Letter to the Editor

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Discrepant results in plasma, but not serum in the Beckman Coulter DxI Access HYPERsensitive hTSH 3rd generation assay affect the management of differentiated thyroid cancer and hyperthyroid patients

Keywords: hyperthyroidism; hypothyroidism; method comparison; thyroid cancer; thyroid-stimulating hormone (TSH); TSH immunoassay.

DOI 10.1515/cclm-2014-0290
Received March 14, 2014; accepted May 21, 2014; previously published online June 18, 2014

To the Editor,

Thyroid-stimulating hormone (TSH) concentrations are reduced in hyperthyroidism, as a normal physiological response during pregnancy, and in hypothyroid patients being treated with high doses of synthetic thyroid hormone like levothyroxine (LT4). About 1% of the general population suffers from hyperthyroidism [1]. At initial presentation, TSH concentrations may be subnormal in patients with subclinical hyperthyroidism and undetectable (<0.01 mU/L) in patients with overt disease. The clinical manifestations of untreated hyperthyroidism and the inappropriately high thyroid hormone actions, or thyrotoxicosis, include weight loss, osteoporosis, cardiovascular complications and even death [1]. Pregnancy has a profound impact on the thyroid gland and in thyroid function tests (TFTs), resulting in decreased TSH throughout pregnancy. The lowest TSH concentrations occur during the first trimester. It is recommended to establish trimester-specific reference intervals for TSH [2]. Reference intervals for TFTs in pregnancy appear to be method-specific [3], but if not available in the laboratory, the following are recommended for TSH: first trimester, 0.1–2.5 mU/L; second trimester, 0.2–3.0 mU/L; third trimester, 0.3–3.0 mU/L [2]. Failure to recognize thyroid dysfunction during pregnancy has adverse outcomes on the mother and the fetus. The majority of patients with hypothyroidism are treated with thyroid hormone replacement, which is monitored regularly by measuring TSH. Suppressed TSH concentrations may indicate that the thyroid hormone dose is too high. Athyrotic patients with a history of differentiated thyroid cancer (DTC) frequently undergo pharmacological suppression of TSH to prevent tumor recurrence. These patients are commonly treated with high doses of thyroid hormone, usually LT4, after thyroid resection and/or radioactive iodine ablation to suppress TSH secretion. LT4 therapy improves survival in both intermediate- and high-risk DTC patients when TSH is suppressed to subnormal ranges and below functional sensitivity of third generation assays (<0.015 mU/L), respectively [4]. Accordingly, the American Thyroid Association and the National Comprehensive Cancer Network guidelines for management of DTC patients recommend suppression therapy tailored to recurrence risk [5, 6]. TSH concentrations should be maintained between 0.1 and 0.5 mU/L for intermediate-risk patients and below functional sensitivity for those at high-risk [6].

Endocrinologists at our institution identified numerous cases of unexpectedly high concentrations of TSH that

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were inconsistent with the clinical picture in hyperthyroid, hypothyroid, and DTC patients on LT4 therapy. We also noticed that despite clinical indication, TSH concentrations were rarely suppressed below functional sensitivity (<0.015 mU/L) in patients undergoing aggressive LT4 therapy or in those with overt hyperthyroidism exhibiting both clinical symptoms and elevated thyroxine. These observations prompted our laboratory to investigate the performance of the Beckman Coulter (Brea, CA, USA) DxI Access HYPERsensitive hTSH 3rd generation assay (DxI-hTSH) in plasma of patients with low TSH.

We evaluated all DxI-hTSH results for a 10-week period from August 3 to October 14, 2011 from both hospitalized and clinic patients. During this time period, 19,100 plasma specimens collected in BD (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) Li-Heparin plasma separator tubes (PSTs) were analyzed in the Vanderbilt University Core laboratory. Physician ordered TSH specimens were typically transported, placed on the Beckman Coulter automated line, centrifuged according to BD specifications for separation of plasma [7], and TSH analyzed on the UniCel DxI 800 (Beckman Coulter) within 4 h of collection. All residual plasma specimens with TSH concentrations <0.5 mU/L were collected for re-analysis on the Cobas e411 3rd generation TSH assay (Roche Diagnostics, Indianapolis, IN, USA). When the results differed across the two platforms by ≥50%, samples were aliquoted, re-centrifuged at 8500 rpm (4400 *g*) for 3 min (STAT spin, Iris Inc) and testing was repeated on the DxI-hTSH assay. The arbitrary cut-off of ≥50% across the two TSH assay platforms was considered appropriate for an initial screen given the inherent imprecision of these assays near the functional sensitivity. Differences between the original and repeat DxI-TSH results were considered clinically significant when they changed by more than 38% or TSH values changed from normal to subnormal (<0.3 mU/L). The desirable specification for total error derived from intra- and inter-individual variation for TSH in plasma is 38.2% [8]. This cut-off would not include patients with result differences due to intrinsic analytical or biological variability. We subsequently evaluated DxI-hTSH results from all specimens collected in BD serum tubes during a 3-week period from November 3–21, 2011 and followed the same steps as with the plasma specimens above. The prevalence of DxI-hTSH results below the functional of the assay (<0.015 mU/L) was determined in serum and plasma through a laboratory information system (LIS) query during two separate 2.5-month time periods, August–October 2011 and November 2011–January 2012. Method comparison analyses were performed using EP Evaluator® 9.0 (Data Innovation LLC, South Burlington, VT, USA). All other statistical analyses were performed with GraphPad Prism 5.0 (GraphPad Software Inc, La Jolla, CA, USA). This study had institutional Review Board approval.

The DxI-hTSH and the e411-TSH assays correlated well in plasma specimens (n=40, slope=1.113, R=0.9934). However, subanalysis of concentrations <0.5 mU/L (n=10) demonstrated up to 91% bias between the two assays and a slope of 1.33. Of the 750 plasma samples with TSH <0.5 mU/L tested on the Roche e411-hTSH assay, 99 (13.2%) had concentrations ≥50% different across the two platforms. These were aliquoted, re-centrifuged and repeated on the DxI-hTSH assay. Among the 99 samples, 64 (8.5% of total results <0.5 mU/L) were deemed with clinical significant differences between the original and repeat analysis on the DxI. The mean TSH concentration in these 64 samples was 0.185 on the DxI-hTSH and 0.041 mU/L on the e411-TSH (Figure 1; p<0.0001, One-way ANOVA, Tuckey's post-test). After re-centrifugation and repeat analysis on the DxI, the mean TSH concentration decreased to 0.076 mU/L (Figure 1; p<0.0001, one-way ANOVA; Tuckey's post-test) but remained significantly higher than the e411-TSH result (p<0.0001). Re-centrifugation did not change the e411-TSH results (p>0.05, data not shown). None of the 64 samples had original DxI results below functional sensitivity and after centrifugation and re-analysis on the DxI-hTSH only 3% were <0.015 mU/L. In contrast, 42% of the samples were <0.015 mU/mL when analyzed on the e411 (p<0.0001, Fisher's exact test).

In order to determine whether contact with the gel polymer in the BD Li-Heparin PSTs caused the discrepant TSH results in plasma, a secondary analysis of patient

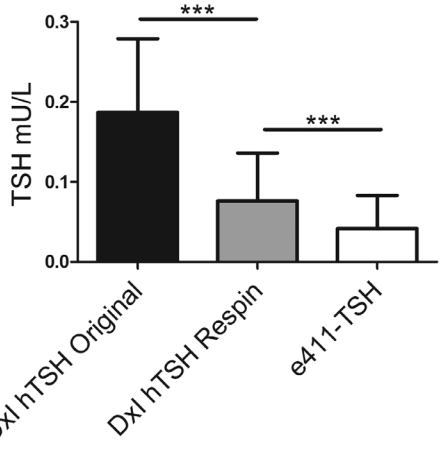

Figure 1 Performance of DxI-hTSH and e411-hTSH assays in plasma samples with original TSH results <0.5 mU/L.
Graphical representation of the average TSH concentration in 64 samples with clinical significant differences (≥38%) between the original DxI-hTSH result (black bar) and the result after respin and reanalysis in the DxI (gray bar). The samples were analyzed unspun using the e411-TSH assay (white bar). Lines represent SD and ***denote p<0.0001.

samples with prolonged storage in their primary PST tubes was carried for 8 days. Samples with TSH <0.5 mU/L were analyzed on the DxI-hTSH and e411-TSH assays. Thirty-one samples, which had concentration differences of ≥50% between the assays, were stored on the gel for an average of 24 h (range: 15–68 h) at 4 °C before repeating the TSH analysis on the DxI. After repeat measurement, specimens were aliquoted into a secondary tube, re-centrifuged and analyzed a third time using the DxI-hTSH assay. No significant difference was found in mean TSH concentrations between the original TSH result and the results after storage on the gel for 24 h (Figure 2; p>0.05, One-way ANOVA, Tuckey's post-test). A significant difference was found between the recentrifuged results and both the original concentrations and the results after the samples were stored on the gel (p<0.0001, One-way ANOVA, Tuckey's post-test). We concluded that prolonged contact with the gel polymer did not affect the results. Discrepancies were only reduced with re-centrifugation.

Since false elevations of TSH were previously observed in plasma but not in serum samples [9], we suspected that the discrepancies would be reduced by switching the TSH specimen type to serum (on November 3, 2011). Matched plasma and serum specimens correlated well on the DxI-hTSH assay (n=20, slope=1.03, R=0.9996). Among 352 serum specimens with TSH results <0.5 mU/L, only 13 had a ≥50% difference between the DxI-hTSH and e411-TSH. After recentrifugation and repeat analysis on the DxI-hTSH assay just three (0.85% of all serum samples <0.5 mU/L) were clinically significant, compared to 8.5% in plasma (p<0.0001, Fisher's exact test). The prevalence of DxI-hTSH results below the functional sensitivity of the assay was determined in serum and plasma. In the time period covered by the LIS query, 19,175 plasma and 15,554 serum specimens were analyzed in our laboratory (Table 1). The rate of TSH suppression was significantly different (p<0.0001, χ^2-test) in serum and plasma. In our institution, only 18 (0.09%) of plasma DxI-hTSH results were <0.015 mU/L, compared to 76 (0.49%) observed after switching to serum (Table 1), which is consistent with the prevalence at other institutions (personal communications).

Here, we report an apparent interference present in plasma specimens collected in BD Li-Heparin PSTs that causes elevated results on the DxI-hTSH, but not the e411-TSH assay. This interference is partially reduced by re-centrifugation of the plasma. A similar interference has been reported for other DxI assays [10]. As in our study, re-centrifugation partially reduces the interference [9] but switching to serum virtually eliminates discrepancies. After the switch, <1% of TSH results were discrepant. Possible sources of interference that could explain the discrepancies, such as new formulations, lots, and the effect of traveling in the automated line, were ruled out during our investigation period. Although not confirmed, one theory is that the false elevations in plasma are caused by fibrin and/or microclots present in the plasma due to incomplete mixing of the PSTs. BD directs that Li-Heparin PSTs be mixed 8–10 times after collection to ensure proper anticoagulation [7]. The issue was communicated and the data was presented to the assay manufacturer.

Unlike previous studies, we investigated the clinical impact of falsely elevated TSH results, particularly at the low end of the assay, when TSH is analyzed in plasma using the DxI-hTSH assay. The diagnoses of the 64 patients with discrepant TSH results were: post-thyroidectomy with LT4 therapy (n=22, 34%), hypothyroidism and LT4 replacement therapy (n=18, 28%), hyperthyroidism (n=14, 22%), pregnancy (n=5, 8%), pharmacological suppression therapy (n=2, 3%), and other (n=3, 5%). From among 11 patients with TSH concentrations within the normal reference range (0.3–5.0 mU/mL) in the DxI-hTSH assay, eight went to subnormal and three were suppressed below functional sensitivity of the Roche e411-TSH assay.

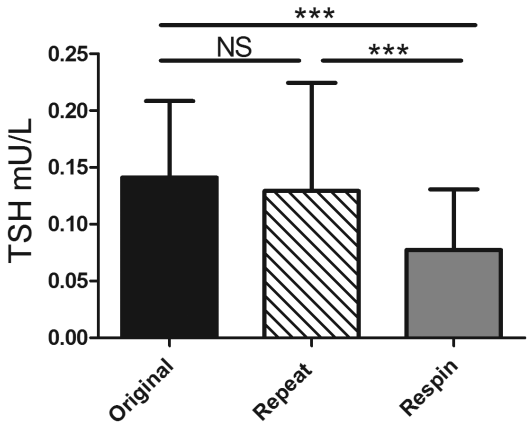

Figure 2 DXI-hTSH concentrations after prolonged contact with gel polymer in BD Li-Heparin PSTs.
Average concentration of TSH in 31 plasma samples with original DxI-hTSH results <0.5 mU/L (black bar), repeat measurement after being contact with the gel barrier for an average of 24 h at 4 °C (striped bar) and subsequent separation and respin (gray bar). Lines represent SD. NS and *** denote p>0.05 (not significant) and <0.0001, respectively.

Table 1 Suppressed TSH results on the Beckman DxI-hTSH assay before and after change in specimen type.

Specimen type	Ordered TSH, n	TSH <0.015 mU/L n, %	p-Value
Plasma	19,175	18 (0.09%)	<0.0001
Serum	15,554	76 (0.49%)	

Among the hypothyroid patients, 10 had a change in LT4 dosage. Falsely elevated TSH results lead to delays in dosage adjustments and/or prolongation of clinical symptoms of hyperthyroidism. Originally the TSH concentrations of the five pregnant patients were within the normal TSH reference interval for the first trimester of pregnancy on the DxI assay (0.04–2.98 mU/L) [3] but two were subnormal after recentrifugation and repeat on the DxI. On the Roche assay, three of the pregnant patients results were subnormal based on its reference interval (0.03–3.4 mU/L) [3], two of which were suppressed below functional sensitivity. In addition, one of the patients without previous history of thyroid disease had experienced severe weight loss but the original DxI-hTSH result suggested normal TFTs. This resulted in a missed diagnosis of hyperthyroidism. Finally, when plasma specimens were used, the DxI-hTSH assay reported incorrect values in patients that were expected to have suppressed TSH. Ten of the 22 patients on TSH suppression therapy showed results below functional sensitivity on the e411-TSH assay, but none were suppressed on the DxI-hTSH assay before or after recentrifugation, including two high-risk DTC patients with TSH suppression target below functional sensitivity. Erroneous TSH elevations in our DTC patients prompted unnecessary treatment changes.

TSH results obtained in BD Li-Heparin PST specimens analyzed using the DxI-hTSH assay should be interpreted with caution in populations with low TSH concentrations, such as pregnant, hyperthyroid, and hypothyroid and DTC patients being treated with LT4. Inaccurate TSH measurements at the low end may result in missed diagnosis of hyperthyroidism, mislead physicians to order unnecessary and costly follow-up testing, and/or prompt inappropriate treatment decisions for pregnant, hypothyroid or cancer patients.

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 funding: None declared.
Employment or leadership: None declared.
Honorarium: None declared.

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