BRIEF REPORT

Biotin interference in TSH, FT4, and FT3 assays based on the LOCI technology: Identifying interference by dilution

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Abstract

Background: Although biotin interferences in TSH, FT3, FT4, and other biotinylated antibody-based assays manufactured by Roche Diagnostics have been well studied, there are relatively few reports on biotin interference in biotin-based assays manufactured by other companies. We investigated biotin interferences in TSH, FT4, and FT3 assays based on the LOCI (luminescent oxygen channeling assay) technology using the Dimension Vista 1500 analyzer (Siemens).

Methods: We prepared four serum pools using leftover specimens. Three serum pools were prepared initially for the original study but the 4th pool was prepared three months later. The aliquots of serum pool one and two were supplemented with various amounts of biotin (50–1200 ng/mL) followed by determination of TSH, FT4, and FT3 concentrations. The aliquots of third pool were also supplemented with biotin to investigate whether 1:3 dilution could identify biotin interference. Aliquots of serum pool four were supplemented with biotin in order to study reproducibility of our original data.

Results: We observed significantly elevated FT3 levels at biotin concentration of 100 ng/mL. In contrast, FT4 levels were falsely elevated but TSH levels were falsely decreased at a biotin level of 500 ng/mL. We also observed nonlinearity in dilution experiment.

Conclusions: We conclude that FT3 assay is most susceptible to biotin interference (threshold: 100 ng/mL) while the FT4 and TSH assays are less affected (threshold: 500 ng/mL). In addition, we also observed nonlinearity upon 1:3 dilution, which may indicate biotin interference (or interference from other compounds).

KEYWORDS

biotin, FT3, FT4, LOCI technology, TSH

1 | INTRODUCTION

Biotin (vitamin B7) previously known as vitamin H is an essential vitamin which acts as a coenzyme for carboxylase reactions in gluconeogenesis, fatty acid synthesis, and amino acid catabolism. Therefore, biotin is essential for life. Biotin also has a favorable safety profile because it is a water-soluble vitamin. The daily recommended allowance of biotin is only 30 microgram but taking biotin supplement in dosage of 5–10 mg/d is popular for healthy hair, nail,

and skin. As a result, approximately 15%–20% Americans regularly takes biotin supplement. Moreover, patients with congenital biotin deficiency may receive biotin supplementation of 40–100 mg/d as a part of therapy. In addition, very high dosage of biotin (300 mg or higher daily) may be used for treating multiple sclerosis and demyelinating pathology.¹

In general, serum biotin levels in individuals taking no biotin supplement vary from 0.4 to 1.2 ng/mL.² Grimsey et al³ studied serum biotin levels after volunteers consumed 5, 10, or 20 mg

biotin and reported that serum biotin levels after taking 5 mg supplement/d varied from 10 to 73 ng/mL. However, after taking 20 mg biotin per day the observed serum biotin levels ranged from 80 to 355 ng/mL. As expected, serum biotin levels would be much higher after taking biotin in pharmaceutical dosages such as 100 mg or 300 mg daily. The plasma biotin concentrations varied from 700 to 900 ng/mL when subjects ingested a single dose of 300 mg biotin supplement, and blood was drawn 2 hour after taking the supplement.⁴

Many automated immunoassays incorporate biotinylated antibodies and streptavidin-coated magnetic beads as a means of immobilizing antigen-antibody complexes to the solid phase. This design offers many advantages including signal amplification and increased sensitivity. However, biotin in high concentrations may significantly interfere with such immunoassays. In general, biotin demonstrates positive interferences using competitive immunoassays but negative interferences using sandwich format. Although individuals taking multivitamins or biotin supplement up to 2.5 mg/d show no interference on biotin-based assays, people taking at least 5 mg biotin/d may show some effects on few biotin-based immunoassays. As expected, with ingestion of higher doses more biotin-based assays are affected.⁵

Interference of biotin in immunoassays is dose-dependent. Most reports deal with the wrong diagnosis of hyperthyroidism due to elevated FT4 (free thyroxine), FT3 (free triiodothyronine) but falsely lower TSH (thyroid-stimulating hormone) value due to biotin interference using immunoassays marketed by Roche or Beckman.⁶⁻⁹ However, the TSH, FT3, and FT4 assays based on the LOCI technology and marketed by Siemens also utilize biotinylated antibody. We studied biotin interference in these assays and also the possibility of identifying such interference by dilution of specimens. We selected TSH, FT3, and FT4 assays for this study because these are most commonly ordered thyroid function tests in our hospital. Moreover, FT3 and FT4 are active forms of thyroid hormones but total T3 represents mostly protein-bound inactive form. Here, we report our findings.

2 | MATERIALS AND METHODS

Biotin was purchased from Sigma Chemical Company (St. Louis, MO), and a standard stock solution of biotin (0.1 mg/mL) was prepared in phosphate buffer saline. Then, this stock solution was diluted to prepare two working solutions (1 µg/mL and 10 µg/mL). The TSH, FT3, and FT4 assays based on the LOCI (luminescent oxygen channeling assay) technology were purchased from Siemens, and assays were run on Dimension Vista 1500 analyzer also obtained from Siemens (Newark, DE). The TSH assay is a homogeneous sandwich chemiluminescent assay based on the LOCI technology and is 3rd generation TSH assay with analytical measurement range of 0.005-100 µIU/mL. However, both FT4 (analytical measurement range: 0.1-8 ng/dL) and FT3 (analytical measurement: 0.50-30 pg/mL) assays are competitive immunoassays.

Four serum pools were prepared by combining specimens where physicians ordered thyroid panel (TSH, FT4, and FT3). Two serum pools were prepared using specimens with high TSH concentrations while two pools were prepared from patients with normal TSH concentrations. Serum pool four containing normal TSH concentration was prepared 3 months after initial study in order to investigate reproducibility of our original data using assay kits of different lot numbers. Serum pools were prepared by combining deidentified leftover specimens from patients after performing all tests ordered by physicians and saving these specimens at 4°C for 1 week as per IRB guidelines. These specimens would have otherwise been discarded.

In the first set of experiments, aliquots of serum pool one (normal TSH) and serum pool two (elevated TSH) were supplemented with various amounts of biotin (50-1200 ng/mL) followed by determination of TSH, FT3, and FT4 using the Dimension Vista 1500 analyzer. Each measurement was performed in duplicate, and the mean value was calculated. In the second set of experiments, aliquots of serum pool three (elevated TSH) were supplemented with biotin (100-1200 ng/mL), and again, TSH, FT4, and FT3 values were measured using the Dimension Vista 1500 analyzer. However, we also determined TSH, FT3, and FT4 concentrations in these aliquots after 1:3 dilution to investigate possible nonlinearity due to biotin interference. Specimens were diluted using dilution solution provided by the Siemens for dilution of TSH specimens. Again, all measurements were performed in duplicate. We also programmed the instrument to take into account the dilution and to calculate expected values.

Three months after initial study, we prepared serum pool four and supplemented aliquots of the serum pool with various amounts of biotin (50-1200 ng/mL) followed by determination of TSH, FT3, and FT4 using the Dimension Vista 1500 analyzer. However, all reagents used for measuring TSH, FT3, and FT4 had different lot numbers than reagent kits that we used for the initial studies. The objective was to verify reproducibility of our original results.

3 | RESULTS

We considered more than 10% difference as analytically significant but 20% difference as clinically significant as proposed by the College of American Pathologists (CAP). We observed more than 10% difference in the FT3 concentration at serum biotin level of only 50 ng/mL while significant clinical interference (more than 20% difference) was observed at serum biotin level of 100 ng/mL. For example, in serum pool one, FT3 value was increased from 2.42 pg/mL to 3.02 pg/mL in the presence of 100 ng/mL biotin, a 25% increase in analyte concentration. In contrast, the FT4 assay showed clinically significant difference in serum biotin concentration of 500 ng/mL. The TSH assay was also impacted at 500 ng/mL serum biotin concentration. For example, in serum pool two, TSH value was reduced from 10.70 µIU/mL to 7.93 µIU/mL in the presence of 500 ng/mL of serum biotin concentration, a 26%

TABLE 1 Biotin interference in thyroid-stimulating hormone (TSH), free triiodothyronine (FT3), and free thyroxine (FT4) assays using Vista 1500 analyzer

Specimen	TSH μ IU/mL Mean of duplicate	FT3 pg/mL Mean of duplicate	FT4 ng/dL Mean of duplicate
Serum pool one	1.85	2.42	1.46
+50 ng/mL biotin	1.85	2.71	1.47
+100 ng/mL biotin	1.76	3.02	1.49
+250 ng/mL biotin	1.66	4.60	1.51
+500 ng/mL biotin	1.29	20.71	1.77
+750 ng/mL biotin	0.09	>30	2.13
+1000 ng/mL biotin	0.022	>30	>8.0
+1200 ng/mL	0.015	>30	>8.0
Serum pool two	10.70	1.78	1.10
+50 ng/mL biotin	10.60	2.15	1.11
+100 ng/mL biotin	10.35	2.47	1.17
+250 ng/mL biotin	9.66	4.58	1.17
+500 ng/mL biotin	7.93	25.78	1.37
+750 ng/mL biotin	1.10	>30	3.48
+1000 ng/mL biotin	0.119	>30	>8.0
+1200 ng/mL biotin	0.058	>30	>8.0

reduction in TSH level (Table 1). In order to verify reproducibility of our initial data, we prepared serum pool four 3 months after the initial study and repeated our original experiments as described for serum pool one and two. We observed similar results which are represented graphically in Figure 1.

We studied possible nonlinearity of dilution to identify biotin interference and obtained best result using 1:3 dilution. We also diluted original serum pool (pool three) to investigate potential matrix effect during dilution. We only observed significant matrix effect on FT3 level as the original value in serum pool three was increased from 2.12 pg/mL to 2.44 pg/mL, a 13% positive bias following dilution. However, with 1:4 dilution matrix bias was more significant (data not shown).

We observed nonlinearity during dilution but such dilution did not eliminate biotin interference due to vulnerability of the FT3 assay to biotin interference. For example, in the presence of 500 ng/mL of biotin, TSH value was reduced from 9.08 μ IU/mL to 7.12 μ IU/mL (21.5% reduction) but calculated TSH value using 1:3 dilution was 8.42 μ IU/mL, which represented only 7.2% reduction, a not clinically significant difference. However, for FT3, the calculated value in the presence of 500 ng/mL after 1:3 dilution was 6.12 pg/mL which was 2.5-fold higher than FT3 value of 2.12 pg/mL in the original pool (Table 2). Nevertheless, this calculated FT3 value was significantly lower than 16.33 pg/mL, a value observed without any dilution, indicating that nonlinearity during dilution is an indicator of biotin interference (or any other interference).

FIGURE 1 Effect of various concentrations of biotin on the LOCI assays for thyroid-stimulating hormone (TSH), free triiodothyronine (FT3), and free thyroxine (FT4). Relative units (TSH: μ IU/mL, FT3: pg/mL and FT4 ng/dL). FT3 values exceed 30 pg/mL (upper limit of the assay) at biotin concentrations of 1000 and 1200 ng/mL but in the graph highest values were shown as 30 pg/mL

TABLE 2 Effect of 1:3 dilution on thyroid-stimulating hormone (TSH), free triiodothyronine (FT3), and free thyroxine (FT4) measurement in the presence of biotin using Vista 1500 analyzer

Specimen	TSH ^a μIU/mL	TSH ^a (1:3 calculated) μIU/mL	FT3 ^a pg/mL	FT3 ^a (1:3 calculated) pg/mL	FT4 ^a ng/dL	FT4 ^a (1:3 calculated) ng/dL
Serum pool three	9.08	8.93	2.12	2.44	1.12	1.15
+100 ng/mL biotin	8.68	8.83	2.91	2.64	1.16	1.29
+250 ng/mL biotin	8.42	8.71	4.53	3.97	1.23	1.40
+500 ng/mL biotin	7.12	8.42	16.33	6.12	1.44	1.53
+1000 ng/mL biotin	1.34	6.88	>30	12.57	4.28	1.66
+1200 ng/mL biotin	0.273	6.57	>30	16.48	>8.0	1.70

^aAll values are mean of duplicate measurements.

4 | DISCUSSION

We selected biotin concentrations that represent serum biotin levels after taking biotin supplement as well as pharmaceutical dosage of biotin (300 mg/d). The FDA report indicates that serum biotin level can reach up to 1200 ng/mL,¹⁰ which is also the highest biotin level chosen for our study. Trambus et al¹¹ reported that some Roche assays, such as troponin T, TSH, and antithyroid antibodies, were extremely sensitive to the lower concentrations of biotin (15.6 and 31.3 ng/mL), whereas the majority of assays were relatively resistant. However, at concentrations ≥ 500 ng/mL, all assays showed significant interference from biotin. Ali et al¹² studied biotin interference in vitro in various biotin-based immunoassays on Vitros 5600 analyzer and observed that biotin at 6.25 ng/mL affected TSH assay causing more than 20% negative bias. Serum biotin level after taking 5 mg supplement/day varied from 10 to 73 ng/mL.³ Therefore, even taking 5 mg biotin, TSH measurement using Vitros 5600 analyzer may be affected.

Li et al investigated the effect of biotin on performance of hormone and nonhormone assays using six healthy adults (two women and four men; mean age: 38 years) who ingested 10 mg biotin for 7 days. The authors reported that biotin ingestion was associated with falsely decreased TSH level but falsely increased FT3 and FT4 levels using Roche assays. The interference in TSH measurement was more significant using Vitros 5600 analyzer (Ortho Clinical Diagnosis), but the TSH assay on the Dimension Vista analyzer was not affected. However, the FT3 assay on the Dimension Vista analyzer was affected.¹³ In another study, the authors resolved erroneous TSH, FT3, and FT4 values obtained by Roche assays by using ADVIA Centaur assays (Siemens Diagnostics) which are not biotin-based.¹⁴

Our study indicates that the FT3 assay based on the LOCI technology is most vulnerable to biotin interference where interference was observed at serum biotin concentration of only 100 ng/mL. This concentration may be achieved in people taking 10 mg biotin supplement per day. However, TSH and FT4 assays based also on the LOCI technology are less susceptible to biotin interference. Based on in vivo study, in order to reach serum biotin concentration 500 ng/mL, a person needs to take at least 100 mg of biotin.⁴ Recent studies indicate that high dose biotin

(300 mg/d) therapy may be effective in treating symptoms of multiple sclerosis because data from clinical trials are encouraging.¹⁵⁻¹⁷ Moreover, patient taking 300 mg biotin supplement is also encountered in clinical practice.¹⁸ Therefore, clinicians must be aware of biotin interferences in TSH, FT3, and FT4 assays based on the LOCI technology.

Avoiding biotin interference is important as we have shown that discordant values of thyroid function tests between original specimen and values obtained after 1:3 dilution (nonlinearity during dilution) may be an indication of biotin interference in a patient taking biotin supplement. Another approach to overcome biotin interference is to wait sufficient time for biotin to be eliminated from the body. Roche Diagnostics recommends waiting for 8 hour before blood collection for patients taking 5 mg of biotin supplement. The American Thyroid Association Guidelines recommend discontinuation of biotin at least 2 days before conducting thyroid function tests.¹⁹ Although biotin interference in biotin-based immunoassays can be completely eliminated by the pretreatment of specimen with streptavidin-coated microparticle to deplete biotin present in specimens, this is a manual, time-consuming process.²⁰ However, best approach to eliminate biotin interference is to use an alternative assay platform that does not use biotin in assay design. Currently, most assays marketed by Abbott Laboratories do not use biotin in assay design and only four assays that are biotin-based showed no biotin interference. DiaSorin Inc. also has no assay that is biotin-based.²¹

We conclude that the FT3 assay on the Dimension Vista analyzer is vulnerable to biotin interference (threshold: 100 ng/mL) but TSH and FT4 assays on the same analyzer are affected at serum biotin concentration of 500 ng/mL or higher.

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