



Case Report

Should functional sensitivity of a new thyroid stimulating hormone immunoassay be monitored routinely? The ADVIA Centaur® TSH3-UL assay experience

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ABSTRACT

Objectives: We emphasize the importance of routine follow-up of subnormal TSH concentrations with QC materials.**Design and methods:** The functional sensitivity (FS) of the ADVIA Centaur® system TSH assay was assessed. We report the values yielded for QC materials in two clinical laboratories.**Results:** The FS was <0.02 mIU/L. The low-TSH QC (a serum pool) showed unacceptable between-lot imprecision (mean 0.0252 mIU/L, CV 22%).**Conclusion:** We do encourage healthcare laboratories to constitute low-TSH serum pools to ensure that the results they report meet 3rd-generation criteria.

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Introduction

Since 1965, thyroid-stimulating hormone (TSH) measurements in serum have gained considerable and significant sensitivity and specificity. The concept of “generations” of sensitivity was suggested by Nicoloff and Spencer [1]; each more sensitive generation representing an improvement of one log concentration over the preceding one. Currently, TSH levels are measured with ultrasensitive immunometric assays which have third-generation potential, i.e. a functional sensitivity (FS) not exceeding 0.02 mIU/L. FS is defined as the lowest concentration corresponding to a between-run coefficient of variation (CV) of 20%, according to the National Academy of Clinical Biochemistry (NACB) guidelines [2]. Third-generation performance is required for detecting subclinical hyperthyroidism [3] or adjusting more carefully suppressive doses in patients receiving exogenous thyroid hormone [4,5]. So, there is a clinical utility in measuring reliably TSH

concentrations between 0.01 and 0.1 mIU/L. This should encourage clinical chemistry laboratories to check the FS announced by the manufacturer.

In this short communication, we emphasize the need to assess the FS before its clinical use, and the importance of routine follow-up of subnormal TSH concentrations with QC materials to check that no change of reliability occurs over time. With this aim in view, we report the assessment of the FS of the ADVIA Centaur® system TSH assay (TSH3-UL, Siemens Healthcare Diagnostics), and the values yielded for QC materials over a period of 5 to 18 months in two French clinical laboratories (Nouvel Hôpital Civil, Strasbourg, and CHU Pontchaillou, Rennes, referred to as Lab 1 and Lab 2, afterwards).

Materials and methods

TSH assay

The ADVIA Centaur® system TSH assay is the most recent TSH assay produced by Siemens and is based upon a two-site sandwich principle with a chemiluminescent reaction of an acridinium ester. Improvement in the analytical signal was achieved thanks to a new generation of acridinium ester and to enhanced light output decreasing the chemiluminescent background. Thus, its FS was improved and the manufacturer claimed a value of 0.008 mIU/L.

Abbreviations: FS, functional sensitivity; TSH3-UL, Siemens third-generation ultrasensitive TSH assay; QC, quality control; TSH, thyroid-stimulating hormone; CV, coefficient of variation; NACB, National Academy of Clinical Biochemistry; WG-STFT, Working Group for Standardization of Thyroid Function Tests.

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Assessment of the FS

Human blood samples from residual sample materials of our daily routine were used to make pools which were kept frozen at -20°C until assayed.

In Lab 1, the FS of the TSH-3UL assay was established by adhering to the NACB protocol [2]. Briefly, five pools were made from samples with a TSH concentration approaching zero, and tested in 30 runs over a period of 6 weeks with 2 lots of reagents (# 215 and # 219) and 2 instrument calibrations. The FS was calculated by plotting with a nonlinear regression model the total CV as a function of the TSH concentration, according to Rawlins and Roberts [6]. Using the best fit reciprocal curve (nonlinear regression) derived on Excel® 2007 (Microsoft) and XLSTAT® 2009.5.01 (Addinsoft), the concentration corresponding to a CV of 20%, i.e. the FS, was obtained.

The FS was checked in Lab 2 with three pools. Pool 2 was made of human serum samples with a TSH concentration below 0.02 mIU/L; and Pool 1 was obtained by diluting Pool 2 to 1/2 with the multi-diluent of the assay; Pool 3 was a mixture of serum samples with TSH concentrations between 0.02 and 0.1 mIU/L diluted to 1/2 with the multi-diluent. They were tested in at least 30 runs over 6 months with 3 lots of reagents (# 215, # 219 and # 222) and 6 instrument calibrations.

Follow-up of the reliability of the TSH3-UL assay

We routinely used lots of reagents, different from either of those tested previously during the assessment study.

In Lab 1, a low TSH level QC material (# 9918) was prepared from a human serum pool and frozen in aliquots, as storage at -20°C has no effect on TSH concentrations [7]. This QC material was used in Lab 1 and 2 to make sure that low values close to the limit of FS were reliable. Lab 2 used two other human pools (# C1 and # C2). Four other commercially available QC materials consisting of lyophilized human-serum-based materials were used: Lyphochek® Anemia control (QC lot # 43170) and Lyphochek® Immunoassay Plus Controls levels 1, 2 and 3 (QC lots # 40231, # 40232, # 40233, respectively) (Bio-Rad Laboratories, Anaheim, CA). They were used over 12 months, from January 2010 to January 2011, in Lab 1. Imprecision data are expressed as mean (mIU/L) and CV (%).

Results

During the initial FS assessment in Lab 1, the mean TSH concentrations and CVs of the 5 pools of human sera were 0.006 mIU/L (CV 63%), 0.013 mIU/L (CV 27%), 0.016 mIU/L (CV 23%), 0.020 mIU/L (CV 19%), and 0.026 mIU/L (CV 12%). The FS was determined at 0.0185 mIU/L as follows: $\text{CV} = 0.005 \times \text{TSH}^{-0.94}$. In Lab 2, TSH concentrations in mIU/L of Pools 1, 2 and 3 were 0.0055 (CV 50%), 0.0120 (CV 20%) and 0.0225 (CV 15%), respectively. The FS was estimated as above at 0.0120 mIU/L. These results, albeit departing from the 0.008 mIU/L quoted by the manufacturer, were still in agreement with NACB guidelines.

The TSH3-UL assay was used in clinical routine in each lab on ADVIA Centaur® analyzers. Between-lot imprecision data of QC materials obtained in Lab 1, are summarized in Table 1.

All levels of Lyphochek® controls showed acceptable CVs (12% for Anemia and 4.2%, 4.5%, 3.7% for Lyphochek 1, 2 and 3, respectively). But the CV for QC # 9918 the serum pool (mean 0.0252 mIU/L, CV 22%) evidenced excessive between-lot variability. Fig. 1A shows the repartition of the results obtained for this human serum pool in Lab 1 over a 5-month period, with 4 different lots of reagents. After the first shift in the assay with lot # 232, the manufacturer was informed of the poor performances in May 2010 and subsequently prompted to check deionized water and assay procedures. Furthermore, the ADVIA Centaur® analyzer was

Table 1

Lab 1 results of between-lot imprecision of QC materials ranging from very low to high levels of TSH over a period of 5 to 12 months.

QC	Pool	Lyphochek			
		Anemia	Level 1	Level 2	Level 3
QC lot #	9918	43,170	40,231	40,232	40,233
n	234	109	527	435	447
Number of reagent lots	4	4	9	7	7
Mean (mIU/L)	0.0252	0.0374	0.397	5.46	31.1
CV	22%	12%	4.2%	4.5%	3.7%

completely overhauled by the manufacturer, in June and July 2010, including the entire replacement of the hydraulic circuit. In August 2010, new reagent lots # 235 and # 238 were assessed, demonstrating that the reproducibility had not been significantly improved. To rule out any technical problem with the analyzer, Lab 2 tested the reproducibility of TSH measurements on the same serum pool with reagent lots # 235-238-239 and 240. Fig. 1B summarizes imprecision of each lot for the various pools used in the two labs. The problem with lot # 238 was evidence on the same QC material, in both labs. Lab 2 continued to use low TSH level QCs (# C1 and # C2) with several other reagent lots (# 244-248-249 and # 253), and did not observe any subsequent shift in 2011. Over a period of 18 months, two shifts were clearly identified with lot # 232 and lot # 238.

Discussion

Despite the fact that the FS was determined in experimental conditions representative of those encountered in routine clinical testing, it was routinely found to vary markedly as a function of reagent lots. We observed poor between-lot reproducibility from April to September 2010, attested from 4 batches of reagents, for control serum samples with a very low TSH value. Therefore, such high result variability undeniably impacted the reliability of the FS of the ADVIA Centaur® TSH3-UL assay, which has clearly undergone some modifications between the assessment and the routine use, owing perhaps to the lack of standardization of reagent lot production in 2010. No other problem has been observed since lot # 239 used in September 2010.

Our results showed that determining the FS during an assessment study is not sufficient to ascertain that a TSH assay permanently qualifies as a third-generation assay. The Working Group for Standardization of Thyroid Function Tests (WG-STFT) emphasized the need for the harmonization of TSH immunoassays, especially for very low TSH concentrations [8]. This was also underlined recently by Owen et al. [9]. Here, we report the need to check the intra-assay harmonization at lower concentrations. To the best of our knowledge, the lowest TSH level control commercially available is Lyphochek® Anemia. But it is not low enough to supervise the stability of subnormal TSH concentrations, and problems may well go undetected, as demonstrated in this study. So we do encourage healthcare laboratories to constitute low TSH serum pools (around 0.02 mIU/L) to be used as QC material, and thus ensure that the results they report are accurate, reproducible and in keeping with third-generation criteria. The second conclusion is to recommend that the manufacturer determine the FS for every batch of reagent before marketing them, in order to improve this key criterion.

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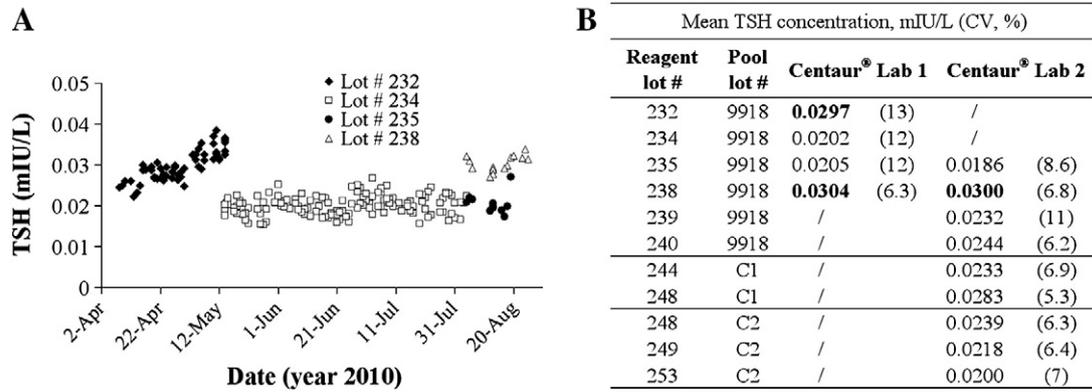


Fig. 1. A) Measurements of the low-TSH-level human serum pool # 9918 with 4 lots of reagents over 5 months in Lab 1, and B) mean TSH concentrations of very low level QC materials (human serum pools) according to reagent lot numbers in Lab 1 and 2. Reagent lot numbers # are filled in chronological order.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at [doi:10.1016/j.clinbiochem.2012.04.017](https://doi.org/10.1016/j.clinbiochem.2012.04.017).

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