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Case report

Heterophile antibody interference affecting multiple Roche immunoassays: A case study

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ABSTRACT

Background: Analysis of many clinically important analytes is dependent on antibody-based assays. However, depending on the design, these assays are vulnerable to interference from endogenous molecules including circulating antibodies and free biotin. In this case report, we describe a patient whose laboratory findings from immunoassay based methodologies, are inconsistent with the clinical presentation.

Case presentation: A 14-year-old male was referred to Pediatric Endocrinology for suspected hyperthyroidism based on critically elevated free thyroxine (fT4) levels although clinical assessment was inconsistent with hyperthyroidism. Because repeat testing was discrepant, Endocrinology questioned the validity of the results prompting consultation with the laboratory to investigate the source of the inconsistent findings. Review of discordant results revealed that fT4 levels measured in laboratories utilizing Roche instrumentation were critically high, while results from laboratories using alternative platforms (i.e. Siemens Centaur) were within normal limits.

Conclusion: After a comprehensive evaluation which included testing of paired specimens on multiple platforms, measurement of serially diluted specimens and a formal evaluation for the presence of heterophile antibodies, it was determined that a heterophile antibody interference was the most likely cause of the aberrant results in this patient.

1. Introduction

Immunologic assays are invaluable in the clinical laboratory, with their application widespread and forming the backbone for many laboratory tests. These assays utilize the specificity and sensitivity of an antibody towards a target analyte to rapidly detect and quantify the analyte of interest [1]. Two commonly used formats are the immunometric (sandwich) and the competitive immunoassays. Used for larger molecules such as TSH, the sandwich immunoassay measures the concentration of an analyte by using a solid phase antibody to immobilize the target antigen while a second tracer antibody binds to a separate epitope on the analyte [1,2]. In contrast, the competitive assay is used for smaller molecules for which antibody binding to two epitopes on the target analyte is not feasible, such as the free thyroid hormones thyroxine (fT4) and triiodothyronine (fT3). The competitive assay measures the concentration of an analyte by the competition of an unknown quantity of analyte with a known quantity of labeled analyte for binding to specific antibodies [1,2]. In both assay formats, manufactures have developed strategies for separating the antigen-reagent antibody complexes which is necessary for quantifying the antigen of interest. For example, some companies, such as Roche, have exploited the high-affinity interaction between biotin and streptavidin as a convenient method to separate the antigen-reagent antibody complexes [2]. Depending on the assay format and the nature of the interferent, the impact on results will likely differ, often in an unpredictable way. Therefore, it is imperative that clinicians be aware of the susceptibility of immunoassays to interferences so they can interpret laboratory results appropriately.

It is well documented that immunoassays are vulnerable to interference with endogenous substances such as heterophile antibodies or circulating therapeutic biotin. Heterophile antibodies interfere with immunoassays by binding to the analyte or to the reagent antibodies [1,3]. Intake of biotin supplements interferes with interaction of streptavidin with biotinylated antigen-antibody complexes [2]. Additionally, endogenous anti-streptavidin antibody has been reported and can also interfere with the streptavidin-biotin interaction widely utilized in many, but not all, immunoassays [4]. As clinical understanding or knowledge of heterophile antibodies is not widespread, unnecessary workup and inappropriate treatment of the patient can occur due to false results [1,5]. Interference with heterophile antibodies

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is generally suspected when laboratory results are discordant with the clinical presentation and are brought to the attention of laboratory consultants [1,5,6]. As some of the most ordered tests in medicine, it is critical that clinicians be aware of possible interferences in thyroid hormone testing by interfering heterophile antibodies, and that laboratory consultants understand the mechanisms of such interferences so suitable analytic interventions can be employed to ensure accurate and reliable results are reported. Here, we report a case of heterophile antibody interference in the measurement of free and total T4, total T3, thyroid stimulating hormone (TSH), and intact parathyroid hormone (iPTH) in a 14- year-old male patient.

1.1. Case presentation

A 14-year-old-male patient was referred to the Pediatric Endocrinology clinic after routine testing ordered by his primary healthcare provider revealed elevated fT4 of 4.35 ng/dL for the provided reference range of 0.93–1.60 and low normal TSH of 1.080 uIU/mL (0.450–4.500). Repeat testing within 1 week showed elevated fT4 at 5.01 ng/dL again with a low normal TSH of 0.948 uIU/mL; additional testing revealed positive thyroid peroxidase antibodies at 115 IU/mL (0–26) and negative thyroglobulin antibodies at < 1.0 IU/mL (0.0–0.9). At his initial evaluation, the patient denied all symptoms and signs of hyperthyroidism including anxiety, jitteriness, insomnia, problems concentrating at school, weight loss, hyperphagia, heat intolerance, pain or tenderness of his eyes, palpitations, or skin lesions. His examination revealed a normal appearing adolescent male without tachycardic or hypertension, no lid lag or retraction, exophthalmos, tongue fasciculations or a peripheral tremor, proximal muscle weakness; his skin was intact. Initial assessment was autoimmune Hashimoto's thyroiditis based on positive thyroid peroxidase antibodies in a patient with biochemical evidence of an elevated fT4. We suspected that this could be hashitoxicosis, which is a transient phase of biochemical hyperthyroidism resulting from release of preformed thyroid hormone due to inflammatory destruction of thyroid cells. It is typically asymptomatic, but if clinically evident, symptoms of hyperthyroidism are usually minimal. However, a significant inconsistency noted at this time was the absence of TSH suppression which, as a result of the negative feedback loop, typically accompanies fT4 elevation. Follow-up testing performed at LabCorp within 2 weeks of his initial visit showed fT4 of 3.36 ng/dL and TSH of 0.740 uIU/mL; thyroid peroxidase antibodies were again positive at 84 (0–26). Testing for Graves' disease was negative.

Table 1 summarizes thyroid hormone testing results in this patient over a two year period. As is true for most patients, the choice of laboratory for clinical testing is dictated by insurance coverage and convenience. As illustrated in Table 1, when this patient had his testing performed at Quest (Siemens), all were within normal limits, as compared to results from LabCorp (Roche) where multiple analytes were out of range, most notably the critically high fT4. In light of the confusing laboratory findings in combination with the fact that the clinical presentation was inconsistent with hyperthyroidism, the clinician consulted with the laboratory director to determine if an analytical issue could explain the results. A comprehensive work-up was initiated and is described below.

1.2. Laboratory tests and investigation

To investigate the discordant laboratory results, a multi-faceted approach was pursued. Based on the fact that thyroid function testing for this patient was within normal limits when testing was performed at Quest (Siemens assays) compared to abnormal results when testing was performed at LabCorp (Roche assays), we hypothesized that a potential interferent was present. To address this question, parallel testing of patient samples was performed on multiple analytic platforms for multiple analytes. In addition to the thyroid specific analytes that were originally assayed, assessment of other markers were included (iPTH, ferratin, Vitamin B12) to determine if multiple assays were affected. Results of this analysis are summarized in Table 2. Analysis of these paired patient specimens revealed that critically high fT4, low normal TSH, elevated total T3 and T4 and undetectable or very low levels of iPTH were reported from laboratories utilizing Roche instrumentation (RWJUH, Mayo Laboratories and LabCorp), while results within acceptable limits were observed when alternative platforms were used. The true concentration of fT4, the most significantly affected analyte, was confirmed by analysis by equilibrium dialysis, considered the gold standard method, which confirmed that this patients fT4 level was within normal limits [1.4 ng/dL (normal range 0.8–2.0)].

Additionally, we requested Mayo perform a heterophile antibody work-up for fT4 and TSH. This investigation involves incubating the patient specimen with a commercial heterophilic antibody blocking reagent and comparing results to an untreated specimen. In addition, analysis for fT4 and TSH was performed on two different immunoassay platforms (Roche e501 and the Siemens Advia Centaur) and found that

Table 1
Summary of thyroid hormone testing results prior to laboratory consultation.

Time of testing	**fT4** LabCorp (Roche)	**fT4** Quest (Siemens)	**TSH** LabCorp (Roche)	**TSH** Quest (Siemens)	**Total T4** LabCorp (Roche)	**Total T3** LabCorp (Roche)
Reference Interval	*0.93-1.6 ng/dL*	*0.90-1.4 ng/dL*	*0.45-4.5 μIU/mL*	*0.50-4.3 μIU/mL*	*4.5-12.0 μg/dL*	*71 - 180 ng/dL*
Initial visit	4.35 ↑		1.11			
1 week	5.01 ↑		0.95			
2 week	3.36 ↑		0.74			
3 month		1.00		1.18		
6 month		1.10		1.89		
12 month		1.30		2.11		
16 month	>7.77 ↑		0.775			
17 month		1.40		3.78		
24 month	7.64 ↑		0.977		14.8 ↑	250 ↑

Table 2
Summary of thyroid function testing in paired patient specimens.

	Lab (assay)	TSH	fT4	Total T4	fT3	Total T3	iPTH
Paired Specimens #1	RWJ (Roche)	0.94	6.36 (↑)	16.2 (↑)	15 (↑)	252 (↑)	3 (↓)
	reference range	*0.35-5.5 μIU/mL*	*0.9-1.8 ng/dL*	*5-12 μg/dL*	*2.3-4.2 pg/mL*	*60-181 ng/dL*	*9-76 pg/mL*
	Mayo (Roche)	0.9	5.3 (↑)	13.5 (↑)	6.6 (↑)	204 (↑)	<6.0 (↓)
	reference range	*0.5-4.3 μIU/mL*	*1.0-1.6 ng/dL*	*5-13.2 μg/dL*	*2.8-4.4 pg/mL*	*60-181 ng/dL*	*15-65 pg/mL*
	Mayo (non-Roche)	1.7	1.3				
	reference range	*0.5-4.3 μIU/mL*	*1.0-1.6 ng/dL*				
	Mayo (Eq Dialysis)		1.6				
	reference range		*0.8-2.0 ng/dL*				
Paired Specimens #2	RWJ (Roche)	0.35	6.42 (↑)				< 2 (↓)
	reference range	*0.35-5.5 μIU/mL*	*0.9-1.8 ng/dL*				*9-76 pg/mL*
	Quest (Seimens)	0.48	1				46
	reference range	*0.5-4.3 μIU/mL*	*0.9-1.4 ng/dL*				*9-69 pg/mL*
	Mayo (Eq Dialysis)		1.4				
	reference range		*0.8-2.0 ng/dL*				
Paired Specimens #3	RWJ (Roche)	1.2	5.81 (↑)	15.2 (↑)	15 (↑)	213 (↑)	< 2 (↓)
	reference range	*0.35-5.5 μIU/mL*	*0.9-1.8 ng/dL*	*5-12 μg/dL*	*2.3-4.2 pg/mL*	*60-181 ng/dL*	*9-76 pg/mL*
	Quest (Seimens)	1.73	1.2	8.5	3.6	114	27
	reference range	*0.5-4.3 μIU/mL*	*0.9-1.4 ng/dL*	*5.7-11.6 μg/dL*	*2.0-4.7 pg/mL*	*105-207 ng/dL*	*9-69 pg/mL*

they were significantly discrepant. Taken together, Mayo concluded that the presence of a heterophile interferent is very likely in this patient's specimens.

To further confirm the presence of an interfering substance, we performed dilutional experiments (Fig. 1). Specifically, patient and control specimens were serially diluted and iPTH and total T4 measured to determine the impact of the suspected interfering substance on specimen dilution. As illustrated in Fig. 1A, a non-linear response was observed when iPTH was measured on diluted patient specimens compared to control. In the patient's undiluted specimen, iPTH was undetectable (< 1.2 pg/mL). However, at a relative concentration of 12.5%, which is equivalent to a 1:8 dilution, iPTH was detected (6.95 pg/mL), showing an inverse relationship of concentration with dilution in contrast to the control specimen which diluted linearly, as would be expected. Interestingly, a linear response was observed when the patient specimen was diluted and total T4 measured (Fig. 1B), illustrating the unpredictable nature of the unknown interferent. Because of the fact that multiple analytes were affected and Roche employs the streptavidin-biotin system in the separation step of their immunoassays, we initially hypothesized that the interference was likely circulating biotin. However, the patient denied taking biotin supplements. Additionally, we measured two unrelated analytes, ferritin (sandwich immunoassay) and Vitamin B12 (competitive immunoassay) using Roche reagents which have analogous assay formulations to TSH and fT4, respectively, and found that the results were comparable between Roche and Siemens (data not shown.) further supporting that circulating biotin was not present.

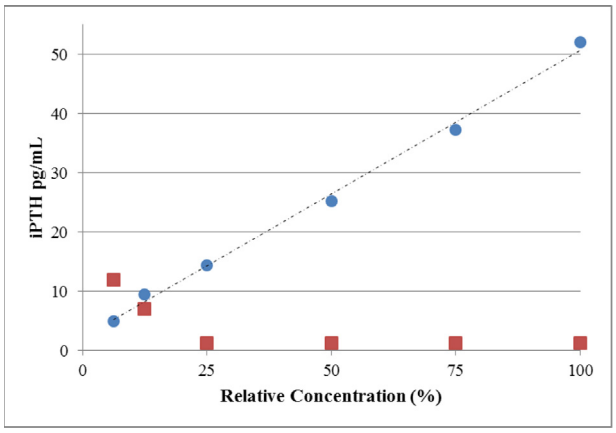
2. Discussion

Heterophile antibodies are well known to interfere with immunoassays, with measurement of hormones, hCG, troponins, and serological markers being particularly vulnerable [1]. This investigation strongly supports the presence of a generalized heterophile antibody in a 14-year-old patient that interferes with multiple Roche immunoassays causing falsely elevated fT4, total T4 and T3, and falsely decreased iPTH. We determined this through non-linear serial dilutions, discrepant testing between platforms, and confirmation of heterophile antibody interference from an investigation by Mayo Laboratories.

Interestingly, while analysis performed using the Roche Cobas e601 platform were affected by the presence of the heterophile antibody, analysis performed using the Siemens Centaur platform were not. Testing performed at Quest and Mayo Laboratories on the Siemens Centaur yielded results that were consistent with the patient's clinical picture. While it is well known that immunoassay results can frequently vary between different platforms, Roche assays have been reported to be affected by multiple types of interferences. Falsely elevated free thyroid hormones in Roche Cobas e602 versus other platforms such as Siemens Centaur, Abbot Architect, and Beckman Dxl have been reported by several groups [4,7–9]. In this case, direct communication with Roche confirmed that the interference identified when using the Cobas e611 was also present across other Roche platforms. Specifically, Roche tested the patient's samples on the MODULAR E170 and Cobas e411 to yield similar results as the Cobas e601, confirming the presence of an unknown interfering substance.

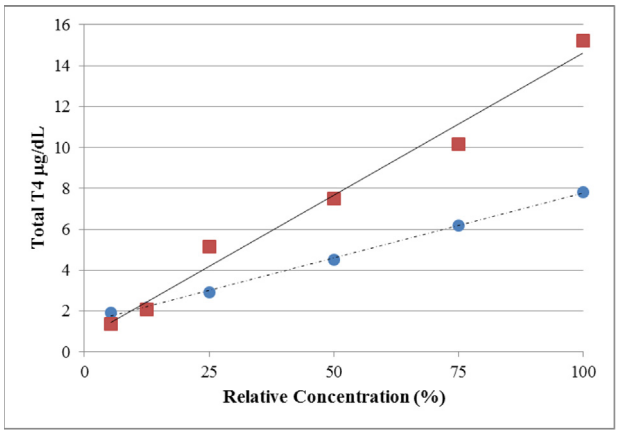
Multiple substances have been reported to interfere specifically with Roche immunoassays. Anti-streptavidin antibodies have been identified as the cause of falsely elevated T4 and falsely decreased TSH using Roche E170 versus Siemens Centaur, which does not utilize streptavidin [4]. Similarly, biotin supplementation has also been widely reported to interfere with streptavidin-biotin based immunoassays [2]. However, circulating biotin was ruled out in the presented case as the patient denied biotin supplementation. Anti-ruthenium antibodies have also been reported to specifically interfere with Roche immunoassays [10] and is another possible mechanism of antibody interference in this patient. Rodriguez-Borja et al., reported through direct communication with Roche, that falsely elevated free T4 and falsely decreased TSH could be due to a streptavidin interfering factor enhanced by a prewash step in the Cobas e602 and MODULAR systems versus the Cobas e411 [8]. However, for our patient, it is unlikely that a prewash step

A.



iPTH		
Relative Concentration (%)	control (pg/mL)	Patient (pg/mL)
100 (undiluted)	52.11	< 2.0
75	37.28	< 2.0
50	25.2	< 2.0
25	14.34	< 2.0
12.5	9.51	6.95
6.25	4.9	11.95

B.



Total T4		
Relative Concentration (%)	control (µg/dL)	Patient (µg/dL)
100 (undiluted)	7.82	15.23
75	6.18	10.18
50	4.49	7.48
25	2.9	5.13
12.5	2.16	2.09
6.25	1.91	1.37

Fig. 1. Linear and non-linear response to specimen dilution. Patient (square) and control (circle) specimens were serially diluted and analyzed for iPTH (1A) and total T4 (1B). 1A. In the undiluted patient specimen, iPTH was undetectable, however at a relative concentration of 12.5%, presumably due to corresponding dilution of the interferent, iPTH becomes increasingly detectable. 1B. A linear response was observed in both the patient and control specimens when total T4 was analyzed. The associated table illustrates the measured concentration at each dilution.

contributed to enhancement of antibody interference as Roche confirmed consistent results using the MODULAR E170 and Cobas e411. Similarly, Zaninotto et al. were unable to further characterize their Roche-specific antibody interference beyond an association with the IgM class [9]. Though we were unable to definitively define the nature of the interfering heterophile antibodies, the interference is likely one that is specific to streptavidin-biotin complexes or to the ruthenium-labeled antibodies which are utilized by multiple Roche immunoassays.

The origin of this patient's heterophile antibodies is unknown. The presence of heterophile antibodies is relatively common, hypothesized to be present in up to 40% of the population, with the vast majority having no analytical impact on antibody based assays [1]. Characterization of heterophilic antibodies reveals they are in general of low affinity with broad specificities. Rheumatoid factor, an IgM or IgA autoantibody, is also widely reported to act as an interfering non-specific heterophilic antibody [11]. These autoantibodies are not only present in patients with autoimmune diseases, but are sometimes present in patients with infections and chronic diseases, as well as a small percentage of healthy people [11,12].

A number of techniques have been utilized to overcome heterophile antibody interference, including use of mouse IgG1 to neutralize human antibodies, precipitating or extracting human antibodies from serum or plasma, and excluding antibodies through size-exclusion [1]. Commercial heterophile blocking reagents are available and have been shown to uncover the presence of heterophile antibodies and reduce false results in immunoassays [13,14]. Blocking antibodies in this investigation were used by Mayo Laboratories to confirm the presence of heterophile antibodies. However, blocking antibodies may not always detect heterophile antibody interference [15]. Development of liquid chromatography-tandem mass spectrometry (LC-MSMS), a more specific, more precise, and more reliable method as compared to immunoassays, has overcome many of the interference issues related to immunoassays [2,16]. However, LC-MSMS requires technical expertise and is not widely available in many clinical laboratories [2,16]. Thus, immunoassays still remain widely used to measure clinical analytes, with heterophile antibody interference a major unpredictable issue that can impact patient care. It is therefore important for both clinicians and clinical laboratory professionals to be aware of and recognize the potential of heterophile antibody interference.

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