



## Comparison of Biotin Interference in Second- and Third-Generation Roche Free Thyroxine Immunoassays

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Dear Editor,

Immunoassays (IAs) based on streptavidin-biotin binding are widely used in clinical laboratory testing owing to the high affinity and stable interaction between streptavidin and biotin (vitamin B7) and the development of various biotinylation methods [1]. Biotin is a water-soluble B-complex vitamin and a coenzyme responsible for carboxyl transfer in essential carboxylases. Circulating serum concentrations of biotin in the general population typically range from 0.1 ng/mL to 0.8 ng/mL [2]. Biotin is rapidly absorbed, reaches peak plasma concentrations within 1–2 hours, and has an effective serum half-life of 15 hours [3]. Oral administration of biotin doses of 10 mg resulted in peak plasma concentrations ranging from 53 ng/mL to 141 ng/mL [3, 4]. Considering a maximum dosage of 10 mg once a day (q.d.; 10 mg is >300-fold the adequate daily intake) via over the counter (OTC) biotin products, the serum biotin concentration would drop below the *in vitro* interference threshold of  $\leq 30$  ng/mL after eight hours [3]. A high biotin concentration in the blood can interfere with IAs based on streptavidin-biotin binding and is known to cause false high results in competitive IAs and false low results in sandwich IAs [1]. Although the recommended daily intake of biotin is low (30  $\mu$ g/day), the use of high-dose biotin supplements (up to 10 mg), which are available OTC, has increased in recent years due to unfounded claims that biotin exerts beneficial effects on hair, nails, and skin [5]. According to

one survey, 7.7% (95% confidence interval [CI], 6.6–8.9%) of an outpatient population (N=1,944) indicated biotin use; measuring biotin in plasma samples from emergency department patients (N=1,442) showed that 7.4% (95% CI, 6.2–8.9%) had a concentration at or above the lowest known threshold (10 ng/mL) for biotin interference in a Roche Diagnostics IA (Indianapolis, IN, USA) [6].

Because the Roche Elecsys assay is based on streptavidin-biotin binding, it may yield incorrect results for samples with high biotin concentration. Measurement of free thyroxine (FT4) using a competitive IA from Roche Diagnostics revealed that high biotin concentrations led to false high results in the Elecsys FT4 II assay. Recently, Roche Diagnostics released the Elecsys FT4 III assay, which is claimed to perform better in the presence of biotin interference. This is the first study to evaluate biotin interference in FT4 assays by comparing the newly released Elecsys FT4 III assay with the previous Elecsys FT4 II assay using a Cobas e602 Analyzer (Roche Diagnostics). The Institutional Review Board of Korea University Anam Hospital, Seoul, Korea, approved this study and waived the need for informed consent (No. 2019AN0400).

Pooled serum samples from 10 subjects with three targeted FT4 concentrations (high: >3 ng/dL; normal: 0.89–1.8 ng/dL; low: <0.8 ng/dL) were prepared by mixing three or four serum samples remaining after routine clinical assays of T3 and FT4.

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**Table 1.** Comparison of relative bias in biotin concentration between Elecsys FT4 II and Elecsys FT4 III

Estimated biotin concentration (ng/mL)	Elecsys FT4 II						Elecsys FT4 III					
	Low FT4		Normal FT4		High FT4		Low FT4		Normal FT4		High FT4	
	FT4 (ng/dL)	Relative bias (%)	FT4 (ng/dL)	Relative bias (%)	FT4 (ng/dL)	Relative bias (%)	FT4 (ng/dL)	Relative bias (%)	FT4 (ng/dL)	Relative bias (%)	FT4 (ng/dL)	Relative bias (%)
<b>0</b>	0.76	0.0	1.34	0.0	5.05	0.0	0.72	0.0	1.27	0.0	4.62	0.0
<b>12.5</b>	0.81	6.0	1.38	3.0	5.24	3.8	0.68	-5.6	1.21	-4.7	4.40	-4.8
<b>25</b>	0.82	6.7	1.42	6.0	5.43	7.4	0.68	-5.0	1.19	-5.9	4.44	-3.9
<b>50</b>	0.86	<b>12.2</b>	1.47	9.7	5.79	<b>14.6</b>	0.66	-8.4	1.17	-7.9	4.42	-4.4
<b>100</b>	0.93	<b>21.6</b>	1.60	<b>19.9</b>	6.46	<b>27.8</b>	0.67	-6.8	1.17	-7.5	4.69	1.5
<b>200</b>	1.08	<b>40.6</b>	1.82	<b>36.0</b>	>7.77	> <b>53.9</b>	0.74	3.2	1.34	5.9	6.43	<b>39.2</b>
<b>400</b>	2.39	<b>212.0</b>	3.91	<b>192.5</b>	>7.77	> <b>53.9</b>	0.95	<b>32.5</b>	1.62	<b>27.7</b>	6.65	<b>43.8</b>

Relative biases >10% are in bold.

Abbreviation: FT4, free thyroxine.

Biotin was purchased from Sigma-Aldrich (St. Louis, MO, USA) to produce a stock solution (4,000 ng/mL) for spiking the pooled serum samples (4 mL for each level). High-, normal-, and low-concentration samples were mixed with the biotin stock solution (9:1, high-biotin pool). For each FT4 range sample, the zero- and high-biotin serum pools were serially diluted to produce biotin-spiked samples (biotin concentration, 12.5, 25, 50, 100, 200, and 400 ng/mL). For both FT4 assays, all samples were analyzed in duplicate using a Roche Cobas e602 Analyzer. All experiments were completed in less than eight hrs without storage. Relative bias was calculated as follows:

$$\text{bias (\%)} = \frac{([\text{biotin-treated analyte}] - [\text{untreated analyte}])}{[\text{untreated analyte}]}$$

To determine the biotin interference concentration, a clinically significant bias was defined as a 10% change in the result. For the Elecsys FT4 II assay, a biotin concentration  $\geq 50$  ng/mL caused a positive bias in low- and high-concentration pooled samples, while the normal-concentration samples showed >10% bias starting at 100 ng/mL of biotin. For Elecsys FT4 III, low- and normal-concentration pooled samples showed a positive bias of 32.5% and 27.7% at 400 ng/mL, respectively. For the high-concentration samples, a positive bias of 39.2% and 43.8% was observed at biotin concentrations of 200 and 400 ng/mL, respectively. The Elecsys FT4 II data sheet indicates a biotin interference threshold of  $\leq 25$  ng/mL. Thus, our findings showed that Elecsys FT4 III is less sensitive to biotin interference and corroborate its revised biotin interference threshold ( $\leq 100$  ng/mL) (Table 1).

In conclusion, Elecsys FT4 III showed decreased biotin interference compared with the previous version of the assay, but an appreciable number of false results were noted. Therefore, labo-

ratories, clinicians, and patients should be aware of the potential for biotin interference in IAs based on streptavidin-biotin binding and take measures to minimize the risk of false results. If biotin interference is suspected, additional methods can be used to reduce the risk of false results, including analysis with an alternative assay that is not based on biotin, use of a sample diluent, or removal of excess biotin using streptavidin-coated beads [7, 8]. Furthermore, based on population pharmacokinetics of exogenous biotin, we recommend that samples from patients receiving therapy with high doses of biotin (i.e., >5 mg/day) should be assayed at least eight hrs following final biotin administration [3, 4, 8].

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## AUTHOR CONTRIBUTIONS

SGY designed the study. JC and SGY contributed to the implementation of the research, analysis of the results, and writing of the manuscript.

## CONFLICTS OF INTEREST

None declared.

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