Thyroid

DOI: 10.1089/thy.2017.0673

Apparent hyperthyroidism caused by biotin-like interference from IgM anti-streptavidin antibodies*

Leo Lam[1] MBChB, BMedSci(Hons); Warwick Bagg[2,3] FRACP, MD; Geoff Smith[4] FRCPA; Weldon Wai Chiu[1] MRCP, FRCPA; Martin James Middleditch[5,6] BSc, Julie Ching-Hsia Lim[7] PhD, Campbell Vance Kyle[1,2,8] FRCPA, PhD

[1] Department of Chemical Pathology, LabPlus, Auckland City Hospital, Auckland, New Zealand

[2] Department of Endocrinology, Auckland City Hospital, Auckland, New Zealand

[3] Medical Programme Directorate, Faculty of Medical and Health Sciences, University of Auckland

[4] Department of Clinical Biochemistry, Southern Community Laboratories Dunedin Hospital, Dunedin, New Zealand

[5] Auckland Science Analytical Services, University of Auckland, New Zealand

[6] School of Biological Sciences, University of Auckland

[7] Department of Physiology, School of Medical Sciences, Faculty of Medical and Health Sciences, University of Auckland, New Zealand

[8] Department of Biochemistry, LabTests, Auckland, New Zealand

***** One of the two patients described in this report have previously presented as an abstract for the 19th European Congress of Endocrinology 2017. A repeat collection of blood was performed for the further characterization of this interference.

Keywords: Immunoassay interference, thyroid function tests, biotin, anti-streptavidin antibody, hyperthyroidism, Graves' disease

Thyroid
Apparent hyperthyroidism caused by biotin-like interference from IgM anti-streptavidin antibodies (DOI: 10.1089/thy.2017.0673)

Abstract:

Background: Exclusion of analytical interference is important when there is discrepancy between clinical and laboratory findings. However, interferences on immunoassays are often mistaken as isolated laboratory artefacts. We characterized and report the mechanism of a rare cause of interference in two patients that caused erroneous thyroid function tests, and also affects many other biotin dependent immunoassays.

Patient findings: Patient 1 was a 77 y female with worsening fatigue while taking carbimazole over several years. Her thyroid function tests however, were not suggestive of hypothyroidism. Patient 2 was a 25 y female also prescribed carbimazole for apparent primary hyperthyroidism. Despite an elevated FT4, the lowest TSH on record was 0.17 mIU/L. In both cases, thyroid function tests performed by an alternative method were markedly different.

Further characterization of both patients' serum demonstrated analytical interference on many immunoassays using the biotin-streptavidin interaction. Sandwich assays (e.g. TSH, FSH, TNT, beta-HCG) were falsely low, while competitive assays (e.g. FT4, FT3, TBII) were falsely high. Pre-incubation of serum with streptavidin microparticles removed the analytical interference, initially suggesting the cause of interference was biotin, however, neither patient had been taking biotin. Instead, a ~100 kDa IgM immunoglobulin with high affinity to streptavidin was isolated from each patient's serum. The findings confirm IgM anti-streptavidin antibodies as the cause of analytical interference.

Summary: We describe two patients with apparent hyperthyroidism as a result of analytical interference caused by IgM anti-streptavidin antibodies.

Conclusion: Analytical interference identified on one immunoassay should raise the possibility of other affected results. Characterization of interference may help to identify other potentially affected immunoassays. In the case of anti-streptavidin antibodies, the pattern of interference mimics that due to biotin ingestion; however, the degree of interference varies between individual assays and between patients.

Thyroid
Apparent hyperthyroidism caused by biotin-like interference from IgM anti-streptavidin antibodies (DOI: 10.1089/thy.2017.0673)
This paper has been peer-reviewed and accepted for publication, but has yet to undergo copyediting and proof correction. The final published version may differ from this proof.

Introduction:

Analytical interference on immunoassays from endogenous antibodies has been reported in 4% of laboratory results (1). With the inclusion of blocking reagents, this is reduced to <1% (1,2); however, its prevalence may be higher in selected assays (3) or in patients with elevated rheumatoid factor (4). These types of interferences are often not easily detected and usually only come to light when there is a discrepancy between clinical and laboratory findings. The detection of interference across multiple immunoassays in a patient remains challenging. As described in cases of high dose biotin ingestion, the combination of falsely high (e.g. FT4, FT3, TBII) and low (e.g. TSH) results may lead to misleading but biologically plausible patterns of laboratory results resembling hyperthyroidism (5,6).

We report two patients with unusual thyroid function tests incongruent to their clinical findings. Neither patient had been taking biotin; however, both demonstrated interference causing falsely high results on competitive assays and falsely low results on sandwich assays. After written consent, we obtained a separate collection of serum from both patients and sought to characterize this interference further.

Patients:

Patient 1 was a 77 y female who presented in 2016 with worsening fatigue on carbimazole 10 mg twice daily for hyperthyroidism. However, clinically she now reported symptoms of hypothyroidism. She had originally been diagnosed with Graves' hyperthyroidism and coeliac disease in 2009. After initial treatment with carbimazole, she was diagnosed with a relapse in 2012. However, the lowest TSH on record was 0.21 mIU/L (reference interval; 0.27-4.2). On examination, there was a small, smooth goiter but no bruit or signs of Graves' ophthalmopathy. Despite symptoms of hypothyroidism, thyroid function tests performed at the time by the Roche Cobas method were: TSH 0.75 mIU/L (0.27-4.2), FT4 12 pmol/L (12-22) and FT3 8.1 pmol/L (3.9-6.8). Thyrotropin binding inhibitory immunoglobulin (TBII) was detected at low levels 3.8 IU/L (< 1.3) using a Roche Cobas assay. Given the unusual presentation, thyroid function tests were repeated on a Siemens Centaur platform, which suggested biochemical hypothyroidism: TSH 37 mIU (0.3-4.0), FT4

7 pmol/L (10-20) and FT3 3.0 pmol/L (3.0-6.5), more in keeping with her symptoms and clinical examination.

Patient 2 was a 25y female who was started on 20 mg/day of carbimazole for apparent hyperthyroidism in 2013 and referred for further assessment. By the time of clinic review, she was in the first trimester of pregnancy and as a result had stopped carbimazole of her own volition. Her symptoms, which were compatible with hyperthyroidism including anxiety, tremor, shortness of breath and loose bowel motions, had gradually improved since pregnancy. On examination there were no signs of thyroid eye disease or goiter; however, a fine tremor was noted. Thyroid function tests performed by the Roche Cobas method at the time demonstrated a high FT4 of 31.9 pmol/L (12-22) and FT3 9.4 pmol/L (3.9-6.8), without suppression of TSH, which was 0.33 mIU/L (0.27-4.2). It was also noted that the lowest TSH on record was 0.17 mIU/L (0.27-4.2). Repeating thyroid function tests using an alternative method (Abbott Architect) demonstrated biochemical euthyroidism and testing of TBII by a radioimmunoassay method (RSR Ltd, United Kingdom), not dependent on biotin-streptavidin interaction, was negative. The overall findings suggested analytical interference, and excluded hyperthyroidism as the cause of her symptoms.

Characterization and identification of interference

Sera from both patients were tested by immunoassays from Roche Cobas (biotin-streptavidin based method) and Siemens Centaur. Their serum was also pre-incubated with streptavidin microparticles (SM) as previously described (7) or with heterophile blocking tubes (HBT; Scantibodies Inc) before retesting by Roche immunoassays. Although differing in magnitude, both patients demonstrated analytical interferences in the same direction on multiple assays, except on the testosterone assay where interference was not detected in patient 2 (Table 1). Varying analytical interference was also observed on immunoassays with initially low or undetectable concentrations following the mixing of additional analyte (Table 2). For all sandwich assays initial results performed on the Roche Cobas appeared to be falsely low while competitive assays appeared to be falsely high. These findings mimic analytical interference caused by ingestion of biotin; however, the degree of interference varied between patient and assay.

Thyroid
Apparent hyperthyroidism caused by biotin-like interference from IgM anti-streptavidin antibodies (DOI: 10.1089/thy.2017.0673)
This paper has been peer-reviewed and accepted for publication, but has yet to undergo copyediting and proof correction. The final published version may differ from this proof.

Thyroid
Apparent hyperthyroidism caused by biotin-like interference from IgM anti-streptavidin antibodies (DOI: 10.1089/thy.2017.0673)

Although pre-incubation with streptavidin microparticles detected interference, neither patient had a history of biotin ingestion. Further, serum biotin was not increased in either patient when tested by an academic research laboratory utilizing tandem-mass spectrometry. We hypothesized an alternative compound with an unusually high affinity to streptavidin as the cause of interference. We eluted this interfering compound from streptavidin microparticles by the addition of citric acid (0.1 M). Following SDS-PAGE, two bands were found (Figure 1). Peptide sequencing of the heavier band (MW: ~100 kDa) identified it as the heavy chain of IgM (See Supplemental Data: Peptide Sequencing). No protein band was eluted following pre-incubation of control patient serum. The findings demonstrate the cause of interference as anti-streptavidin antibodies of IgM isotype in both patients.

Discussion

We describe two patients with unusual thyroid function tests with persistent analytical interference in specimens collected 4 and 7 years since first presentation. Neither had a history of biotin use; however, pre-incubation of each patient's serum with streptavidin microparticles removed the interfering compound. In each patient's serum a ~100 kDa protein with affinity for streptavidin was isolated. Peptide sequencing of this protein confirmed IgM anti-streptavidin antibodies. Similar to interference from biotin, a large number of assays were affected, with falsely high results on competitive assays (e.g. FT4, FT3, TBII, Digoxin) and falsely low results on sandwich assays (e.g. TSH, FSH, Troponin T, HCG). However, the degree of interference otherwise varied unpredictably between assays and patients.

In both patients, analytical interference led to unnecessary treatment with carbimazole, anxiety for the patients and significant confusion for clinicians involved. Carbimazole was discontinued in patient 1 with resolution of the fatigue. Both patients were biochemically euthyroid using immunoassays not utilizing the biotin-streptavidin interaction. They and their doctors were advised that future results from immunoassays using biotin-streptavidin interaction should be interpreted with caution and testing by alternative platforms should be sought.

Thyroid
Apparent hyperthyroidism caused by biotin-like interference from IgM anti-streptavidin antibodies (DOI: 10.1089/thy.2017.0673)
This paper has been peer-reviewed and accepted for publication, but has yet to undergo copyediting and proof correction. The final published version may differ from this proof.

Analytical interferences on immunoassays can be broadly categorized as interferences which alters the measurable concentration of the analyte (e.g. effect of TBG on Total T4 or macroprolactin on prolactin assays) or interferences which alter antibody binding or the assay reaction (8). The causes of this latter category include heterophile antibodies, human anti-animal antibodies, paraproteins, biotin or endogenous antibodies which target reagents (e.g. anti-ruthenium antibodies). In these cases, altered antibody binding may result in analytical interferences on multiple immunoassays. When these interferences are suspected, a range of laboratory procedures can be used to detect analytical interference in immunoassays. In addition to what has been described in this report, serial dilution, the presence of rheumatoid factor, polyethylene glycol precipitation and pre-adsorption with protein A/G may be helpful in selected cases (8). However, no single procedure is sufficiently sensitive and robust to detect all causes of analytical interference. The laboratory findings of unexplained apparent high serum estradiol or drugs such as digoxin (Table 1), which had not been prescribed to the patient, may be helpful as a rapid screening test to detect analytical interferences caused by biotin or anti-streptavidin antibodies.

Recently, multiple laboratories including ours (7), have described the use of streptavidin microparticles in the detection of analytical interference caused by biotin ingestion (9,10). As we demonstrate in this case report, treatment with streptavidin microparticles is also effective at detecting interference caused by anti-streptavidin antibodies. The presence of anti-streptavidin antibodies competes with biotinylated reagents used in immunoassays. As the binding of biotinylated reagents to streptavidin is required to retain signal generating antibody complexes, the presence of anti-streptavidin antibodies leads to a reduction in signal observed in immunoassays in a similar manner to ingested biotin (Figure 2). As the signal intensity is directly proportional to the concentration in sandwich assays, a reduction in signal caused by biotin or anti-streptavidin antibodies translates to artificially low sandwich immunoassay results. In contrast, signal intensity is inversely proportional to concentration on competitive assays, resulting in artificially increased results.

While a history of biotin ingestion or supplement use may identify the cause of interference, this information is often absent or unreliable. Unfortunately, most laboratories do not have the ability to measure serum biotin directly; in these instances, interference caused by biotin could be differentiated from anti-streptavidin antibodies by the significant fluctuations between consecutive laboratory results. This is due to the dissipating interfering effects of biotin within several hours of ingestion with the complete normalization of thyroid hormones and TSH results within 48 hours (7, 11). Alternatively, the combination of heterophile blocking reagents, protein A/G or polyethylene glycol methods were successful in detecting the interfering effects of anti-streptavidin antibodies in five of six patients (Supplemental Table 2), including the two patients from this report.

Multiple immunoassay interference leading to apparent biochemical hyperthyroidism has been described in all previous case reports of analytical interference suspected to be caused by anti-streptavidin antibodies (Supplemental Table 2). While the antigenic source of these antibodies at this stage has not been determined, this interference is likely significantly underrecognized. As demonstrated in a recent study, using a semi-automated research assay which detects IgG anti-streptavidin antibodies, 0.6% of specimens tested for anti-CCP have been identified to be falsely reported as positive (12). It should be noted while the prevalence for anti-streptavidin antibodies has been established for the IgG isotype, to our knowledge, this is the first report of IgM as the isotype of anti-streptavidin antibodies. Protein A/G methods used to detect interference from endogenous antibodies have weak or no affinity towards human IgM, so may not identify such antibodies (13).

The biotin-streptavidin interaction is widely used in clinical immunoassays due to its specificity, flexibility, and high affinity. Analytical interference caused by biotin is increasingly recognized to affect major platforms including analysers from Beckman Coulter, Immunodiagnostic Systems, Vitros, Siemens as well as Roche (see ref 5 for analyzer and assay specific details). Similar to biotin ingestion, interference caused by anti-streptavidin antibodies can affect immunoassays on other platforms (14). Our report demonstrates anti-streptavidin antibodies can mimic interference caused by biotin. This differential diagnosis should be included when interference from biotin is considered,

especially if the patient denies taking biotin. In these cases, clinical correlation and collaboration with the laboratory are critical in the interpretation of results.

Corresponding author: Campbell Kyle

Email: CampbellK@adhb.govt.nz

Running title: Interference from anti-streptavidin antibodies

Acknowledgements: We would like to acknowledge Mr. Eric Thorstensen, Liggins Institute, Faculty of Medical and Health Sciences, University of Auckland for his help with the measurement of serum biotin.

Author's Disclosure Statement: No conflicts of interests

Funding: None

Ethical approval: Consent was obtained from both described patients.

Contributorship: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

Thyroid
Apparent hyperthyroidism caused by biotin-like interference from IgM anti-streptavidin antibodies (DOI: 10.1089/thy.2017.0673)
This paper has been peer-reviewed and accepted for publication, but has yet to undergo copyediting and proof correction. The final published version may differ from this proof.

Thyroid
Apparent hyperthyroidism caused by biotin-like interference from IgM anti-streptavidin antibodies (DOI: 10.1089/thy.2017.0673)
This paper has been peer-reviewed and accepted for publication, but has yet to undergo copyediting and proof correction. The final published version may differ from this proof.

References

1. Bjerner J, Nustad K, Norum LF, Olsen KH, Bormer OP 2002 Immunometric assay interference: incidence and prevention. Clin Chem 48:613-621

2. Preissner CM, Dodge LA, O'Kane DJ, Singh RJ, Grebe SKG 2005 Prevalence of Heterophilic Antibody Interference in Eight Automated Tumor Marker Immunoassays. Clin Chem 51:208-210

3. Preissner CM, O'Kane DJ, Singh RJ, Morris JC, Grebe SKG 2003 Phantoms in the Assay Tube: Heterophile Antibody Interferences in Serum Thyroglobulin Assays. The J Clin Endocrinol Metab 88:3069-3074

4. Marks V 2002 False-positive immunoassay results: a multicenter survey of erroneous immunoassay results from assays of 74 analytes in 10 donors from 66 laboratories in seven countries. Clin Chem 48:2008-2016

5. Piketty ML, Polak M, Flechtner I, Gonzales-Briceno L, Souberbielle JC 2017 False biochemical diagnosis of hyperthyroidism in streptavidin-biotin-based immunoassays: the problem of biotin intake and related interferences. Clin Chem Lab Med 55:780-788

6. Elston MS, Sehgal S, Du Toit S, Yarndley T, Conaglen JV 2016 Factitious Graves' disease due to biotin immunoassay interference - A case and review of the literature. J Clin Endocrinol Metab 101:3251-3255

7. Lam L, Kyle CV 2017 A simple method to detect biotin interference on immunoassays. Clin Chem Lab Med 55:e104-e106

8. Tate J, Ward G. Interferences in Immunoassay 2004 Clin Biochem Rev 25:105-120

9. Trambas C, Lu Z, Yen T, Sikaris K 2017 Depletion of biotin using streptavidin-coated microparticles: a validated solution to the problem of biotin interference in streptavidin-biotin immunoassays [Published online ahead of print January 1 2017]. Ann Clin Biochem DOI: 10.1177/0004563217707783

10. Piketty ML, Prie D, Sedel F, Bernard D, Hercend C, Chanson P, Souberbielle JC 2017 High-dose biotin therapy leading to false biochemical endocrine profiles: validation of a simple method to overcome biotin interference. Clin Chem Lab Med 55:817-825

11. Kummer S, Hermsen D, Distelmaier F 2016 Biotin Treatment Mimicking Graves' Disease. N Engl J Med 375:704-706.

12. Berth M, Willaert S, De Ridder C 2018 Anti-streptavidin IgG antibody interference in anti-cyclic citrullinated peptide (CCP) IgG antibody assays is a rare but important cause of false-positive anti-CCP results. Clin Chem Lab Med DOI:10.1515/cclm-2017-1153. [Epub ahead of print]

13. Darcy E, Leonard P, Fitzgerald J, Danaher M, O'Kennedy R. Purification of Antibodies Using Affinity Chromatography. In: Walls D, Loughran ST, eds. Protein Chromatography: Methods and Protocols. Humana Press, Totowa, NJ, pp2011:369-382.

14. Rulander NJ, Cardamone D, Senior M, Snyder PJ, Master SR 2013 Interference from anti-streptavidin antibody. Arch Pathol Lab Med 137:1141-1146

Thyroid
Apparent hyperthyroidism caused by biotin-like interference from IgM anti-streptavidin antibodies (DOI: 10.1089/thy.2017.0673)
This paper has been peer-reviewed and accepted for publication, but has yet to undergo copyediting and proof correction. The final published version may differ from this proof.

Table 1: Detection and characterization of interference of two patients with anti-streptavidin antibodies

	Patient 1					Patient 2				
Assay (Reference Interval)	**Roche**			**Siemens**	**Direction of**	**Roche**			**Siemens**	**Direction of**
	Neat	**SMT**	**HBT**	**Neat**	**interference**	**Neat**	**SMT**	**HBT**	**Neat**	**interference**
Competitive assays										
FT4 (12-22 pmol/L)	28	16	22	16	↑	>100	22	28	21	↑↑↑
FT3 (3.9-6.8 pmol/L)	9.0	4.7	6.7	4.3	↑	19.5	5.3	7.8	5.2	↑↑↑
Estradiol pmol/L (< 180 pmol/L*)	243	< 92	115	76	↑↑↑	377	219	218	130	↑
Testosterone (0-1.8 nmol/L)	3.3	< 0.09	0.2	0.3	↑↑↑	1.0	1.2	1.0	1.0	—
25OH-Vit D (50-150 nmol/L)	45	44	62	NA	—	98	95	116	NA	—
TBII (< 1.3 IU/L)	7.1	< 0.3	NA	NA	↑↑↑	3.2	< 0.3	NA	NA	↑↑↑
Digoxin nmol/L	NA	NA	NA	NA		3.07	NA	NA	NA	↑↑↑

Thyroid
Apparent hyperthyroidism caused by biotin-like interference from IgM anti-streptavidin antibodies (DOI: 10.1089/thy.2017.0673)
This paper has been peer-reviewed and accepted for publication, but has yet to undergo copyediting and proof correction. The final published version may differ from this proof.

Thyroid
Apparent hyperthyroidism caused by biotin-like interference from IgM anti-streptavidin antibodies (DOI: 10.1089/thy.2017.0673)
This paper has been peer-reviewed and accepted for publication, but has yet to undergo copyediting and proof correction. The final published version may differ from this proof.

(0.6-2.0 nmol/L)									
Sandwich Assays									
TSH (0.27-4.2 mIU/L)	0.23	0.68	0.42	0.85	↓↓	0.40	0.78	0.61	0.73	↓
LH (> 15 IU/L*)	26	33	27	31	↓	4.2	6.1	4.5	4.5	↓
FSH (> 20 IU/L*)	41	58	42	75	↓	1.9	6.3	2.9	6.5	↓
PTH (1.7-7.3 pmol/L)	0.80	4.0	1.8	NA	↓↓↓	< 0.64	2.1	0.75	NA	↓↓↓

Table 2: Interference from anti-streptavidin antibodies by mixing studies

Assay	Patient 1			Patient 2		
	Neat	SM	Direction of interference	Neat	SM	Direction of interference
Sandwich						
HS Troponin T (< 15 ng/L)	189	294	↓	300	296	—
NT-pro-BNP (< 35 pmol/L)	31	31	—	33	32	—
HCG (Beta+total) (< 14 IU/L)	211	334	↓	165	326	↓↓
Competitive						
Progesterone (< 6 nmol/L)	37	28	↑	37	24	↑

Thyroid
Apparent hyperthyroidism caused by biotin-like interference from IgM anti-streptavidin antibodies (DOI: 10.1089/thy.2017.0673)
This paper has been peer-reviewed and accepted for publication, but has yet to undergo copyediting and proof correction. The final published version may differ from this proof.

Thyroid
Apparent hyperthyroidism caused by biotin-like interference from IgM anti-streptavidin antibodies (DOI: 10.1089/thy.2017.0673)
This paper has been peer-reviewed and accepted for publication, but has yet to undergo copyediting and proof correction. The final published version may differ from this proof.

Figure Legends

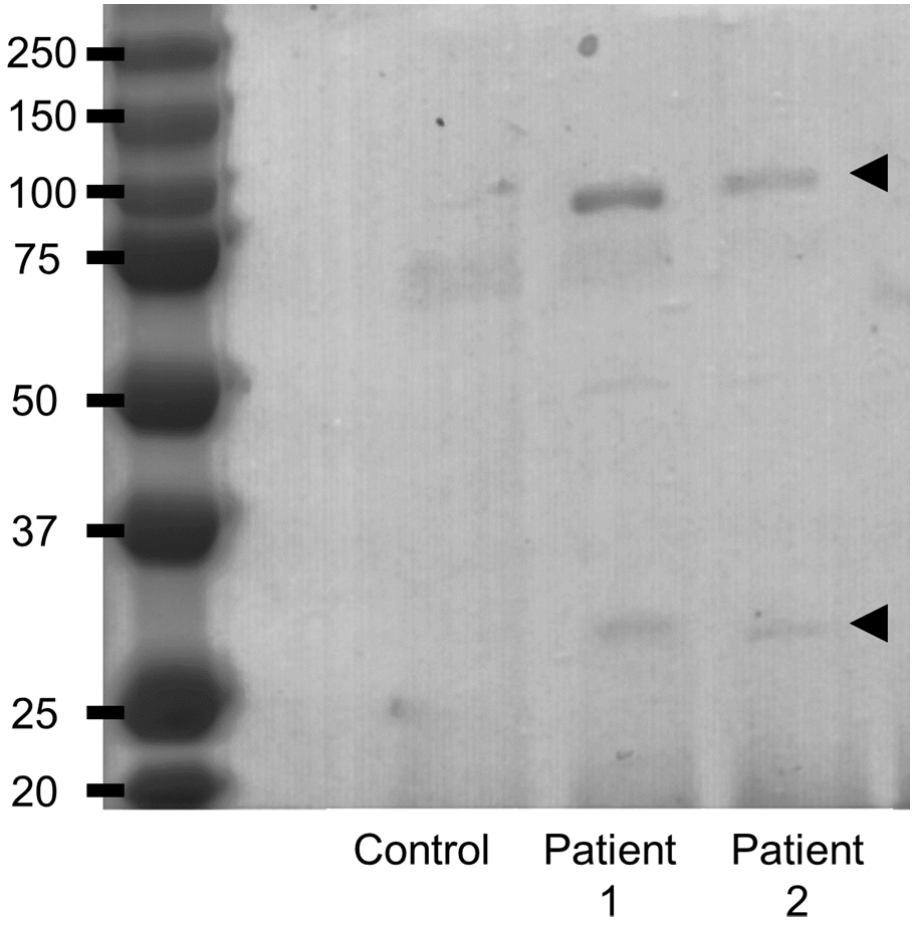

Figure 1: Molecular weight of protein bound to streptavidin microparticles in two patients with anti-streptavidin antibodies.

SDS-PAGE of protein eluted from streptavidin microparticles identified a heavy (~100kDa) and light band (25-37kDa) corresponding to IgM heavy chain and light chain (arrow heads). Elution of protein was carried out by incubation of streptavidin microparticles with 0.1M citric acid at 56^{o}C for 15 minutes following 3 washes with PBS. Protein was visualized by staining the gel with Coomassie blue. IgM heavy chain was confirmed in both patients by proteolytic digestion and peptide sequencing (See Supplemental Data: Peptide Sequencing).

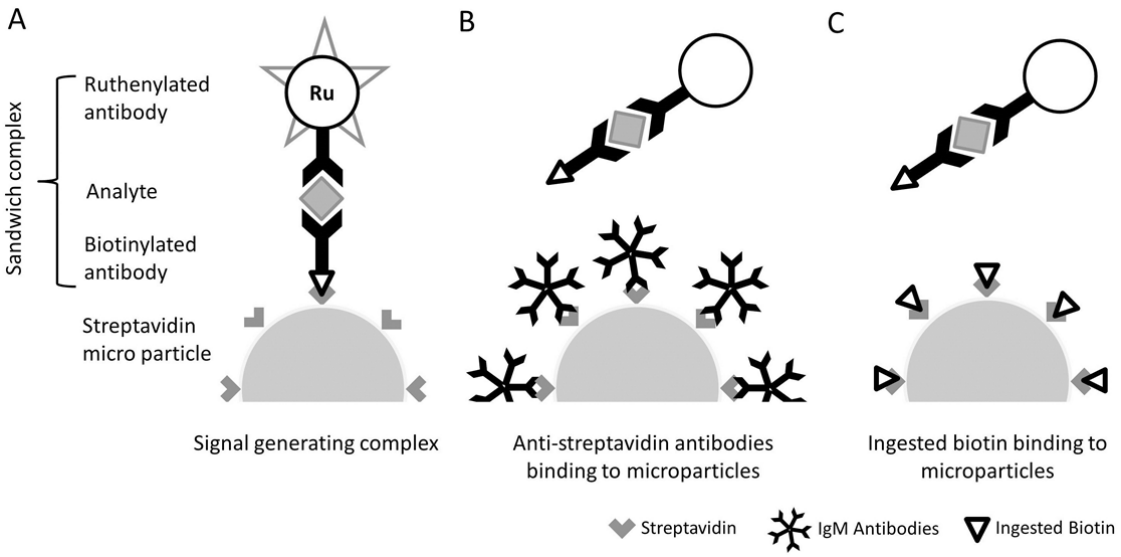

Figure 2: Mechanism of interference caused by anti-streptavidin antibodies and biotin on sandwich assays

Signal can only be generated when biotinylated reagents bind to streptavidin microparticles on Roche Cobas electrochemiluminescence immunoassays (A). Anti-streptavidin antibodies and biotin compete with biotinylated reagents preventing the normal assay reaction due to the formation of other streptavidin complexes (B & C). Both biotin and anti-streptavidin antibodies reduce the number of signal generating complexes leading to falsely low results on sandwich assay (e.g. TSH) and falsely high results on competitive assays (e.g. FT4 and FT3). These interferences can be removed by treating the serum with streptavidin microparticles before assaying.

Thyroid
Apparent hyperthyroidism caused by biotin-like interference from IgM anti-streptavidin antibodies (DOI: 10.1089/thy.2017.0673)
This paper has been peer-reviewed and accepted for publication, but has yet to undergo copyediting and proof correction. The final published version may differ from this proof.

Thyroid
Apparent hyperthyroidism caused by biotin-like interference from IgM anti-streptavidin antibodies (DOI: 10.1089/thy.2017.0673)
This paper has been peer-reviewed and accepted for publication, but has yet to undergo copyediting and proof correction. The final published version may differ from this proof.

Supplemental Data: Peptide Sequencing

Peptide sequencing of gel bands:

Individual gel bands were cut in to small $1mm^3$ pieces and destained with a 50:50 (v/v) solution of acetonitrile and 50 mM NH_4HCO_3 before being dehydrated in acetonitrile. The gel pieces after drying were reduced in 10mM dithiothreitol (Bio-Rad) for 20 minutes at 56^oC and incubated in a solution containing 50 mM iodoacetamide (GE Healthcare) and 50 mM NH_4HCO_3 at room temperature for 30 minutes in the dark. The gel pieces were dehydrated in acetonitrile, dried and digested overnight in a solution containing 12.5ng/uL trypsin (Promega, Madison, WI, USA) and 50mM NH_4HCO_3. Digests were acidified to pH 3 by the addition of 10% formic acid (Scharlau).

Digested specimens were injected onto a 0.3x 10mm trap column packed with Reprosil C18 media (Dr Maisch) and desalted before being separated on a 0.075 x 150 mm picofrit column (New Objective) packed in-house with Reprosil C18 media using a 45 minute gradient. The picofrit spray was directed into a TripleTOF 6600 Quadrupole-Time-of-Flight mass spectrometer (Sciex) and MS/MS performed on the most abundant multiply-charged peptides using a total cycle time of ~2 seconds. The mass spectrometer and UHPLC system were under the control of the Analyst TF 1.7 software package (Sciex).

The resulting data were searched against a database containing the Uniprot human sequences using ProteinPilot version 5.0 (Sciex). Search parameters were as follows: Sample Type, identification; Search Effort, Thorough; Cys Alkylation, Iodoacetamide; Digestion, Trypsin; ID Focus, Biological modifications and Amino Acid substitutions.

References:

1 Khieng V, Stevens C. Vitamin D Toxicity?: a case study. *N Z J Med Lab Sci.* 2010:44-50

2 Rulander NJ, Cardamone D, Senior M, Snyder PJ, Master SR. Interference from anti-streptavidin antibody. *Arch Pathol Lab Med.* 2013; 137:1141-1146

3 Mattman A, DeMarco ML, Wong S, Holmes DT, Lee J. Grave Clinicopathologic Correlation: A Case of Hyperthyroxinemia. *J Appl Lab Med.* 2016; 1:310-314

4 Peltier L, Massart C, Moineau MP, Delhostal A, Roudaut N. Anti-streptavidin interferences in Roche thyroid immunoassays: a case report. *Clin Chem Lab Med.* 2016; 54:e11-e14

Thyroid
Apparent hyperthyroidism caused by biotin-like interference from IgM anti-streptavidin antibodies (DOI: 10.1089/thy.2017.0673)
This paper has been peer-reviewed and accepted for publication, but has yet to undergo copyediting and proof correction. The final published version may differ from this proof.

Supplemental Tables:

Supplemental Table 1: Streptavidin microparticle treatment – Control experiments

	A. Roche		B. Siemens					
Assay	**Control**	**Control (SM)**	**Control**	**Control (SM)**	**#1**	**#1 (SM)**	**#2**	**#2 (SM)**
FT4 (pmol/L)	19	19	16	16	16	15	21	19
FT3 (pmol/L)	5.3	5.1	5.0	4.9	4.3	4.3	5.2	5.1
TSH (mIU/L)	0.83	0.82	0.87	0.91	0.85	0.85	0.73	0.73
Testosterone (nmol/L)	9.7	10.8	11	12	0.3	0.4	1.0	1.1
FSH (IU/L)	4.7	4.5	5.0	4.9	75	61	6.5	7.6

A. Results of immunoassays on Roche Cobas on control patient (Control) before and after streptavidin microparticle preincubation (SM).

B. Results of immunoassays on Siemens Advia Centaur on control patient and on two patients described in text (#1, #2), before and following treatment with streptavidin microparticles (SM).

Thyroid
Apparent hyperthyroidism caused by biotin-like interference from IgM anti-streptavidin antibodies (DOI: 10.1089/thy.2017.0673)

Supplemental Table 2: Description of possible analytical interference from anti-streptavidin antibodies in previous case reports

Clinical presentation and initial biochemistry findings	Clinical Consequence	Tests to detect interference	Interference detected	Ref
36 y F fatigue and weight loss Vitamin D ↑↑↑ Other affected assays: PTH↓↓↓, FT4↑, TSH↓↓	- No adverse clinical consequence reported	HBT Streptavidin[1]	No Yes	(1)
61 y M increasing fatigue following treatment for biochemical findings of hyperthyroidism TT4↑↑, TSH[2] ↓↓↓. Other affected assays: Roche: ↑↑Cortisol, ↓↓LH, ↓↓↓FSH, ↓↓Prolactin	- Treated with methimazole.	HBT Protein A Streptavidin Mouse Ig	Yes Yes Yes No	(2)
16 y F fatigue and coldness FT4 ↑, FT3 ↑, TSH ↓, anti-TSHR ↑↑	- No adverse clinical consequence reported	HBT Streptavidin	Yes Yes	(3)
17 y F with amenorrhea, FT4 ↑↑, FT3↑↑, TSH ↓↓↓, anti-TSHR↑↑↑, Anti-TPO↑↑↑ and Anti-TG↑↑	- Treated with Thiamazol	HBT Streptavidin[1] PEG	No Yes Yes	(4)

Four previous case reports of possible analytical interference from anti-streptavidin antibodies. In all cases, initial results were identified on a Roche analyser. [1]Testing performed by Research & Development at Roche Diagnostics. [2]Interference was also

demonstrated on Ortho Vitros assay. ↓ - Falsely low. ↑ - Falsely high. N.I. – No interference detected.

Thyroid
Apparent hyperthyroidism caused by biotin-like interference from IgM anti-streptavidin antibodies (DOI: 10.1089/thy.2017.0673)
This paper has been peer-reviewed and accepted for publication, but has yet to undergo copyediting and proof correction. The final published version may differ from this proof.

Peptide Sequencing Table 1: Peptide sequences of protein bound to streptavidin microparticles from patient #1 matching to IgM heavy chain.

Confidence Score	Sequence	Modifications	ppm error	Obs MW	Theor MW
Ig mu chain C region OS=Homo sapiens GN=IGHM PE=1 SV=3 sp|P01871|IGHM_HUMAN					
55	ATGFSPR		-0.96	734.3705	734.3712
99	DGFFGNPR		-0.76	908.4135	908.4141
99	DVMQGTDEHVVCK	Oxidation(M)@3; Carbamidomethyl(C)@12	0.67	1532.6610	1532.6599
99	EGKQVGSGVTTDQVQAEAK	Glu->pyro-Glu@N-term; GG(K)@3	0.29	2026.9923	2026.9919
99	EGKQVGSGVTTDQVQAEAKESGPTTYK	Carbamidomethyl@N-term; Carbamidomethyl(K)@19	1.41	2908.4094	2908.4050
99	EKNVPLPVIAELPPK		0.96	1642.9674	1642.9658
56	EQLNLR	Glu->pyro-Glu@N-term	-2.65	753.4115	753.4133
99	ESDWLGQSMFTCR	Gly->Ser@6; Carbamidomethyl(C)@12	0.12	1645.6866	1645.6865

Thyroid
Apparent hyperthyroidism caused by biotin-like interference from IgM anti-streptavidin antibodies (DOI: 10.1089/thy.2017.0673)
This paper has been peer-reviewed and accepted for publication, but has yet to undergo copyediting and proof correction. The final published version may differ from this proof.

98	ESGPTTYK	Glu->pyro-Glu@N-term	-1.71	863.4011	863.4025
99	ESGPTTYKVTSTLTIK	Carbamidomethyl(K)@8	0.94	1781.9427	1781.9410
99	FTCTVTHTDLPSPLK	Carbamidomethyl(C)@3	1.82	1715.8584	1715.8552
95	GFPSVLR		-0.77	774.4383	774.4388
99	GGKYAATSQVLLPSK	Carbamidomethyl@N-term	1.35	1575.8640	1575.8621
98	GQPLSPEK		0.46	854.4503	854.4498
99	GQPLSPEKYVTSAPMPEPQAPGR	Carbamidomethyl(K)@8; Oxidation(M)@15	1.38	2509.2307	2509.2271
99	GVALHRPDVY		0.17	1125.5931	1125.5931
99	GVALHRPDVYLLPPAR		-1.65	1773.0018	1773.0050
99	LICQATGFSPR	Carbamidomethyl(C)@3	-1.60	1248.6265	1248.6285
99	NVPLPVIAELPPK		1.92	1385.8308	1385.8282
99	PDVYLLPPAR		-0.16	1139.6339	1139.6339

Thyroid
Apparent hyperthyroidism caused by biotin-like interference from IgM anti-streptavidin antibodies (DOI: 10.1089/thy.2017.0673)
This paper has been peer-reviewed and accepted for publication, but has yet to undergo copyediting and proof correction. The final published version may differ from this proof.

99	PKGVALHRPDVYLLPPAR	Carbamidomethyl@N-term	0.27	2055.1750	2055.1741
99	QIQVSWLR	Gln->pyro-Glu@N-term	-1.63	1011.5485	1011.5502
99	QTISRPK	Gln->pyro-Glu@N-term	-4.25	811.4518	811.4552
99	QVGSGVTTDQVQAEAK	Gln->pyro-Glu@N-term	-0.30	1599.7734	1599.7740
99	QVGSGVTTDQVQAEAKESGPTTYK	Carbamidomethyl(K)@16	1.70	2537.2288	2537.2246
99	REGKQVGSGVTTDQVQAEAK	Arg->Asn@1	1.35	2045.0052	2045.0026
99	SKLICQATGFSPR	Carbamidomethyl(C)@5	0.11	1463.7556	1463.7555
99	TSAPMPEPQAPGR	Dethiomethyl(M)@5	-2.31	1289.6334	1289.6365
99	VSVFVPPR		-0.34	899.5226	899.5229
99	VSVFVPPRDGFFGNPR		0.62	1789.9275	1789.9264
99	VTSTLTIK		-2.24	861.5153	861.5172

Thyroid
Apparent hyperthyroidism caused by biotin-like interference from IgM anti-streptavidin antibodies (DOI: 10.1089/thy.2017.0673)
This paper has been peer-reviewed and accepted for publication, but has yet to undergo copyediting and proof correction. The final published version may differ from this proof.

Peptide Sequencing Table 2: Peptide sequences of protein bound to streptavidin microparticles patient #2 matching to IgM heavy chain.

Confidence Score	Sequence	Modifications	ppm error	Obs MW	Theor MW
Ig mu chain C region OS=Homo sapiens GN=IGHM PE=1 SV=2 tr|A0A087WYJ9|A0A087WYJ9_HUMAN; tr|A0A075B6N9|A0A075B6N9_HUMAN					
99	AATSQVLLPSK		1.62	1113.6412	1113.6394
99	AIPPSFASIFLTK		-0.74	1390.7850	1390.7860
99	ALHRPDVY		-1.94	969.5013	969.5032
99	ASIFLTK		-7.09	778.4533	778.4589
99	CTVTHTDLPSPLK	Carbamidomethyl(C)@1	-6.76	1467.7292	1467.7391
99	CVPDQDTAIR	Carbamidomethyl(C)@1	-2.00	1173.5425	1173.5448
99	DGFFGNPR		-2.46	908.4119	908.4141
99	DVMQGTDEHVV	Oxidation(M)@3	-4.82	1244.5283	1244.5343
99	DVMQGTDEHVVCK	Carbamidomethyl(C)@12	-1.26	1516.6	1516.66

Thyroid
Apparent hyperthyroidism caused by biotin-like interference from IgM anti-streptavidin antibodies (DOI: 10.1089/thy.2017.0673)
This paper has been peer-reviewed and accepted for publication, but has yet to undergo copyediting and proof correction. The final published version may differ from this proof.

				631	50
99	EGKQVGSGVTTDQVQAEAK		-1.92	1930.9557	1930.9596
99	EKNVPLPVIAELPPK	Carbamidomethyl@N-term	-1.05	1699.9855	1699.9873
99	EQLNLR		-4.57	771.4204	771.4239
99	ESDWLGQSMFTCR	Gly->Ser@6; Carbamidomethyl(C)@12	0.82	1645.6879	1645.6865
99	ESGPTTYK		-6.03	881.4079	881.4131
99	ESGPTTYKVTSTLTIK		-9.46	1724.9031	1724.9197
99	FASIFLTK		-5.39	925.5223	925.5273
99	FTCTVTHTDLPSPLK	Cys->Ser@3; Dehydrated(T)@4	1.30	1624.8483	1624.8461
99	GFPSVLR		0.95	774.4395	774.4388
99	GGKYAATSQVLLPSK	Carbamidomethyl@N-term; Tyr->Phe@4	-4.44	1559.8602	1559.8672
99	GLTFQQNASSMCVPDQDTAIR	dHex(1)Hex(5)HexNAc(4)NeuAc(1)(N)@7; Oxidation(M)@11;	3.06	4413.8105	4413.7979

Thyroid
Apparent hyperthyroidism caused by biotin-like interference from IgM anti-streptavidin antibodies (DOI: 10.1089/thy.2017.0673)
This paper has been peer-reviewed and accepted for publication, but has yet to undergo copyediting and proof correction. The final published version may differ from this proof.

Thyroid
Apparent hyperthyroidism caused by biotin-like interference from IgM anti-streptavidin antibodies (DOI: 10.1089/thy.2017.0673)
This paper has been peer-reviewed and accepted for publication, but has yet to undergo copyediting and proof correction. The final published version may differ from this proof.

		Carbamidomethyl(C)@12			
99	GQPLSPEK		-11.63	854.4398	854.4498
99	GVALHRPDVY		-0.75	1125.5923	1125.5931
99	GVALHRPDVYLLPPAR	Carbamidomethyl@N-term	-2.55	1830.0217	1830.0265
99	GVTTDQVQAEAK		-1.65	1245.6180	1245.6201
99	HRPDVYLLPPAR		-1.57	1432.7916	1432.7939
99	HTDLPSPLK		-4.82	1006.5399	1006.5447
99	KVTSTLTIK		-5.75	989.6064	989.6121
99	LICQATGFSPR	Carbamidomethyl(C)@3	1.42	1248.6302	1248.6285
57	LLPSK		-5.08	556.3557	556.3585
66	LSPEK		-9.23	572.3117	572.3170
99	NVPLPVIAELPPK	Cation:Na(E)@9	3.19	1407.8147	1407.8102

Thyroid
Apparent hyperthyroidism caused by biotin-like interference from IgM anti-streptavidin antibodies (DOI: 10.1089/thy.2017.0673)
This paper has been peer-reviewed and accepted for publication, but has yet to undergo copyediting and proof correction. The final published version may differ from this proof.

99	NVPLPVIAELPPKVSVFVPPR	Carbamidomethyl(K)@13	1.89	2324.3665	2324.3621
99	NVSLVMSDTAGTCY	Oxidation(M)@6; Carbamidomethyl(C)@13	-0.65	1532.6477	1532.6487
99	PDVYLLPPAR		-0.89	1139.6329	1139.6339
98	PKGVALHRPDVY		-6.70	1350.7317	1350.7408
99	PKGVALHRPDVYLLPPAR	Lys->Allysine(K)@2	1.30	1997.1238	1997.1211
99	PLPVIAELPPK		-1.04	1172.7157	1172.7169
97	PLSPEK		-3.35	669.3676	669.3698
99	QATGFSPR		-3.35	862.4269	862.4297
99	QIQVSWLR	Gln->pyro-Glu@N-term	3.32	1011.5535	1011.5502
64	QLNLR	Gln->pyro-Glu@N-term	-7.34	625.3502	625.3547
99	QNGEAVK	Gln->pyro-Glu@N-term	1.41	727.3511	727.3501
68	QTISR		1.39	603.3350	603.3340

99	QTISRPK	Gln->pyro-Glu@N-term	-4.38	811.4517	811.4552
99	QVGSGVTTDQVQAEAK	Gln->pyro-Glu@N-term	-5.33	1599.7657	1599.7740
99	QVGSGVTTDQVQAEAKESGPTTYK	Carbamidomethyl(K)@16	4.09	2537.2351	2537.2246
99	SAPMPEPQAPGR		-4.43	1236.5867	1236.5922
98	SDISSTR		-5.44	764.3622	764.3665
99	SKLICQATGFSPR	Carbamidomethyl@N-term; Carbamidomethyl(C)@5	-5.33	1520.7688	1520.7770
99	SMCVPDQDTAIR	Carbamidomethyl(C)@3	-5.24	1391.6101	1391.6173
99	SPADVFVQWMQR		-2.36	1462.6992	1462.7028
99	SQVLLPSK		-0.66	870.5168	870.5175
99	STGKPTLY		-3.00	865.4520	865.4545
99	STGKPTLYN		-5.41	979.4922	979.4974
99	STGKPTLYNVSLVMSDT	Oxidation(M)@14;	3.07	2217.0	2217.02

Thyroid
Apparent hyperthyroidism caused by biotin-like interference from IgM anti-streptavidin antibodies (DOI: 10.1089/thy.2017.0673)
This paper has been peer-reviewed and accepted for publication, but has yet to undergo copyediting and proof correction. The final published version may differ from this proof.

	AGTC	Carbamidomethyl(C)@21		361	93
99	STGKPTLYNVSLVMSDTAGTCY	Oxidation(M)@14; Carbamidomethyl(C)@21	5.45	2380.1055	2380.0928
88	SVFVPPR		-1.67	800.4531	800.4545
69	SVLR		-2.27	473.2951	473.2962
99	TCVVAHEALPNR	Methyl(T)@1; Carbamidomethyl(C)@2	-0.59	1379.6971	1379.6980
99	TSAPMPEPQAPGR	Dethiomethyl(M)@5	-5.07	1289.6299	1289.6365
99	TSQVLLPSK		-4.11	971.5611	971.5651
96	TSTLTIK		-4.74	762.4451	762.4487
99	TVTHTDLPSPLK		-4.79	1307.7021	1307.7085
99	VFAIPPSF		-3.09	876.4719	876.4745
99	VFAIPPSFAS		0.11	1034.5438	1034.5437
99	VFAIPPSFASIFLTK		4.18	1636.9297	1636.9229

Thyroid
Apparent hyperthyroidism caused by biotin-like interference from IgM anti-streptavidin antibodies (DOI: 10.1089/thy.2017.0673)
This paper has been peer-reviewed and accepted for publication, but has yet to undergo copyediting and proof correction. The final published version may differ from this proof.

99	VFAIPPSFASIFLTKSTK	Carbamidomethyl(K)@15	5.73	2010.1305	2010.1190
99	VGSGVTTDQVQAEAK		-4.72	1488.7351	1488.7419
99	VPLPVIAELPPK		-2.45	1271.7822	1271.7853
99	VQHPNGNK		1.21	892.4527	892.4515
78	VQWMQR		-3.37	846.4142	846.4171
99	VSVFVPPR		0.12	899.5231	899.5229
99	VTSTLTIK		1.23	861.5182	861.5172
99	VVAHEALPNR		-13.57	1104.5891	1104.6040
99	YAATSQVLLPSK		1.24	1276.7043	1276.7028
66	YFAH		-4.66	536.2358	536.2383
99	YVTSAPMPEPQAPGR	Carbamidomethyl@N-term	4.47	1656.8004	1656.7930

Thyroid
Apparent hyperthyroidism caused by biotin-like interference from IgM anti-streptavidin antibodies (DOI: 10.1089/thy.2017.0673)
This paper has been peer-reviewed and accepted for publication, but has yet to undergo copyediting and proof correction. The final published version may differ from this proof.