Letter to the Editor

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Anti-streptavidin antibodies mimicking heterophilic antibodies in thyroid function tests

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To the Editor,

Hereby we report a rare interference that affected the thyroid function tests in two patients seen in our outpatient clinic between May and June 2017. In our laboratory, thyroid-stimulating hormone (TSH) is measured with a two-site electrochemiluminescent immunoassay (ECLIA) with ruthenium label, whereas free thyroxine (FT4) and free triiodothyronine (FT3) are measured by a competitive ECLIA also using a ruthenium label, both assays being run on a Cobas 8000® e602 module (Roche Diagnostics®, Basel, Switzerland). Sheep polyclonal antibodies are used for FT4 and FT3 measurement and mouse antibodies for TSH. For TSH, the limit of detection (LOD) is 0.005 mU/L and within-run precision is 8.6% and 4.0% at concentrations of 0.03 and 3.96 mU/L, respectively; for FT4, LOD is 0.5 pmol/L and within-run precision is 5.0% and 2.7% at concentrations of 1.6 and 87.8 pmol/L, respectively; for FT3, LOD is 0.6 pmol/L and within-run precision is 3.0% and 1.1% at concentrations of 1.5 and 46.8 pmol/L, respectively.

The first patient was a pregnant 34-year-old woman with autoimmune thyroiditis. The patient was taking L-thyroxine 50 µg/day. Her medical history showed that she underwent ovarian cyst removal in 2005 and pituitary microprolactinoma treated with cabergoline. The examination of her clinical file revealed normal thyroid function tests (TSH, FT4 and/or FT3) since December 2014. However, in May 2017, FT4 (reference range [RR] 12.0–22.0 pmol/L) and FT3 (RR 3.1–6.8 pmol/L) concentrations were both increased (59.5 and 13.3 pmol/L, respectively), whereas her TSH level was normal at 0.78 mU/L (RR 0.27–4.20 mU/L). The second patient was a 12-year-old girl who was taking depakine as treatment for epilepsy since 2008. No other drugs or vitamins were taken by the patient. During her clinical follow-up in June 2017, her TSH level was rather low at 0.51 mU/L (age-specific RR 0.7–5.33 mU/L), whereas FT4 (RR 10.4–24.0 pmol/L) and FT3 (RR 3.1–8.0 pmol/L) concentrations were both increased (45.2 and 16.12 pmol/L, respectively). No previous thyroid function tests results were available on her clinical file. The results are shown in Table 1.

The pattern of normal TSH associated with elevated FT4 and FT3 may be related to central hyperthyroidism due to a TSH-producing adenoma, resistance to thyroid hormones, recent L-thyroxine administration, or drugs (e.g. amiodarone, heparin) [1]. However, these two patients were judged as euthyroid by their physician and had values that were discordant with their previous results. Therefore, an interference involving our assay was suspected [2, 3]. To confirm and characterize this interference, different procedures were undertaken on the serum of each patient.

We first challenged the serum of the two patients against the Diasorin Liaison XL® (Saluggia, Italy), a different method available for FT4 measurement in our

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Table 1: Comparison of TSH, FT4 and FT3 values between the Cobas E602, the Liaison XL, the Ortho Vitros 5600 and the Cobas E602 (new formulation) immunoassays.

	Cobas E602 (FT4II)	Liaison XL	Ortho Vitros 5600	Cobas E602 PZ (FT4III) new assay formulation
Patient 1				
TSH, mU/L	0.78 (0.27–4.20)	NA	1.38 (0.46–4.68)	NA
FT4, pmol/L	59.5 (12.0–22.0)	14.5 (10.3–21.9)	17.6 (10.0–28.2)	21.81
FT3, pmol/L	13.3 (3.1–6.8)	NA	4.94 (4.3–8.1)	NA
Patient 2				
TSH, mU/L	0.51 (0.7–5.33)	NA	1.04 (0.46–4.68)	NA
FT4, pmol/L	45.2 (10.4–24)	13.33 (10.3–21.9)	17.7 (10.0–28.2)	17.71
FT3, pmol/L	16.12 (3.1–8.0)	NA	8.24 (4.3–8.1)	NA

PZ, Penzberg; NA, not applicable.

laboratory. This method uses chemiluminescent technology involving mouse polyclonal antibodies labeled with isoluminol. For FT4, LOD is 1.3 pmol/L and within-run precision is 2.4% and 2.4% at concentrations of 5.02 and 38.61 pmol/L, respectively. In contrast to the increased values obtained with the Cobas 8000® assays, FT4 values were within the normal range with the Liaison XL® platform (Table 1). The Vitros 5600 assay available in another laboratory was also used as a comparison method for TSH, FT4 and FT3. This latter assay uses chemiluminescent technology involving luminol-derived molecule and peracid salt as luminescent materials (Vitros 5600®; Ortho Clinical Diagnostics®, Raritan, NJ, USA). Sheep anti-T4 and anti-T3 were used for the FT4 and FT3 measurements and mouse monoclonal for anti-whole TSH and anti-TSH β-subunit. For TSH, LOD is 0.014 mU/L and the within-run precision is 3.3% and 1.4% at concentrations of 0.17 and 2.21 mU/L, respectively; for FT4, LOD is 0.9 pmol/L and the within-run precision is 2.4% and 1.6% at concentrations of 6.5 and 47.6 pmol/L, respectively; for FT3, LOD is 0.8 pmol/L and the within-run precision is 2.2% and 1.1% at concentrations of 4.0 and 24.1 pmol/L, respectively. All results were within normal ranges with this assay (Table 1). The absence of streptavidin-biotin immobilization system on the Liaison XL® first made us think about a biotin interference. However, neither patient was overtly taking biotin nor did they have detectable concentrations of biotin.

We next performed a dilution test (the serum of the first patient was serially diluted at titers of 1/2, 1/4, and 1/8) in 0.9% NaCl on the Roche Cobas E602 (FT4 and FT3) and Diasorin Liaison XL® (FT4) (there was not enough volume to perform the dilution test in the other patient) [4]. A lack of parallelism would argue for the possible presence of an interfering agent. After dilution of the patient sample, the FT4 and FT3 dilution pattern was very linear on the Cobas module, but a complete lack of linearity was observed for FT4 on the Diasorin Liaison XL® (Table 2). This argued for the presence of a potential interference [3].

Thereafter, the serum of both patients was treated with heterophilic blocking tubes (HBT; Scantibodies Laboratory, Inc., Santee, CA, USA), which contain binders that can inactivate heterophilic antibodies. After this procedure, FT4 and FT3 levels were significantly decreased but without reaching the reference intervals for FT4 (patient 1) and FT3 (patient 2) on the Roche Cobas E602. Interestingly, FT4 levels remained much the same on the Diasorin Liaison XL® after the HBT treatment in each patient (Table 2). Interference by heterophilic antibodies was highly suspected at first, as we found a clear reduction of hormonal values in the HBT study.

An aliquot was sent to Roche Diagnostics (Penzberg, Germany) to confirm our data. Surprisingly, the additional investigations performed by Roche Diagnostics revealed the presence of anti-streptavidin antibodies.

With such misleading results, we performed an additional test recently published by Piketty et al. [5]. Briefly, streptavidin beads (0.72 mg/mL in HEPES-bovine serum albumin buffer, pH 7.4; Roche) were incubated for 1 h at room temperature with the serum of the first patient obtained during her regular follow-up 3 months later, and this treated serum was reassayed on the Cobas E602 after removal of beads by centrifugation. This method was designed to identify biotin interference (high affinity to streptavidin), but we made the assumption that anti-streptavidin antibodies would also be cleared by this procedure. Interestingly, we observed a clear reduction in the FT4 and FT3 levels in comparison to the untreated serum (Table 2). Furthermore, we confirmed that this kind of interference can last for a period of time even if a clear decline was observed after 3 months [6, 7].

Roche Diagnostics also analyzed the two samples with a third-generation FT4 assay not yet available in the market and found normal results (Table 1).

Table 2: Hormonal results obtained from the dilution tests (1/2, 1/4, and 1/8) and before and after treatment with heterophilic tubes (HBT) and streptavidin beads on the Cobas E602 (TSH, FT4 and FT3) and the Liaison XL (FT4).

	Cobas E602 (FT4II)			Liaison XL
	TSH, mU/L	FT4, pmol/L	FT3, pmol/L	FT4, pmol/L
Patient 1				
Before HBT	0.78 (0.27–4.20)	59.5 (12–22)	13.3 (3.1–6.8)	14.5 (10.3–21.9)
After HBT	NA	23.84	5.99	15.16
Dilution 1/2	NA	24.02	5.56	15.07
Dilution 1/4	NA	14.24	2.95	14.58
Dilution 1/8	NA	10.43	1.94	15.01
Before streptavidin beads[a]	NA	21.29	7.74	NA
After streptavidin beads[a]	NA	14.37	5.44	NA
Patient 2				
Before HBT	0.51 (0.7–5.33)	45.2 (10.4–24)	16.12 (3.1–8.0)	13.33 (10.3–21.9)
After HBT	0.75	18.90	8.63	12.51

NA, not applicable. [a]Serum of the first patient obtained during her regular follow-up 3 months later.

Interferences affecting both FT4 and FT3 have been previously related to anti-ruthenium antibodies [8], biotin [9], anti-streptavidin antibodies [6, 7, 10], heterophilic antibodies [11] or thyroid autoantibodies [12]. In our study, interferences were more likely to be due to anti-streptavidin antibodies than heterophilic antibodies.

Streptavidin is a protein produced by *Streptomyces avidinni*, which has the ability to bind to biotin with very high affinity (affinity constant of 10^{15} L/mol) [13]. The biotin-streptavidin interaction has been extensively employed in both sandwich (e.g. Roche, Ortho or Siemens) and competitive (e.g. Roche, Beckman Coulter, Siemens) *in vitro* immunoassays as an immobilizing system [5, 13]. We only found three reports reporting such interference with streptavidin [6, 7, 10]. The method comparison with another technique not using the streptavidin-biotin immobilization system (e.g. Diasorin or Abbott) appeared to be a valuable option in our two cases. We still do not know why these patients developed antibodies against streptavidin or streptavidin-like proteins and why the HBT significantly reduced this interference on the Roche Cobas E602. We also could not exclude the presence of heterophilic antibodies along with antibodies against streptavidin. Manufacturer's improvements have already led to a significant reduction in the number of assay interferences [14, 15] and it seems that the next generation of FT4 assay will prevent this rare streptavidin-related interference observed in our two cases.

Fortunately, clinicians were quickly warned about the presence of interference and further investigations were stopped. In conclusion, it is important to keep in mind that all immunoassays are prone to interference and that a continuous discussion between clinicians, biologists and manufacturers remains essential in avoiding unnecessary investigations and inappropriate treatments [6, 7, 12].

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