Review

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False biochemical diagnosis of hyperthyroidism in streptavidin-biotin-based immunoassays: the problem of biotin intake and related interferences

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Abstract: Immunoassays are now commonly used for hormone measurement, in high throughput analytical platforms. Immunoassays are generally robust to interference. However, endogenous analytical error may occur in some patients; this may be encountered in biotin supplementation or in the presence of anti-streptavidin antibody, in immunoassays involving streptavidin-biotin interaction. In these cases, the interference may induce both false positive and false negative results, and simulate a seemingly coherent hormonal profile. It is to be feared that this type of errors will be more frequently observed. This review underlines the importance of keeping close interactions between biologists and clinicians to be able to correlate the hormonal assay results with the clinical picture.

Keywords: anti-streptavidin antibody; biotin; immunoassay; interference.

Introduction

Immunoassays are widely used and developed in clinical laboratories, because of performant technologies that also allow high throughput measurements. Their full automation confers many analytical advantages: good sensitivity, precision, and rapid measurement of serum hormone levels as well as of many other analytes. Although generally robust, all immunoassays remain vulnerable to an occasional endogenous analytical error in some patients. Worse, some of these interferences may simulate a coherent hormonal profile, with the unfortunate combination of falsely low and falsely elevated test results. This is related to the two different assay formats in use in immunoassays, in which inhibition of immune complexes separation, whatever the cause, will lead to opposite erroneous results. This opposite impact may mimic an endogenous endocrine pathological condition, and lead to a seemingly consistent biochemical diagnosis of hyperthyroidism [1–3], as well as vitamin D intoxication [4], or parathyroid hormone (PTH) suppression [5]. Some new therapeutic protocols involving high doses of biotin (300 mg) in neurology have very recently attracted our attention to this problem [1–3, 6]. Therefore there is a concern that this type of double-sided errors could be more and more frequently encountered.

Precise understanding of the analytical principles is necessary in the correct interpretation of laboratory results and suspicion of erroneous results. The mechanisms involved and the cases reported will be examined, along with the strategies to be employed to suspect and overcome them.

Mechanisms of interference in the two assay formats

The “sandwich” immunometric assay design is the principle used to measure large molecules, such as thyroid stimulating hormone (TSH), pituitary glycoprotein hormones, human chorionic gonadotropin (HCG), PTH, insulin-like growth factor-1 (IGF1), insulin, thyroglobulin, C-peptide. These assays are also termed “two-site immunoassays” because of a double epitopic recognition. TSH (for example) is “sandwiched” between two different antibodies: one is labeled with a signal to be measured (luminescent or fluorescent compound, enzyme, isotope). The other one, named the “capture antibody” will allow

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the separation of the immune complexes on a solid phase. The higher the concentration of TSH, the higher the signal linked to the solid phase will be. Thus, the calibration curve is an increasing curve, because the signal bound to the solid phase is directly proportional to the concentration of TSH in the sample. This assay format conveys good sensitivity, specificity and robustness.

The sandwich format is not suitable for small molecules, whose structures are too small to allow two antibodies to bind without steric hindrance [all steroids, T3 and T4, either total or free fraction, 25 hydroxy vitamin D (25OH D)]. Many assays of antibodies are also designed according to the "competitive" format [antibody to TSH receptor (TRAb), anti-TPO, anti-thyroglobulin]. Taking cortisol measurement as an example, the sample is incubated with an anti-cortisol antibody and a tracer (cortisol labeled with a measurable signal: enzyme, fluorescent or luminescent compound, isotope). A competition occurs between the cortisol of the sample and the labeled cortisol for the antibody binding sites. These cortisol molecules bound to the antibody are captured on a solid phase likewise in the sandwich assays. Unlike sandwich assays, the higher the cortisol concentration in the sample, the lower the signal-labeled cortisol bound to the antibody. Thus the calibration curve is a decreasing curve, because the signal bound to the solid phase is inversely proportional to the cortisol concentration in the sample.

At the end of the incubation, in both assay formats, the hormone bound to the antibody reagent is separated from the reaction milieu. Then the signal bound to these immune complexes is measured. The choice of the methodology of separation of immune complexes is specific to each reagent manufacturer, for each analyte. To help separating out the reagent antibodies, the streptavidin-biotin interaction provides an efficient and convenient method (biotinylated antibody or biotinylated antigen are separated on a streptavidin-linked solid phase). For each assay, a calibration curve is constructed using definite concentrations of the analyte. Any interfering substance that prevents the separation of immune complexes will potentially affect multiple immunoassays: biotin, for example, will result in falsely elevated or depressed hormone levels, in competitive (Figure 1A) or non competitive sandwich assays (Figure 1B), respectively, in immunoassays involving streptavidin-biotin separation.

Depending on the design of the immunoassays considered, both positive and negative analytical errors may occur for the same patient, the combination of which may mimic a pathological hormonal condition. Therefore, elevated free T3 (FT3), free T4 (FT4) and TRAb concentrations associated with a low TSH level can be expected, as well as a high cortisolemia together with a blunted adrenocorticotropic hormone (ACTH) concentration, an elevated concentration of 25OH vitamin D associated with a suppressed PTH level (Table 1). Even if in most cases, clinical presentation is not consistent with the biochemical hormonal profile, this phenomenon may lead to delayed therapy because of confusing biochemical picture due to erroneous results, inappropriate explorations and generate much stress for the patients. In these circumstances, the discrepancy between the clinical picture and the laboratory results prompted reassessment of the hormone assays, and led to identification of an analytical artifact in the case reported: either biotin therapy, or anti-streptavidin antibody.

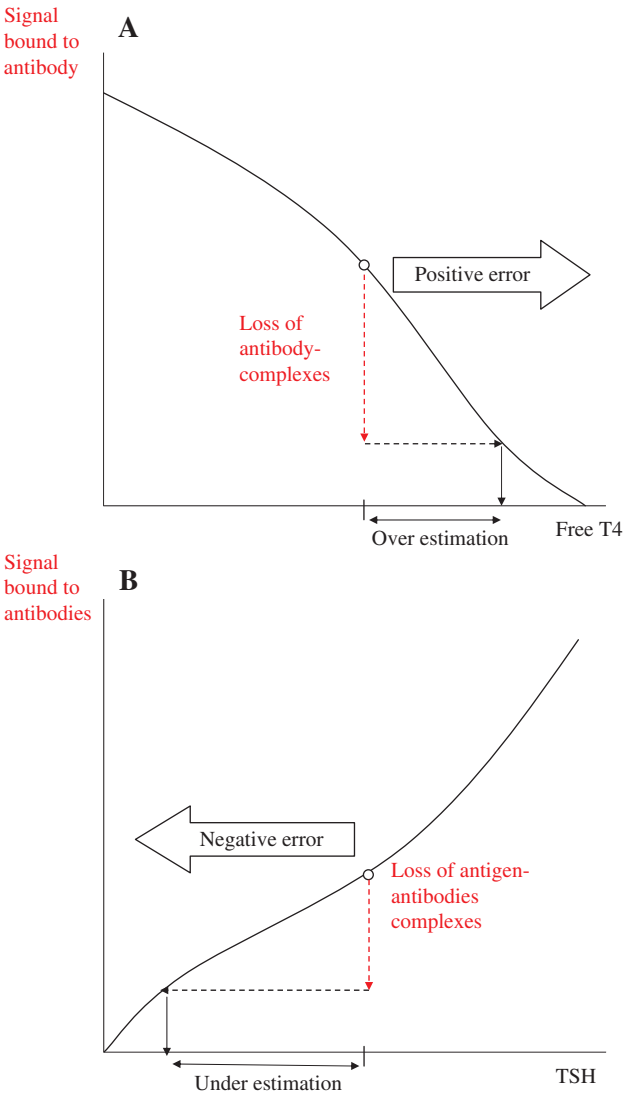

Figure 1: Mechanisms of interference. Inhibition of immune complexes separation leading to a loss of antigen-antibodies complexes, induces an overestimation of the analyte concentration in a competitive assay (A) and underestimation of the analyte concentration in a sandwich assay (B).

Table 1: Potential errors in hormone testing, due to biotin interference.

Biological presentation	Erroneous diagnosis	Potential risk/adverse consequence	Already reported (if Yes, see Table 3)
Thyrotropic axis – Low TSH – High FT3, FT4 – High antibodies	– Hyperthyroidism – Graves' disease	– Anti-thyroid drug therapy – Inappropriate management	Yes
Calcium/phosphate metabolism – High 25OHD – Low PTH	– Vitamin D intoxication – PTH suppression	– Inappropriate explorations – Inappropriate stopping of vitamin D supplementation, even in a normocalcemic patient – Delay in initiating appropriate therapy	Yes
Corticotropic axis – High cortisol – Low ACTH	Hypercortisolism	– Inappropriate exploration – Inappropriate management in case of corticotherapy	Yes
Gonadotropic axis – High testosterone/estradiol – Low FSH, LH	– Excessive peripheral secretion of gonadic steroids or occult gonadic steroids intake	Inappropriate examinations	Yes
Somatotropic axis – Low IGF1 – Low GH	Pituitary GH deficiency	– Inappropriate explorations – Possible initiation of inappropriate GH treatment in a child with short stature	No
Pregnancy – Low hCG	Absence of pregnancy	Delay in pregnancy monitoring	No
Glucose metabolism – Low insulin – Low C-peptide	Possible diagnosis of insulin-dependent diabetes in hyperglycaemic patient	– Inappropriate complementary explorations – Possible initiation of inappropriate therapy – Missed diagnosis of hyperinsulinism	No
Prolactin – Low prolactin	NA	– Delayed diagnosis in patients with a true prolactin adenoma	No

NA, non applicable.

Biotin therapy

Immunoassays involving streptavidin-biotin interaction are now widely used by many reagent manufacturers. This is due to the high-affinity, stable interaction of streptavidin with biotin, together with the various chemical and enzymatic biotinylation methods available for use in different assay designs, in which biotinylation does not alter a molecule's properties. A solid phase coated with streptavidin can be conveniently used in different immunoassays. On the other hand, biotin supplement use has expanded over the years, ranging from medically prescribed therapies, to vitamin purchased on the internet [7, 8]. For these reasons, we have to pay attention to biotin therapy.

Biotin intake

Biotin is a water-soluble B-complex vitamin, also termed vitamin B8 (or B7) or vitamin H, and is a coenzyme responsible for carboxyl transfer in five essential carboxylases; therefore biotin is involved in many metabolisms. The recommendations for adequate intake in adults has been estimated to be 30 µg/day [9], with blood concentration ranging from 0.12 to 0.36 nmol/L [10]. This usual dietary intake is not expected to be high enough to affect immunoassays based on the streptavidin-biotin binding [11]. However, patients with inherited metabolic diseases like propionic acidemia, biotinidase deficiency, (and patients with parenteral nutrition) receive higher dosage of biotin, with daily oral doses in case of inherited metabolic diseases ranging from 10 to 40 mg per day [12, 13]. Supraphysiological biotin administration is also used in some auto-medication aimed at reducing hair loss or fortifying hair and nails (up to 20 mg per day). It is sometimes listed as an unnamed supplement to improve hair, nails and skin, and is not considered as a medication by the patient, so not worth mentioning [8]. More recently, very high dose of biotin (300 mg per day) have been proposed in some clinical protocols in multiple sclerosis and demyelinating pathologies [14].

Table 2: Examples of five widely used hormone immunoassays, using streptavidin-biotin interaction.

Hormone assays	Company (analyzer)				
	Beckman Coulter (Access, DXi, DxC)	Immuno diagnostic system (Isys)	Ortho Clinical Diagnostic (Vitros)	Roche (Cobas, Elecsys, Modular)	Siemens (Dimension Vista, Exl)
FT3	✔			✔ (286)	✔ (205)
FT4	✔			✔ (82)	✔ (205)
Total T3				✔ (41)	
Total T4				✔ (409)	
TSH			✔ (20.5)	✔ (102)	✔ (2050)
TRAb				✔ (41)	
SHBG	✔			✔ (246)	
Thyroglobulin	✔			✔ (327)	
PTH		✔	✔ (20.5)	✔ (205)	
25OH vit D		✔ (300)	✔ (61)	✔ (286)	
Cortisol			✔ (41)	✔ (123)	
ACTH				✔ (246)	
Testosterone			✔ (41)	✔ (123)	
Estradiol			✔ (20.5)	✔ (147)	✔
FSH			✔ (41)	✔ (246)	✔
LH			✔ (20.5)	✔ (205)	✔
Prolactin			✔ (41)	✔ (164)	✔
IGF1		✔ (300)			
GH		✔ (300)		✔ (123)	
C peptide				✔ (246)	
Insulin				✔ (246)	

A "✔" mark indicates that the streptavidin biotin interaction is used as immune complexes separation methodology. Biotin concentrations (nmol/L) above which an erroneous result can happen are indicated for each assay, when information is given in the reagent notices (i.e. concentration leading to bias above ±10% of the target). In theory, the analytes for which erroneous results occur at the lowest biotin concentration, are those that will be most frequently impacted facing biotin supraphysiological intake. Dark rectangle, assay non available in the tests menu.

Hormone immunoassays possibly impacted by biotin interference

The Table 2 presents five widely used automated hormone immunoassays involving the streptavidin-biotin interaction. It can be noticed that the various hormone assays in the different tests menu, sometimes from the same manufacturer, do not use systematically the streptavidin-biotin methodology, and therefore vary in their susceptibility to biotin interference. Looking, for example, at free thyroid hormones and TSH assays, interference of biotin may lead to various misleading biochemical profiles: inappropriate secretion of TSH with Beckman Coulter assays (FT4 and FT3 erroneously high results); isolated suppressed TSH (TSH erroneously low result) for Ortho Clinical Diagnostics assays, and a coherent hyperthyroid profile, with Roche Diagnostics and Siemens Dimension Vista assays (both high free thyroid hormones and low TSH erroneous results).

Considering an external quality assessment widely used in France (Probioqual, June 2016 results), the streptavidin-biotin interaction (from the five immunoassays mentioned above) is used in 500/953 immunoassays for TSH measurement, 509/810 for FT4, 442/709 for FT3, 242/513 for PTH, 221/535 for 25OH vitamin D: this technology for immune complexe separation represents about 50% of hormone immunoassays in use.

Besides automated routine immunoassays, many ELISA research assays use streptavidin-coated microwells plates that are susceptible to give completely erroneous results when used in biotin-treated patients. Given the widespread use of biotinylated compounds as reagents in various biotechnologies, the biotin interference deserve large information as Research teams are not always familiar with laboratory medicine, and may be unaware of this problem.

Magnitude of change in concentration: a dose-dependent interference, with large difference in susceptibility between assays

Dose-dependent positive and negative interferences have been reported related to biotin intake (Table 3). The

sandwich assay format is theoretically less vulnerable than competitive assay format because in the former, antibody reagents are in excess, unlike in the competitive format. However, this notion has to be taken with caution, since it also depends on the sample volume to be used in a particular assay, that brings a variable amount of interfering factor (the higher the sample volume used, the higher the amount of biotin present in the reaction mixture). The assay design is also important: in one or two steps, with or without a washing step. Some reagent manufacturers

Table 3: Reported interferences due to biotin therapy.

Result (alternative method or reassayed off biotin)		Biotin daily dose, clinical conditions	Duration of artifact	Clinical consequences	Ref. (year)
FT4, pmol/L	**77** (11)	10 mg (organic acidosis), newborn, hypothyroid	>2 days	Delay in treating congenital hypothyroidism	[12] (1996)
TSH, mUI/L	**38.4 (>209)**				
PTH, pg/mL	48 (**786**)	10 mg (end stage renal disease)	15 days	Delay in appropriate care	[5] (2009)
FT4, pmol/L	**75.9** (15.5)	40 mg (propionic acidaemia)	ND	None	[11] (2012)
FT3, pmol/L	**14.0** (4.5)				
TSH, mUI/L	0.62 (3.96)				
FT4, pmol/L	**>77** (normal[a])	30 mg (organic acidosis), newborn	>25 h	None	[13] (2012)
FT3, pmol/L	**24.9** (normal[a])				
TSH, mUI/L	3.75 (normal[a])				
FT4, pmol/L	**99** (normal[a])	300 mg (multiple sclerosis)	ND	ND	[1] (2015)
FT3, pmol/L	**12.3** (normal[a])				
TSH, mUI/L	**<0.01** (normal[a])				
FT4, pmol/L	**100.4** (18.0)	300 mg (multiple sclerosis)	<2 weeks	None	[2] (2016)
Total T3, nmol/L	**>9.7** (2.4)				
TSH, mUI/L	**0.02** (0.78)				
TRAb, IU/L	**36** (<1)				
Thyroglobulin, ng/mL	3.9 (21)				
FT4, pmol/L	**>100** (17)	300 mg (multiple sclerosis, menopausal woman)	<3 days[b]	None	[3] (2016)
FT3, pmol/L	**11.6** (4.4)				
TSH, mUI/L	**0.02** (1.95)				
TRAb, U/L	**>40** (2.3)				
LH, U/L	**2.0** (33)				
FSH, U/L	**2.5** (89)				
Estradiol, pg/mL	**296** (13.6)				
Testosterone, ng/mL	**5.9** (0.4)				
Cortisol, nmol/L	**765** (207)				
Vitamin B12, pmol/L	**>1400** (165)				
Folate, nmol/L	**>45** (18.1)				
Ferritin, ng/mL	**10** (100)				
FT4, pmol/L	**50.1** (13.2)	300 mg (X-linked adrenoleuco-dystrophy)	ND	None	[6] (2016)
TSH, mUI/L	**0.07** (2.34)				

Values indicated in bold were out of the age and method-related reference range. ND, not described. FT3, TT3: all results have been converted to pmol/L and nmol/L, respectively. FT4, all results have been converted to pmol/L. PTH, all results have been converted to pg/mL. [a]Detailed data not given in the case report. [b]Normalization of FT4 only described (69 pmol/L vs. 14 pmol/L after 3 days off biotin).

provide information concerning the biotin concentration above which an erroneous result can be encountered (see Table 2). Regretfully, it is not specified which biotin daily doses correspond to those concentrations, that do not allow to foresee (and prevent) any wrong result. As a matter of fact, if these data are obtained by in vitro spiking experiments, they might not reflect what happens in vivo (see below). Whatever the method used to study the interference, it seems that the magnitude of change in concentration is very different among the different analytes [13].

However, when facing very high concentration of biotin in multiple sclerosis, almost every competitive and sandwich assay will be equally dramatically artifacted, as evidenced in recent case reports [2, 3] (Table 3). When multiple immunoassays are prescribed and assayed using the same streptavidin-biotin methodology, many other erroneous results are to be expected: it may be useful to look for falsely lowered (sandwich assays) and falsely increased concentrations (competitive assays). Some other analytes concentrations are likely to be erroneous, for as many as 12 assays demonstrating interference have been reported in one recent case involving biotin megadose, in sandwich as well as competitive assays [3].

Measurement of serum free biotin concentration was performed in one case, using a microbial growth assay: the free biotin was assayed in a patient receiving 10 mg biotin, suffering from end-stage renal disease [5]. The free biotin concentration measured was 4.8 µg/L, i.e. 19.6 nmol/L, and led to a dramatically erroneous PTH concentration: 48 pg/mL, instead of 786 pg/mL. This may seem surprising as, according to the reagent notice, no artifact should occur up to 205 nmol/L of serum biotin concentration (see Table 2, Roche PTH assay). This might be related to metabolites (such as bisnorbiotin, biotin sulfoxide [10]) and/or in vivo biotinylated compounds, that may also participate in the artifact [5]. As a matter of fact, it is known that biotin derivatives may bind to avidin, although less tightly [10]. Therefore assay of serum free biotin may not be a contributing element to evaluate the extent of an error [5]. For the same reason, in vitro spiking experiments with free biotin might fail to reproduce the magnitude of the artifact observed in patients treated with biotin.

Time response curve following ingestion of biotin

The main route of excretion of biotin (either intact or as biotin metabolites) is renal [10]. The time response curve revealed that the extent of change in immunoassay results was maximum 2 h post a 30 mg biotin ingestion, and was significant up to 25 h [13]. As expected, the time interval is longer for biotin 300 mg dose in neurology, with a calculated half-life varying between 7.8 and 18.8 h, and maximum biotin concentration averaging 823 ± 303 µg/L (3374 ± 1242 nmol/L) [15]. No accumulation of biotin in blood is expected in case of repeated administration [15]. Elston et al. observed a normalization of the FT4 result 3 days after cessation of 300 mg biotin administration [3]. The time needed to recover a biotin concentration compatible with the other tests is not known.

The interference is most likely amplified in chronic kidney disease. In a case of end-stage renal failure, the PTH erroneous result remained and lasted up to 15 days after withdrawal of a treatment with 10 mg biotin [5].

Therefore, as recommended by some manufacturers, it is desirable to stop biotin before having a blood sampling, preferably more than 24 h [8]. This precaution can be enough in biotin auto-medication, but will be insufficient for the high doses prescribed in neurology, or in case of renal disease. Unfortunately, in case of intravenous nutrition, it may be difficult to withdraw biotin from the nutrient solution.

In summary, the relationship between biotin daily dose, serum biotin concentration, and time of sampling on the one hand, and the magnitude of interference on the other hand deserve further investigations. Sampling time protocols specific to the different clinical situations involving biotin could be useful to help avoiding an erroneous result.

Anti-streptavidin antibody

The same mechanism of interference can be expected from the presence in some sera of an anti-streptavidin antibody [16, 17]. Unlike biotin, this interference is endogenous, therefore non-transient: given the possibility of interference for an extended time, a cautioning of the patient is necessary, 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 [16]. The seroprevalence of this antibody may be very low, but is to be evaluated. The mechanism of appearance is still not elucidated. Streptavidin is produced by *Streptomyces avidinni*: it is unknown in what circumstances it could lead to an immunization.

In the cases reported, 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,

whereas the TSH concentration may be apparently lowered, but not fully suppressed, as should be recorded in a real hyperthyroid patient sample. The lack of suppression of TSH, facing dramatically high "thyroid hormone" concentrations will be noticed and evoke the syndrome of inappropriate secretion of thyrotropin, as reported in every case [4, 16, 17] (Table 4).

What can be done to suspect and overcome these interferences?

The possibility of an analytical artifact may be inferred from various signs [18]:

- Lack of coherence with the clinical presentation; e.g. a thyrotoxic biochemical profile in a patient who is clinically euthyroid. The anomaly may extend to several other endocrine investigations to heighten suspicion of assay interference [3].
- Comparison of physiologically dependent variables: Lack of the usual balance between the hormone and its regulating factor, evoking, for example, the very rare syndrome of inappropriate secretion of TSH [8, 16, 17].
- Extremely unusual analyte concentration.
- Markedly different results given by different analytical methods: as mentioned above, these analytical errors are method-dependent, because they impact specifically streptavidin-biotin based immunoassays.

When an interference is suspected, hormone laboratories are used to look for an interference due to an heterophilic antibody. The heterophilic antibody interference has been well-known for decades. It can affect any immunoassay [19], although in most cases this interference concerns sandwich assays, provoking a positive error [20], unlike in the biotin interference. In some extremely rare cases, multiple artifacted hormone assays have been reported due to an heterophilic antibody [20, 21]. Although less notorious, the biotin artifact is also worrying, as a seemingly coherent hormonal profile can be recorded because multiple, both positive and negative erroneous results are generated; this may lead to inappropriate therapy.

Interference testing can be done, to objectivate an analytical problem. The linearity can be tested with a dilution test, provided that an appropriate diluent exists, when a measurable analyte concentration is expected [16]. It is also needed that the immunoassay is reliable on a diluted sample (some immunoassays do not support the sample dilution because of matrix effect, or "resist" to dilution (e.g. free thyroid hormones assays, [22])). When feasible, the dilution test will demonstrate a negative interference (higher concentrations in the diluted sample than in the undiluted sample: sandwich format) and/or a positive interference (conversely, lower than expected concentrations in the diluted sample, competitive format). Excepting biotin, which is exogenous (and can be evidenced by inquiry), precise identification of the interfering substance may require a highly specialized lab.

A neutralization of biotin (or of anti-streptavidin antibody) can be obtained with the aid of a high-capacity streptavidin solid phase, to "block" the interference [5, 16]. Results obtained after removal of the interfering factor are not reportable, and only indicate that the original values are not reliable.

The biotin interference is a method-specific interference, that can be simply overcome by comparing the results with an alternative method: markedly differing

Table 4: Reported interferences due to anti-streptavidin antibody.

Result (alternative method)		Duration of artifact	Clinical consequences	Ref. (year)
Total T4, nmol/L	**179**	≥ 2 years	Yes, inappropriate anti-thyroid drug	[16] (2013)
TSH	1.59			
25OH Vitamin D, nmol/L	> **250** (101)	ND	None	[4] (2010)
PTH, pg/mL	**0.94** (16)			
FT4, pmol/L	**27** (14)			
TSH, mUI/L	0.35 (0.79)			
FT4, pmol/L	**25.6**(13.3)	≥ 18 months	Yes, inappropriate anti-thyroid drug	[17] (2016)
FT3, pmol/L	**13.8** (4)			
TSH mUI/L	0.83 (2)			
Anti-TPO, TRAb, anti-TG	All **positives** (negatives[a])			

Values indicated in bold were out of the age and method-related reference range. ND, not described. FT3, all results have been converted to pmol/L. FT4, all results have been converted to pmol/L. PTH, all results have been converted to pg/mL. [a]Detailed data not given in the case report.

results given by different analytical methods are obtained, provided that a different methodology is chosen for comparison.

The appearance of this type of interference and the increasing frequency of its occurring has urged the reagent companies to reformulate their assays aiming at minimizing the risk of such misleading results. These attempts will hopefully be successful; they could involve modified assay protocols whose design prevents inhibition of immune complexes separation (e.g. Barbesino recorded a normal Sex Hormone Binding Globulin (SHBG) result in a patient treated with 300 mg of biotin, although this assay used streptavidin-biotin, whereas all others assays were grossly artifacted [2]).

Will non-immunoassay-based techniques allow improvement of such pitfalls? Unfortunately, very powerful and reliable measurements such as LC-MSMS, are not yet adapted to high throughput sandwich assays. Moreover, LC-MSMS is a complex method that requires technical expertise. If LC-MSMS are more and more used throughout the world for small molecules assays, such as steroids and vitamin D metabolites, it is unlikely that large molecules measurements (such as glycoproteins sandwich assays) will be replaced in a foreseeable future. Therefore sandwich immunoassays, despite their drawbacks, will still be in general use, at least for the next coming years.

Clinical and therapeutic information are crucial to suspect and detect such interferences. Specifically, the notion of biotin therapy (including parenteral administration), has become a key question: nowadays, clinicians should actively ask their patient about biotin intake [2, 8] because the patient will not always mention it. It is desirable that the laboratories now give a systematic warning concerning biotin interference together with any result of a method involving the streptavidin-biotin interaction. Ideally, it is also wished for health information systems to be able to generate an automatic flag concerning biotin administration to alert the laboratory [11].

Conclusions

Due to their easy and fast automation, many hormones are very frequently assayed. Sometime errors can occur with some serum, and their investigation is always needed to keep on improving immunoassay technologies. Laboratorians (as well as research teams) have to be aware of such cases, particularly biotin artifact, leading to both positive and negative errors in streptavidin-biotin based immunoassays. Other cases have to be kept in mind, where both low and high erroneous results may also be encountered, when occurs an inhibition of the signal to be measured [23].

Investigations for possible interference that can be undertaken in any laboratory include testing for linearity, retesting off biotin, and confirmation using an alternative method. As the diversity of available methods is declining, with high volume analytical platform being implemented, the referral to an alternative method may be difficult; nevertheless it must remain readily accessible. It may be feared that the biotin interference will be regularly encountered, because of the widespread use of streptavidin-biotin technology, and of the more frequent administration of biotin in supraphysiological doses.

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 [4, 5, 7].

Despite progress in immunoassays 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.

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References

1. Decoudier B, Delemer B, Maarouf A, Tourbah A, Marot D. Une hyperthyroïdie énigmatique … de l'importance des interférences dans les dosages hormonaux. 32e SFE congress. oct 2015, Poster P172. Ann Endocrinol 2015;76:428.
2. Barbesino G. Misdiagnosis of Graves' disease with apparent severe hyperthyroidism in a patient taking biotin megadoses. Thyroid 2016;26:860–3.

3. Elston MS, Sehgal S, Du Toit S, Yarndley T, Conaglen JV. Facticious Graves' disease due to biotin immunoassay interference – a case and review of the literature. J Clin Endocrinol Metab 2016;101:3251–5.
4. Khieng V, Stevens C. Vitamin D toxicity? a case study. NZ J Med Lab Sci 2010;64:44–50.
5. Meany DL, Jan De Beur SM, Bill MJ, Sokoll LJ. A case of renal osteodystrophy with unexpected serum intact parathyroid hormone concentrations. Clin Chem 2009;55:1737–41.
6. Simó-Guerrero O, Giménez-Pérez G, Recsens-Garci A, Villà-Blasco C, Castells-Fusté I. False overt hyperthyroidism by interference in immunoassays. Endocrinol Nutr 2016;63:431–2.
7. Waghray A, Milas M, Nyalakonda K, Siperstein AE. Falsely low parathyroid hormone secondary to biotin interference: a case series. Endocr Pract 2013;19:451–5.
8. Seaborg E. Thyroid month January 2016: beware of biotin. In endocrine news. Washington, USA: Endocrine society, 2016:42. Available at: http://endocrinenews.endocrine.org/January-2016-thyroid-month-beware-of-biotin.
9. National Research Council. Dietary reference intakes for Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic acid, Biotin, and Choline. National Academy of Sciences. Washington, DC: National Academy press, 1998:374–89.
10. Zempleni J, Wijeratne SS, Hassan YI. Biotin. Biofactors 2009;35:36–46.
11. Kwok JS, Chan IH, Chan MH. Biotin interference on TSH and free thyroid hormone measurement. Pathology 2012;44:278–80.
12. Henry JG, Sobki S, Arafat N. Interference by biotin therapy on measurement of TSH and FT4 by enzymeimmunoassay on Boehringer Mannheim ES700 analyser. Ann Clin Biochem 1996;33:162–3.
13. Wijeratne NG, Doery JC, Lu ZX. Positive and negative interference in immunoassays following biotin ingestion: a pharmacokinetic study. Pathology 2012;44:674–5.
14. Sedel F, Papeix C, Bellanger A, Touitou V, Lebrun-Frenay C, Galanaud D, et al. High doses of biotin in chronic progressive multiple sclerosis: a pilot study. Mult Scler relat Disord 2015;4:159–69.
15. Peyro Saint Paul L, Debruyne D, Bernard D, Mock DM, Defer GL. Pharmacokinetics and pharmacodynamics of MD1003 (high dose biotin) in the treatment of progressive multiple sclerosis. Expert Opin Drug Metab Toxicol 2016;12:327–44.
16. Rulander NJ, Cardamome D, Senior M, Snyder PJ, Master S. Interference from anti-streptavidin antibody. Arch Pathol Lab Med 2013;137:1141–6.
17. Peltier L, Massart C, Moineau MP, Delhostal A, Roudaut N. Anti-streptavidin interferences in Roche Thyroid immunoassays: a case report [letter]. Clin Chem Lab Med 2016;54:e11–4.
18. Selby C. Interference in immunoassay. Ann Clin Biochem 1999;36:704–21.
19. Tate J, Ward G. Interferences in immunoassay. Clin Biochem Rev 2004;25:105–19.
20. Gulbahar O, Degertekin CK, Akturk M, Yalcin MM, Kalan I, Atikeler GF, et al. A case with immunoassay interferences in the measurement of multiple hormones. J Clin Endocrinol Metab 2015;100:2147–53.
21. Mongolu S, Armston AE, Mozley E, Nasruddin A. Heterophilic antibody interference affecting multiple hormone assays: is it due to rheumatoid factor? Scand J Clin Lab Invest 2016;76:240–2.
22. Faix JD. Principles and pitfalls of free hormone measurements. Best Pract Res Clin Endocrinol Metab 2013;27:631–45.
23. Ohba K, Noh JY, Unno T, Satoh T, Iwahara K, Matsushita A, et al. Falsely elevated thyroid hormone levels caused by anti-ruthenium interference in the Elecsys assay resembling the syndrome of inappropriate secretion of thyrotropin. Endocr J 2012;59:663–7.