

## **Interference from Anti-Streptavidin Antibodies in a patient with toxic nodular thyroid disease. Thyroid conundrum, a test for a test**

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### **Introduction:**

Measurements of thyrotropin and of free thyroxine and triiodothyronine are widely used diagnostic methods for thyroid function evaluation. However, some serum samples will demonstrate a nonspecific binding with assay reagents that can interfere with the measurement of these hormones. Several case reports have described the presence of such interferences resulting in reported abnormal concentrations of thyroid hormones inconsistent with the patient's thyroid state<sup>1</sup>. Misdiagnosis, subsequent inappropriate treatment, and adverse consequences for the patient can result from interferences yielding false results<sup>2-4</sup>.

Automation of immunoassays has allowed the rapid measurement of serum hormone levels and other analytes, aiding in the accurate diagnosis of disease. However, endogenous antibody interference in immunoassays can yield false results. Heterophile antibodies (HAb) are human poly-specific antibodies targeted against animal antigens, the most common being human anti-mouse antibodies (HAMA). Heterophilic antibody interference against reagent antibodies has been well documented, and other interfering antibodies have also been reported<sup>5-7</sup>.

Although interferences have been well documented. The streptavidin-biotin interaction provides an efficient and convenient method to manipulate assay components and is currently used in several immunoassay platforms. To date, there have been limited reports in the literature of interference from endogenous or nonspecific anti-streptavidin antibodies; however, such antibodies would potentially affect multiple diagnostic platforms.

We report results for a patient who presented with spurious thyroid function test and was diagnosed with thyrotoxicosis due to autonomous toxic nodule. Subsequent assessment showed abnormal thyroid function results in discordance with his clinical picture. His workup revealed interference from a non-specific streptavidin immunoglobulin.

### **Case study:**

A 68 year old man presented to the emergency department with rapid atrial fibrillation in the absence of an infective or ischaemic precipitant. On examination, he was normotensive, but tachycardic with an irregular heart rate of 100 beats/minute. Oxygen saturation was normal and he was afebrile. Cardio-respiratory examination was normal and his ECG indicated atrial fibrillation with no acute ischaemic changes. Examination of the thyroid demonstrated asymmetrical enlargement of the left hemithyroid, without clinical evidence of retrosternal extension or ophthalmopathy.

His past medical history included type 2 diabetes, a haemochromatosis carrier state (with H63D heterozygous mutation), osteoarthritis and gastroesophageal reflux disease. His initial thyroid function tests demonstrated severe thyrotoxicosis with a suppressed TSH of <0.02 mIU/L (NR 0.27-4.2mIU/L), FT4 >100 pmol/L (NR 12-22pmol/L) and FT3 of T3 23.8 pmol/L (NR 3.0-7.8pmol/L) with negative TRAb < 1.0 U/L (NR < 1).

A technecium-99 labelled thyroid scintigraphy scan demonstrated increase uptake in the lower pole of the left thyroid lobe with almost complete suppression of the remainder of the thyroid, consistent with a large left sided autonomous nodule. A thyroid ultrasound showed multiple thyroid nodules of increased vascularity, the dominant nodule measuring 4.1 cm maximally. The patient was subsequently discharged on carbimazole at a dose of 15 mg TDS and referred for outpatient follow up. Progress TFT results showed in Table 1.

**Table 1. Progress thyroid function results using Roche Elecsys assay.**

Roche Date	TSH (0.27-4.2mIU/L)	T4 (12-22pmol/L)	T3 (3.0-7.8pmol/L)	Carbimazole Dosage
11/12/15	<0.02	>100	22.2	15mg TDS
08/01/16	1.09	40	25.1	15mg BD
04/03/16	4.79	36.2	22.2	15 mg BD

The discrepancies between free thyroxine and TSH suggested the possibility that one or more of the laboratory results did not accurately represent his biologic status as he was clinically hypothyroid. Hence, sample was repeated using a Nonspecific Antibody Blocking Tube (NABT) (Table 2), however, results remained unchanged. Rheumatoid factor was negative at 13 mIU/L (RR <15 mIU/L) and there was no history of biotin use.

Carbimazole was ceased for one week and a repeat TFT done using a different platform (abbott Architect) showed similar results with a TSH of 29.8 mIU/L, FT4 of 36.2 pmol/L and FT3 of 22.2 pmol/L. In addition, TSH dilution did not show linear changes.

Hence, the sample sent for assessment using non-streptavidin base platform and results shown in Table 3. To identify the cause of interference, another sample was sent to Roche Germany to be preincubated with streptavidin microparticles. Further testing concluded that the interference was due to non-specific streptavidin immunoglobulin.

**Table 2. Antibody Binding tubes (Scantibody)**

Roche Elecsys	TSH (0.27-4.2mIU/L)	T4 (12-22pmol/L)	T3 (3.0-7.8pmol/L)
Neat Sample	0.61	>100	26.2
Post-Scantibody incubation	0.62	>100	25.4

**Table 3.**

Repeat TFT	Use ruthenium-streptavidin		Don't Use ruthenium-streptavidin	
	Roche	Abbott	Advia Centaur	
TSH (mIU/L)	0.57	3.23	3.02	
T4 (pmol/L)	> 100	12.1	13.08	
T3 (nmol/L)	26.2	4.6	5.04	

**Discussion:**

There are many causes of interference in immunoassays causing erratic patient results.<sup>8,9</sup> These erroneous results potentially lead to unnecessary, expensive and possibly harmful investigations and treatment. A method-specific interference due to anti ruthenium antibodies in the Roche free thyroxine (fT4) and free triiodothyronine (fT3) assays has been described previously<sup>10,11</sup>. However, few case reports reported the impact of anti- streptavidin antibodies interference<sup>12,13</sup> and cause falsely elevated TSH. Streptavidin is produced by

*Streptomyces avidin*; it is unknown in what circumstances it could lead to an immunization. Unlike biotin, this interference is endogenous, therefore non-transient<sup>13</sup>. In the cases reported<sup>12,13</sup>, the interfering substance will have a more pronounced effect in competitive assay, and a less-remarkable effect on a sandwich assay: for example, FT3 and FT4 concentrations will be overestimated.

In our case report, the diagnosis with underlying primary thyroid disorder was a masking factor at the beginning of treatment; however the subsequent thyroid function results indicated a lack of the usual balance between the hormone and its regulating factor. The patient's specimen demonstrated interference across manufacturer platforms, affecting all streptavidin-mediated diagnostic assays tested. Platforms and assays that do not employ streptavidin did not demonstrate nonlinear dilution or elevated competitive and suppressed sandwich results.

One notable element of this case is that the interference caused reciprocal changes in TSH and T4. It is important for the clinical laboratory to recognize that a single interference can yield opposite effects in different assays, depending on whether a sandwich or competitive format is used. A traditional algorithm for investigating immunoassay interference from species heterophiles or human anti-mouse antibodies would have failed to resolve these results because the interference was not fully removed by Heterophile blocking. This case also demonstrates the value of having diverse immunoassay platforms available to aid in the investigation of problematic or suspicious results, as interference against common laboratory assays.

Although it appears a streptavidin antibody is a rare occurrence, this interference may easily go undetected and a seroprevalence study may be indicated to evaluate the magnitude of this problem.

#### **Conclusion:**

Despite progress in immunoassay technologies, the problem of unwanted interference has yet to be overcome. Critical analysis of the hormone results, together with an open and permanent communication between laboratory and clinical staff, remain the best strategy to avoid clinical mismanagement due to unsuspected interference. Given the striking effect of streptavidin antibody on thyroid function laboratory tests and the possibility of interference for an extended time, a cautioning of the patient is necessary and to provide the information on the potential effect of this antibody for future medical examinations, and avoid the risk of future inappropriate treatment or investigations.

The incidence of thyroid disease, and the frequency with which patients have their thyroid status assessed, has driven much attention and information on the streptavidin- biotin separation interference. However, this interference is not restricted to thyroid testing. If FT4, TSH and FT3 assays seem particularly prone to this interference, PTH and phosphocalcic examination is another field where these pitfalls are worrying<sup>14,15,16</sup>

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