Letter to the Editor

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Interferences in free thyroxine concentration using the Roche analytical platform: improvement of the third generation?

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To the Editor,

Automated immunoassays are widely used in laboratory practice allowing the measurement of different hormonal parameters with a good sensitivity and a high throughput. They are generally robust but remain vulnerable to interferences causing either false-positive or false-negative results [1]. Fifty percent of analytical interferences may induce clinical consequences including inappropriate investigation and treatment. More than half of commercial immunoassays use biotin-streptavidin interaction to separate immune complexes [2]. This technique is convenient, thanks to a high-affinity interaction not affected by multiple washing or extreme pH [3]. The covalent binding of biotin to thyroid hormones does not alter the immunologic specificity [4]. The separative method is, however, sensitive to anti-streptavidin antibodies (ASA) and the presence of high biotin concentrations in the sample. Each immunoassay is differently impacted depending on the sample volume, the washing steps and the design of the assay (sandwich or competitive, in one or two steps) [1].

Recently, Roche Diagnostics launched a third generation for the quantification of free thyroxine (FT4) where T4 and biotinylated T4 compete for a polyclonal sheep antibody labeled with a ruthenium complex. The separation of immune complexes is obtained by the addition of streptavidin-coated paramagnetic particles. The third generation includes a pretreatment step with a blocking protein reducing the ASA interference. The threshold above which the biotin concentration impacts the FT4 assay is increased (100 ng/mL as compared to 20 ng/mL for the second generation) [5].

In this letter, we report two cases of interference with a comparison of results obtained using immunoassays from different manufacturers and different generations of FT4 assay on a Roche analytical platform.

A 37-year-old woman visited her general practitioner with different complaints including heart palpitations, insomnia, tiredness and weight loss. Thyroid palpation revealed no goiter. The family history revealed that her father had Hashimoto's thyroiditis and her sister, Grave's disease. She took no medication or vitamin supplementation. Thyroid testing was performed using a Roche Cobas e602 module (Roche Diagnostics, Basel, Switzerland) which showed the following results: FT4 25.4 pmol/L (normal range [NR]: 12–22, second generation), free triiodothyronine (FT3) 9.9 pmol/L (NR: 3.6–6.8) and thyroid-stimulating hormone (TSH) level 0.38 mIU/L (NR: 0.27–4.2). The anti-TSH antibodies (TRAb) were measured using a competitive radioimmunological assay (Brahms Thermo Scientific, Henningsdorf, Germany) not involving a streptavidin-biotin interaction and were undetectable (<1.0 IU/L – NR: <1.5 IU/L). The patient was referred to the endocrinology department and the thyroid function tests worsened at the second biological control performed 2 months later, with an FT4 raised at 48.7 pmol/L. Interference caused by ASA was suspected due to the discordant biochemical pattern with high FT4 and normal TSH [1]. The sample was treated with a high-affinity agarose streptavidin resin (Pierce Biotechnology, Thermo Scientific, Waltham, MA, USA) to remove potential interfering ASA, as described by Rulander et al. [3]. The FT4 concentration measured using the second-generation

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Roche after this pretreatment step was 18.3 pmol/L. The original sample was analyzed using the third-generation Roche, the Advia Centaur (Siemens Healthcare Diagnostics, Beersel, Belgium) as well as the Lumipulse G600II (Fujirebio, Gent, Belgium) assays. All results were within the NR provided by each manufacturer (Table 1).

Thyroid testing was requested for a newborn girl 2 weeks old. She had several episodes of severe hypoglycemia, and raised lactate and transaminase levels. The thyroid function (TSH, second generation FT4, FT3) measured using a Roche analytical platform was compatible with severe hyperthyroidism: TSH <0.01 mIU/L (NR: 0.72–11), FT4 >100 pmol/L (NR: 11.5–28.3 pmol/L) and FT3 19.3 pmol/L (NR: 3–9.3 pmol/L [6]). The concentration of TRAb was not raised neither for the baby nor for her mother. The thyroid ultrasound and clinical examination did not reveal any thyroid abnormalities. As a high dose of biotin (50 mg/kg/day) had been administered to the newborn girl for a suspicion of metabolic disease, an interference due to exogenous biotin was suspected. The same sample was therefore analyzed on an Advia Centaur. The results were almost normal with a TSH at 0.19 mIU/L (NR: 0.3–4) and an FT4 at 16.9 pmol/L (NR: 9–26). A washout period of 48 h was advised before a new venipuncture was realized to check the thyroid tests on the Roche platform. The TSH and FT4 were then found to be 0.88 mIU/L and 18.9 pmol/L, respectively.

The impact of exogenous biotin using different generations commercialized by Roche and other analytical platforms was therefore tested subsequently on two volunteers (one 39-year-old woman and one 44-year-old man) who gave their written informed consent. They ingested 100 mg of biotin. Venipunctures were performed before (0 h) and 1, 2 and 5 h after the biotin intake. TSH and FT4 (second and third generation) were measured using the Roche assay. For both volunteers, an important raise in the measured FT4 was observed irrespective of the test generation in the 1 h sample with a progressive decrease. The TSH concentration also decreased rapidly with levels that did not get completely normalized within 5 h. All samples were treated with a suspension of microparticles coated with streptavidin (Sigma-Aldrich, St. Louis, MO, USA) to remove biotin interference as described by Piketty et al. [7]. The FT4 results obtained with the second-generation Roche assay on the treated samples were within the reference range. The FT4 concentrations measured on original samples using the Lumipulse G600II were unaffected by the presence of biotin (Table 2).

Several interferences due to ASA [3, 8], and more frequently biotin intake [9, 10], have been reported in the literature as affecting different endocrine assays [11]. In both cases, the separation of immune complexes based on a streptavidin-biotin interaction was altered, causing either falsely low results for sandwich assays (i.e. TSH) or falsely high results for competitive tests (i.e. FT4). Interferences caused by ASA seem to be infrequent according to the few cases reported in the literature [2]. Competitive assays are generally more affected by ASA because the reagent antibodies are present in a limited amount [3]. The resulting biochemical pattern typically suggests a TSH producing adenoma or a resistance to thyroid hormones, which are rare pathologies [1]. Contrary to biotin intake, ASA interference is not transient and could potentially impact immunoassays for at least 2 years [3]. Procedures such as sequential dilutions or precipitation with polyethylene glycol are frequently used to detect interferences. However, they cannot be applied in the case of FT4 assays because of the risk of equilibrium displacement between the free and the protein-bound T4 fractions. The use of high-affinity agarose for streptavidin or streptavidin-coated beads [11] allows the efficient removal of such interfering antibodies or every streptavidin-binding compound.

Table 1: ASA interference: free thyroxine (FT4) levels measured using the Cobas e602/Roche second generation and third generation (with or without pretreatment), the Advia Centaur/Siemens as well as the Lumipulse G600II/Fujirebio.

	Cobas e602/Roche (2nd gen.)	Cobas e602/Roche (2nd gen.)	Cobas e602/Roche (3rd gen.)	Advia Centaur/ Siemens	Lumipulse G600II/Fujirebio
FT4, pmol/L					
Pretreatment	None	High-capacity streptavidin agarose [3]	None	None	None
Concentration, pmol/L	48.7	18.3	18	15.5	15.5
Normal range	12–22	12–22	12–22	9–26	10–20

ASA, anti-streptavidin antibodies.

Table 2: Biotin interference: free thyroxine (FT4) and thyroid-stimulating hormone (TSH) concentrations obtained for two volunteers at different times following a 100-mg biotin dose using the Cobas e602 second generation and third generation (with or without pretreatment) as well as the Lumipulse G600II.

	0 h	1 h	2 h	5 h
FT4, pmol/L				
V1				
Cobas e602/Roche (2nd gen.)	18.1	49.3	23.3	18.7
Cobas e602/Roche (2nd gen.) + pretreatment [7]	18.4	17	17.5	17.7
Cobas e602/Roche (3rd gen.)	18.1	69.3	28.3	22.1
Lumipulse G600II/Fujirebio	14.8	12	13.9	13.9
V2				
Cobas e602/Roche (2nd gen.)	15	27.3	26.5	19.7
Cobas e602/Roche (2nd gen.) + pretreatment [7]	17.2	17.1	20.7	20.7
Cobas e602/Roche (3rd gen.)	17.7	39.2	37.9	23.3
Lumipulse G600II/Fujirebio	13.9	14.7	14.3	14.7
TSH, mIU/L				
V1				
Cobas e602/Roche	3.79	0.04	0.16	2.31
V2				
Cobas e602/Roche	1.53	0.03	0.03	0.25

V1, volunteer 1; V2, volunteer 2.

An adequate design can protect the assay from such interferences. FT4 measurement using the Advia Centaur is less susceptible to ASA or exogenous biotin because the binding between avidin and biotin is already preformed before the addition of the sample. The quantification of FT4 on the Lumipulse G600II is theoretically not affected by such interferences because the separation of immune complexes is not based on a biotin-streptavidin interaction. Our case report confirms that the modification introduced by Roche in the third-generation FT4 assay reduces its sensitivity to ASA.

The biotin intake in the normal Western diet is estimated to be 30–70 µg/day, a dose not sufficient to affect the streptavidin-biotin-based immunoassays [12]. Over the recent years, interferences started to be reported because biotin megadoses (10–300 mg/day) became common for medical (rare metabolic diseases, progressive multiple sclerosis) [13] and non-medical purposes (cosmetic benefits) [4]. The importance of the problem is difficult to estimate because patients taking high doses of biotin for cosmetic use may not report it to their physician [12]. The extent of biotin impact on immunoassays is dose-dependent and varies from test to test [7, 14]. Different washout periods ranging from 8 h to several days are recommended in the literature to avoid biotin interference [2].

The assessment of FT4 levels following the ingestion of 100 mg biotin in two volunteers demonstrated a maximal interference 1 h post dose. These results corroborate those published in the literature. The pharmacokinetics of biotin is described by a two-compartment model with a rapid absorption, distribution and renal excretion as unchanged biotin and metabolites. The median time for reaching the maximal plasma biotin concentration has been estimated to occur at 1.25 h following a dose of 100 mg [15].

For both volunteers, the FT4 levels did not completely normalize within 5 h. Surprisingly, the interference was more pronounced for the third-generation Roche Diagnostics compared to the second. Unfortunately, the level of biotin and metabolites could not be measured to check whether their concentrations were above the threshold provided by Roche for biotin interference (i.e. >100 ng/mL, third generation) [5]. Peyro Saint Paul et al. showed that a 100-mg single oral dose biotin leads to a serum biotin peak of 494.9 ± 161 ng/mL, 1.25 h after intake [15]. It is therefore highly conceivable that biotin levels in our two volunteers exceeded the biotin threshold values provided by Roche Diagnostics.

Katzman et al. recently studied the prevalence of biotin intake in a US outpatient population and revealed that approximately 7% of patients either reported biotin supplementation or presented a serum biotin concentration susceptible to interfere on the Roche Diagnostics immunoassay [16]. Given the increase in biotin use for non-medical reasons and the importance of misdiagnosis caused by interferences in immunoassays, a study of the biotin intake epidemiology in Europe would be

helpful. Information about the potential interference of biotin should be provided by laboratories to patients (for example, in blood sampling centers) and to clinicians.

This study was performed in accordance with the ethical guidelines, and written informed consent was obtained from both the volunteers.

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