the electrochemiluminescence reaction of a new generation of acridinium ester. This new acridinium ester displays lower non-specific binding and exhibits enhanced light output and greater reactivity for labelling proteins and antibodies than the ester incorporated in previous TSH and FT4 assays.

The TSH3-UL assay is based upon a two-site sandwich principle and the FT4 test is a competitive immunoassay.

2.2. Analytical performance evaluation

Samples

Residual material from daily routine sampling was used. Human blood samples were collected into serum separating tubes and stored, until later use, at -20°C. This procedure has been shown to have no effect on TSH and FT4 concentrations for several years [8].

Precision study

Experiments were performed using low, medium and high levels of ligands provided by the manufacturer (ref. 9815171, 9815172 and 9815173). Aliquots of ligands were stored frozen (-

20°C) and thawed immediately before analysis. The intra-assay imprecision was determined by performing replicate measurements ($n = 20$) in a single run. The inter-assay imprecision was obtained by analyzing ligands for 30 non-consecutive days over a minimal period of 3 months with 2 or 3 reagent lots. New calibrations were performed at weekly intervals.

Method comparison

Serum samples were analyzed on the same day with the ADVIA Centaur and the Cobas e 411 (Roche Diagnostics GmbH, Mannheim, Germany) TSH and FT4 assays. The software program MedCalc version 12.2 (Mariakerke, Belgium) was used for statistical analysis, which included Deming regression analysis and Bland-Altman agreement plots.

2.3. Reference ranges

Reference ranges of TSH and FT4 were obtained from 379 healthy subjects (187 nonpregnant women and 192 men, mean age 44 years, range 15-72 years) from the western ($n = 137$), northern ($n = 111$) and eastern ($n = 131$) regions of France. Normal subjects were selected appropriately according to the NACB guidelines [2]. Enrolled individuals were undergoing no treatment, except oral contraception, showed no clinical evidence or history of thyroid diseases, as confirmed by accurate clinical examination (no visible or palpable goitre), and had no detectable levels of anti-TPO antibodies (DYNOTEST anti-TPO_n RIA, Brahms Diagnostica GmbH, Berlin, Germany or Access TPO antibody, Beckman Coulter, CA, USA). Informed consent was obtained from all subjects before testing. TSH values were logtransformed before analysis to obtain a set of normally distributed values [2]. Normal distributions of FT4 and log-transformed TSH concentrations were checked with the Chi-Square test, and the 2.5th and 97.5th percentiles then calculated using the normal distribution method (MedCalc).

3. Results and discussion

The precision studies showed that for TSH concentrations from 0.365 mIU/L to 18.43 mIU/L, the intra-assay CV ranged from 2.0% to 2.3% and the inter-assay CV from 3.4% to 4.4%. These precision results were similar to previously published data obtained with the Elecsys 2010 system [9] or the Olympus AU3000i system [10] for example, and were better than those obtained with the earlier TSH3 assay with the Centaur analyzer [11,12]. For FT4 concentrations from 8.62 pmol/L to 51.74 pmol/L, the intra-assay CV ranged from 3.0% to 5.2% and the inter-assay CV from 2.5% to 7.2%. FT4 precision performances were lower than those obtained with the Elecsys 2010 [9,13] and Olympus AU3000i systems [10] but higher than the Centaur FrT4 assay precision performance [11]. Therefore the Centaur TSH3-UL and FT4 thyroid assays showed acceptable precision performance, exceeding that achieved by the former TSH3 and FrT4 assays.

The relation between the Advia Centaur TSH3-UL and TSH cobas e 411 assays is shown in Fig. 1A and B. The Deming regression analysis (Fig. 1A) shows a slope of 0.926, and an intercept of -0.017 mIU/L. The 95% confidence interval (852 to 1.000) indicates that the slope is not statistically different from 1.0, and the 95% confidence interval of the intercept (-142 to 107) shows no statistical difference from zero. This suggests that there is no statistical difference between the two methods. The bias plot representing the relation between the difference and the mean of measurements obtained with both methods is shown in Fig. 1B. A negative bias of 7% was observed for TSH measurements obtained with the Centaur assay which yielded lower values than the cobas e 411 assay. Since the TSH3-UL

assay is more recent than the cobas assay, different International Reference Preparations (IRPs) have been used to calibrate these two TSH assays. Indeed, standardisation of the TSH3-UL method is traceable to the WHO 3rd IRP 81/565, and that of the cobas e 411 TSH assay to the WHO 2nd IRP 80/558. However, the regression analysis results and the negligible 7% negative bias revealed good correlation and agreement between the Advia Centaur TSH3-UL and the cobas e 411 TSH assays.

The relation between the FT4 Advia Centaur and the FT4 cobas e 411 assays is shown in Fig. 1C and D. The regression analysis (Fig. 1C) shows a slope of 1.052, and an intercept of 1.745 pmol/L. The 95% confidence interval of the slope (0.955 to 1.149) shows no statistical difference from 1.0. The 95% confidence interval of the intercept (0.438 to 3.051) shows a statistical difference from zero indicating that both methods differ at least by a constant amount. Bland-Altman bias plots are presented in Fig. 1D. A positive bias of 17% was observed for FT4 values obtained with the Centaur system, which yielded higher values than the cobas e 411 analyzer. Two major difficulties are encountered when measuring FT4 [14]. First, FT4 represents only a minute fraction (~0.02%) of total T4. In addition, the equilibrium between bound and free T4 must be minimally disturbed with minimal amounts of serum protein-bound T4 being displaced into the free phase by dilution of the serum when direct assay ingredients are added for FT4 measurements. FT4 results provide an estimation of the free form and are still susceptible to method-dependent artefacts. Our observation of a non negligible bias is concordant with other studies that have also reported considerable differences between FT4 assays [4,14,15,16]. Despite this fact, the agreement between the Advia Centaur and cobas e 411 FT4 assays is acceptable.

TSH values for the 379 healthy subjects were normalized using a log transformation before analysis (Fig. 2A). TSH data yielded a reference range of 0.636 - 3.24 mIU/L (95% CI: 0.598 to 0.676 - 3.05 to 3.45). Reference ranges of TSH for each site are presented in Figure 2C.

Mean TSH concentrations showed no significant difference among the western, northern and eastern regions of France (ANOVA I on log-transformed data). Nor were statistical differences observed in TSH concentrations between women and men, or between individuals aged 18-43 or 44-72 years (data stratified according to Estaquio et al. [17]).

The FT4 values were Gaussian-distributed (Fig. 2B) and the reference range was found to be 10.5 - 18.9 pmol/L (95% CI: 10.2 to 10.8 - 18.6 to 19.2) using the Advia Centaur system. No age or gender effect was observed for FT4 results. Nevertheless, mean FT4 concentrations were different between sites (ANOVA I, Newman-Keuls *post-hoc* comparison, $P < 0.001$, Fig. 2C). This difference can be attributed to the well-described west-to-east gradient in the population risk of iodine deficiency for both genders in France [18]. Lower iodine concentrations among residents living in the east compared to those living in the northern and western regions of France is associated with a higher prevalence of thyroid hyperfunction [19]. Nevertheless, mean FT4 concentrations were lower in Strasbourg indicating potential unaccounted-for confounders (iodine, hormonal and smoking status).

4. Conclusion

The new TSH and FT4 assays run on the Advia Centaur analyzer demonstrate several performance characteristics that contribute to their reliability. Good precision, reproducibility and inter-method correlation are important criteria for their use in the clinical laboratory. The reference ranges of TSH and FT4 reported here were obtained in a large population of healthy subjects are in accordance with the reference intervals stated by the manufacturer for the exploration of thyroid function.

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Captions

Figure 1. Comparison between the Advia Centaur and cobas e 411 TSH and FT4 assays. TSH (A) linear regression and (B) bias plot with a mean difference of -7% . FT4 (C) linear regression and (D) bias plot with a mean difference of 17% .

Figure 2. Relative frequencies of A) TSH (log-transformed) and B) FT4 concentrations, indicating Gaussian distributions of values, all French sites combined ($n=379$). C) Adult reference ranges of TSH and FT4, analyzed for each site.

Figure1

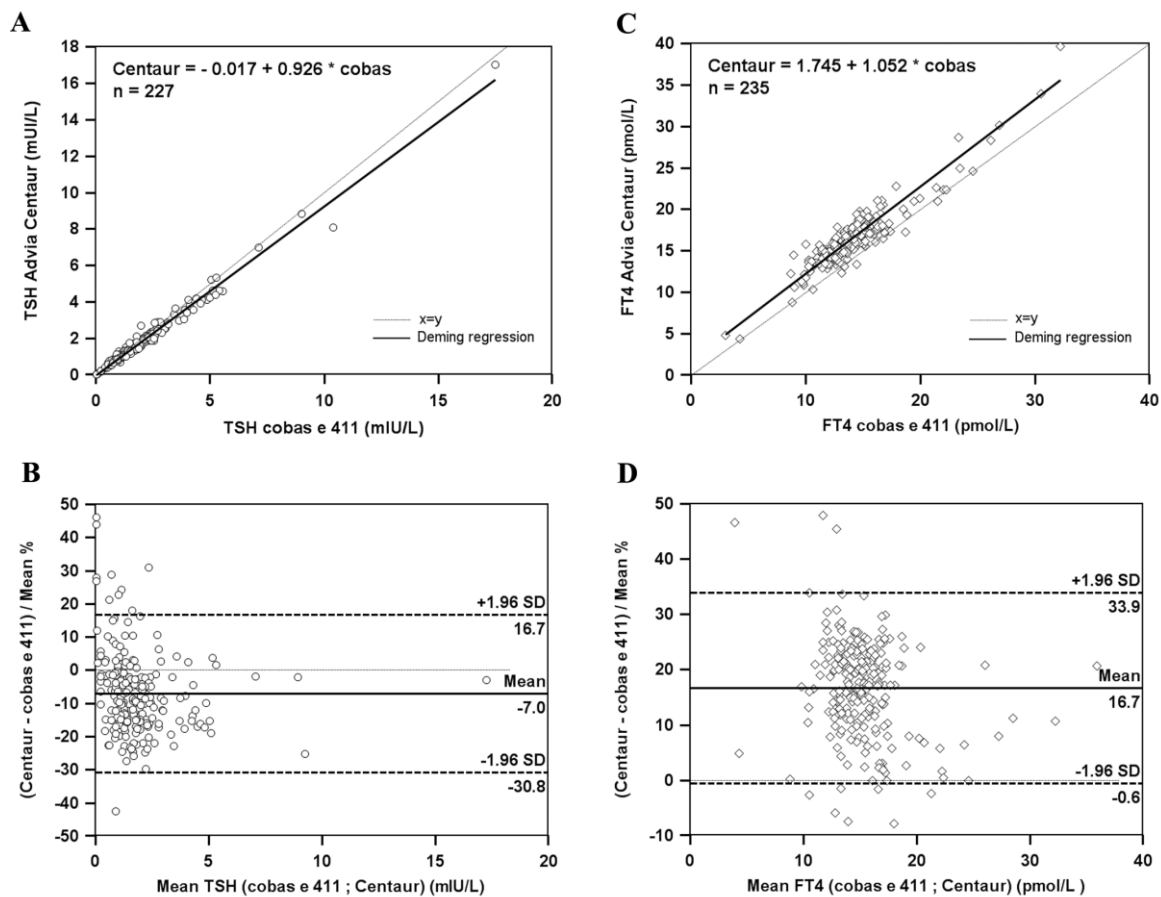


Figure2

