



# Sense and nonsense concerning biotin interference in laboratory tests

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## REVIEW

## ABSTRACT

**Introduction:** Biotin supplementation (mainly OTC preparations) has gained popularity. There are concerns about biotin interference in immunoassays and potential misdiagnosis, especially since the discovery of high dose therapy in MS. This review summarizes the dangers of biotin usage and possible countermeasures.

**Methods:** Immunoassays design determines whether positive or negative analytical errors may occur. Techniques using biotinylated reagent and biotin binding proteins may generate errors. In sandwich immunoassays, biotin causes lowered results. Competitive immunoassays are more vulnerable: biotin usage causes false increased results. The interference is platform dependent. Parameters vary in their susceptibility: a combination of false positives and negatives mimicking a coherent profile is dangerous, e.g. combining falsely lowered TSH with falsely elevated FT4/FT3 mimicking hyperthyreosis. Other susceptible parameters are thyroglobulin, DHEA-S, estradiol, testosterone, ferritin, progesterone, Vitamin D, Vitamin B12, PSA, PTH, LH, FSH, Troponins I and T, Pro-BNP. Digoxin and PSA may also be affected. Tumor markers and  $\beta$ -hCG are robust. Inserts of serological markers of HIV, hepatitis B and C warn for biotin interference.

**Results:** Manufacturers have made assays less vulnerable for biotin interference. In doubtful cases, it is helpful to determine testosterone in females and estrogen in males. Both are elevated if biotin interference is present. Biotin supplementation should be discontinued. However, this is impossible in MS patients needing biotin, as interrupting this medication is discouraged.

**Conclusions:** Solutions to overcome this interference are: informing patients prior to analysis (avoiding peak biotin values when sampling), choice of appropriate immunoassays, and use of biotin removing steps prior to analysis.

## ARTICLE HISTORY

## KEYWORDS

Biotin; interference;  
immunoassays

## Introduction

Immunoassays are widespread laboratory assays, most frequently used for hormone, oncologic and cardiologic measurement. This type of assay has become indispensable in modern medicine, mainly because of its property of high throughput, robustness and fully automated measurement. Immunoassays use high specific antibodies to target and quantify specific molecules of interest. This type of assay was first developed in 1959 in the form of a radioimmunoassay (RIA). The original competitive RIA required purification of antibody. Later on, 'cold' (non-radioactive) immunoassays were developed. Non-competitive (sandwich) immunoassays have been developed. The first immunoassays were all heterogeneous, meaning that a separation of bound from free is needed. When the free phase consists of labeled antigens, the immunoassay is competitive. In contrast, when this consists of labeled antibodies, it is a sandwich immunoassay. In 1976, another important feature in the progress of the immunoassay was first described: the use of chemiluminescence. This is nowadays used by most commercial automated immunoassay platforms [1]. Understanding the basic principles of the different assays is essential to gain insight in the possible dangers of interference. Therefore, we have included here a short repeat of the basics of the competitive versus noncompetitive/sandwich immunoassays. In the noncompetitive assays, the analyte of interest becomes trapped between a biotinylated and a labeled antibody. The measured signal is directly proportional to the analyte concentration

in the sample. In a competitive immunoassay a labeled analyte analog is being measured after binding to the biotinylated antibody. The measured signal is hence inversely proportional to the concentration of target analyte in the sample. In both immunoassays, the target analyte becomes separated from the reaction environment by binding to a streptavidin-based solid phase. This occurs through binding of biotinylated antigen or antibody. From the previous information, it may be clear that the design of the immunoassay determines whether positive or negative analytical errors may occur. In a competitive assay, excess biotin leads to falsely elevated results, in a noncompetitive assay, lowered results may be expected [2]. Figure 1 displays the mechanism of biotin interference in competitive and sandwich immunoassays. This type of immunoassays are the ones that are most used, but of course other systems are possible. Devices using a magnetic bead-based capture system are also relatively common and do not have this issue.

In other words, the usage of biotin is very popular and widely distributed in modern immunoassays. Its usefulness lies in the fact that it is a small molecule (244,31 g/mol) that binds with exceptional strength and specificity to streptavidin. The latter is an 56 kDa homotetramer, able to bind up to 4 biotin molecules. The dissociation constant of the complex amounts to no less than  $1,3 \times 10^{-15}$  M. An important advantage is the fact that this complex has high thermostability and is resistant against extreme pH, denaturing agents and enzymatic

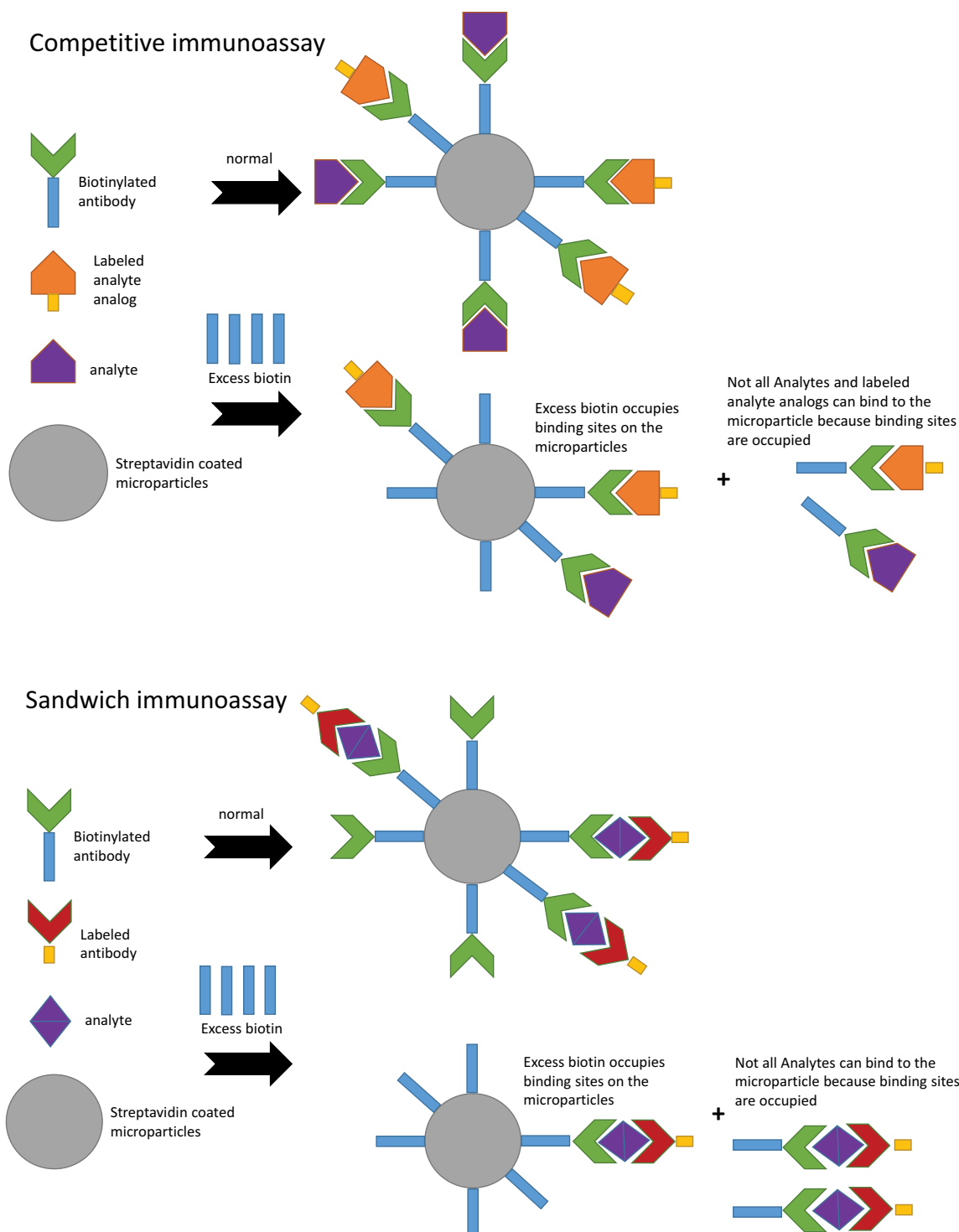


Figure 1. Display of the mechanism of biotin interference in immunoassays.

degradation [3]. The fact that this technology has never been patented also plays an important role in the widespread use of this technology.

Recently however, an important drawback has come to light. More and more reports are being published concerning the interference of biotin in immunoassays. Severe cases of misdiagnosis and unnecessary treatment have been reported. Due to the hype of using biotin in supplements for hair and nails and its utility in slowing down the progression of MS, the worldwide consumption of biotin has risen sharply. But is this all so innocent or do we have to pay more attention to the possible dangers?

### Who is at danger?

Biotin is a water soluble vitamin/micronutrient and a coenzyme to 5 carboxylases. It is also covalently bound to lysine residues in histones. Because of this, it plays a role in the epigenetic regulation of genes, the structure of chromatin and cell signaling. Mammals cannot produce biotin and are consequently fully dependent on intake through the nutrition. Normal daily intake in western countries ranges from 35 to 70  $\mu\text{g}$  per day, which lies above the daily recommended 5 to 35  $\mu\text{g}$ . Our western intake pattern results in serum concentrations

ranging from 0.12 to 0.36 nmol/L [4]. Rarely, people can be deficient in biotin. This shortage occurs in biotinidase deficiency (1/60089 living births) and severely malnourished children. Treatment of such a deficiency consists of 5 to 30 mg biotin per day. Biotin-thiamin-responsive basal ganglia disease is a condition that affects the nervous system. This is also treated with high dose biotin, namely 5 to 10 mg per day. In conclusion, it is unnecessary for healthy subjects to use biotin supplements. However, marketers have made convenient use of the property of biotin to promote protein synthesis and thereby also keratin production. Supplements are available in all sizes and weights with concentrations ranging from 50 µg in multivitamin preparations to 20 mg in preparations specifically targeting growth and quality of hair and nails. Proof of these claims is however lacking, with only very little scientific evidence of any beneficial effect [5,6].

On the other side of the spectrum, there are the patients with multiple sclerosis (MS). MS is an immune-mediated disease of which there are two forms: relapse remitting and progressive MS. Relapse remitting MS is characterized by unpredictable acute episodes of neuronal dysfunction, followed by more stable periods. In progressive MS there is a continuous clinical deterioration and neurodegradation without any stable periods in between. Most patients (85%) present with relapse remitting MS, which then eventually evolves into the progressive form of the disease. Since there is no medication yet that can cure the disease or prevent deterioration, there is a great unmet need for a treatment [7]. In this context, 'Qizenday' or MD1003' from the French company MedDay is currently in phase III clinical trial. They developed a 100 mg immediate release oral formulation of the biological active isomeric (3aS, 4S, 6aR)-configuration (d(+)) biotin. The endpoint of the clinical trial is slowing down the progression of the disease. For this, high doses of 100 to 300 mg per day are administered. The mechanism is based on counteracting the virtual hypoxia phenomenon. This is accomplished due to an increase in energy production through the TCA cycle and enhanced myelin repair. The terminal half-life (T<sub>1/2</sub>) is estimated at 22 hours and peak plasma concentrations were reached at a T<sub>max</sub> of 2 to 2.5 hours. Up to today, conditional approval by EMA (European Medicines Agency) has been denied. This is due to insufficient knowledge concerning the pharmacokinetic profile. Expectations are that the trial will end in 2020, followed by a New Drug Application (NDA) at the U.S. Food and Drug Administration (FDA) and a Marketing Authorization Application (MAA) in Europe at the EMA [8,9]. An important note here is that although there is no commercial preparation on the market yet, magistral preparations are already in use.

### Which analytical platforms require extra attention?

Every analytical technique that uses biotinylated reagent and biotin binding proteins is at risk for generating biotin-related analytical errors. In a sandwich immunoassay, the measured signal is directly proportional to the concentration of target analyte in the sample. In a competitive

immunoassay, the measured signal is inversely proportional to the concentration of target analyte in the sample. In the latter assay, usage of biotin will cause false increased results. In a sandwich immunoassay, the results will be lowered. The interference is therefore platform dependent. Analyzers subject to this interference are Roche Elecsys using ElectroChemiluminescence Immunoassays (ECLIA), Beckman Coulter chemiluminescent assays and Ortho Clinical Diagnostics Vitros using an immunometric assay. Also to consider are the Siemens Dimension Vista 1500 analyzers using Luminescent Oxygen Channeling Assay (LOCI), however the concentration of biotin required to alter results is relatively high. Siemens Advia Centaur is a random access immunoassay system. Most immunoassays are not biotin-streptavidin based and the majority of those that do utilize beads prebound to biotinylated antibody. The same is true for the Siemens Immulite. Unfortunately, some assays of these two Siemens platforms do have a vulnerable design [10]. Platforms using technologies other than biotin-streptavidin linkage such as Abbott Architect and Alinity and Diasorin Liaison can here be used without issues. Diasorin uses chemiluminescence technology (CLIA) with a paramagnetic microparticle solid phase (MP). Abbott uses Chemiluminescent Microparticle Immunoassay (CMIA) also with MP. So both systems rely on a magnetic bead-based capture system. Another possibility is Tosoh's AIA-PACK assays. They also use a biotin-free magnetic bead-based capture system. An important note is the fact that not all analyzers are equally at risk. Also the individual parameters vary in their susceptibility. This is due to the fact that the streptavidin-biotin methodology is not always used systematically for every assay on an analyzer, even if from the same manufacturer. Furthermore, the streptavidin, anti-biotin antibody and biotinlabeled reagent can be combined in the manufacturing process. These assays that use the so-called pre-bound reagents overcome the issue of biotin interference [11]. Roche Elecsys is the only one that uses biotin-streptavidin linkage for every test [12,13].

### Which analytes are vulnerable?

Not all parameters are equally vulnerable. In addition to that, the susceptibility of the parameter is also dependent on the analyzer on which it is determined. As mentioned earlier, the interference may induce both false positive (competitive assays) and negative results (sandwich assays), depending on the type of immunoassay. The biggest danger lies therefore in the fact that an unfortunate combination of both false positives and negatives may perfectly mimic a coherent hormonal profile. The best known example is the combination of a falsely lowered TSH with falsely elevated FT3 and FT4 mimicking a coherent hyperthyroid profile [14,15]. However, not all of these assays mentioned are equally vulnerable and this is platform-dependent. If we continue on the example of the thyroid hormones, only Roche and Siemens generate a complete coherent hyperthyroid profile. The same sample determined with the Beckman Coulter assay will generate erroneously high FT3 and FT4 results. Analyzed with

the Ortho Diagnostics assay, we will get an erroneous low TSH [4,13]. The example of the thyroid hormones may be the best known, it is certainly not the only one. This of course makes it a very complex problem and not always easy to know which parameters to look out for. Other parameters that are well known to be susceptible are thyroglobulin, DHEA-S, estradiol, testosterone, ferritin, progesterone, Vitamin D, Vitamin B12, PSA, PTH, LH and FSH. Of these, progesterone and estradiol are important in fertility check-up and IVF [16,17]. In general, it is assumed that the sandwich assay is somewhat less vulnerable in comparison to the competitive assay. This is because in the sandwich assay, antibody reagents are in excess. However also other factors may have an influence on the sensitivity, such as the sample volume used, whether or not there are one or two steps or there is a washing step. And we have not yet mentioned the most important influencing factor here, namely the *in vivo* present concentration of biotin. The amount of biotin present in the sample is directly related to the degree of interference. However, this is also difficult to interpret, seeing the fact that the magnitude of concentration needed to influence the results is different among different analytes and different manufacturers. Taken all together, thyroid hormones, LH, FSH and troponins I and T are the assays that are most vulnerable and can already show biased results at concentrations of approximately 30 ng/mL. Troponin I negative interference has been reported from concentrations of 0.5 mg/L biotin. Herein lies the potential danger of missing an acute myocardial infarction. This is also true for Troponin T. The problem with this aforementioned parameters has today been partially counteracted by the manufactures with the launch of newer generation reagents who are more robust. This results in the elevation of the interference threshold from 30 to approximately 100 ng/mL. Also grossly affected are PSA, ACTH, pro-BNP, DHEA-S, testosterone and insulin. TDM monitoring, namely digoxin, can be problematic. An overestimation up to 300% has already been reported. This could have the very undesirable result of inappropriate dose-adjustment. For this set of parameters, erroneous results can appear from 500 ng/mL, which corresponds to intake of 100 to 300 mg biotin per day.

Besides the hormonal assays, the oncologic and cardiologic assays also deserve the necessary attention. Tumor markers (AFP, CA125, CA19-9, CA15-3 and CEA) and Beta-hCG have shown to be relatively robust, meaning the results remain unaffected at low concentrations and the magnitude of error is modest at doses of 300 mg per day [14]. In addition, one may also ask questions about the reliability of POC hCG devices. It may seem surprising, but there is no problem with the test line. It's the control line that provides an indication of the problem, since this line is absent in a number of brands. The theory behind this is based on the fact that in these brands, the anti-IgG antibodies have been replaced by immobilized streptavidin and a biotinylated blue dye. When biotin is present in the urine, streptavidin will be saturated and the biotinylated dye will be unable to bind and hence to form the control line. A different technique is used to immobilize the anti-hCG capture antibodies. The test line will hence not be influenced, but since the control line is absent, the test is

invalid and cannot be used to determine pregnancy [18] [14] (Table 1).

And what about serological markers? Recent research in this area suggests that the extent of biotin interference may be broader than what is commonly thought. The inserts of the serological markers of human immunodeficiency virus (HIV), hepatitis B virus (HBV) and hepatitis C virus (HCV) of multiple manufacturers contain warnings for biotin interference. Anti-hepatitis B surface antibodies (anti-HBs) are determined using a sandwich immunoassay. Anti-hepatitis B core total antibody (anti-HBc) and anti-hepatitis B e-antibody (anti-Hbe) are measured using a competitive immunoassay. The latter two assays are expected to generate false positive results after biotin intake, the first assay is expected to generate a decreased signal. An experiment was performed in which healthy volunteers were vaccinated against HBV. They underwent blood sampling at baseline, 1.5 h and 24 h after intake of 100 mg biotin. At baseline, all were negative for anti-Hbe and anti-HBc, with anti-HBs levels that correspond to a vaccinated status. 1.5 h after ingestion, all volunteers showed a decrease in anti-HBs values, which in several cases even corresponded to values under the immunological cutoff. Additionally, the anti-HBc and anti-HBs levels all showed a decreased measured signal (the signal is inversely proportional to the concentration of the antibodies). A biotin spiking experiment in which sera of patients positive for anti-HIV or anti-HCV were used, showed several false negative cases. Logically, the higher the concentration of biotin used, the lower the measured indexes were. The previous data confirm that virological parameters are equally affected and could potentially lead to misdiagnosis, which could have serious consequences for patients as well as partners [19].

Conclusively, when determined on a platform using biotin-streptavidin linkage, results should be interpreted with the necessary alertness. Patients using supplements require extra attention, and the reliability of their results depends on the dose taken and the specific parameter measured. Generally, the concentration of biotin present in multivitamin preparations will be too low to produce a clinically significant shift in results. Samples from patients taking very high doses such as in MS, will be affected with great probability and artifact almost all results generated. To be able to report correct results, these samples require previous treatment to neutralize the biotin. Samples from these patients will after all not only contain biotin, but also its metabolites. These will additionally contribute to the interference [10,20]. In summary, any influence on the results is only to be expected with intake from 5 to 10 mg, always taking into account the difference in sensitivity between the variety of assays and analyzers. The biggest risk was established on the Roche platform.

### What do EMA and the FDA say?

In November 2017, the FDA published a safety warning to make clinicians, patients and labs aware of the possible



**Table 1.** Summary of the different parameters with corresponding platforms, their susceptibility to biotin and an indication of whether they show positive or negative bias [28].

Parameter	Platform(s)	Positive (+) or Negative (-) Interference
AFP	ROCHE; SIEMENS; ORTHO	-
ACTH	ROCHE	-
Anti-HAV IgM	ROCHE	-
<b>Anti-TPO</b>	ROCHE	+
Anti-Thyroglobulin	ROCHE	+
<b>Anti-TSH</b>	ROCHE	+
<i>Anti-HBc</i>	ROCHE	-
Anti-HCV	ROCHE	-
C-Peptide	ROCHE	-
CA125	ROCHE; SIEMENS; ORTHO	-
CA15-3	ROCHE; ORTHO	-
CA19-9	ROCHE; SIEMENS; BECKMAN COULTER; ORTHO	-
Calcitonin	ROCHE	-
CEA	ROCHE; SIEMENS; ORTHO	-
Cortisol	ROCHE; ORTHO	+
CK-MB	ROCHE; SIEMENS; ORTHO	-
Anti-CCP	ROCHE	-
DHEA-S	ROCHE; SIEMENS	+
<b>Estradiol</b>	ROCHE; SIEMENS; ORTHO	+
<b>Folate</b>	ROCHE; SIEMENS; ORTHO	+
FT4	ROCHE; SIEMENS; BECKMAN COULTER	+
FT3	ROCHE; SIEMENS; BECKMAN COULTER	+
FSH	ROCHE; SIEMENS; ORTHO	-
HCG	ROCHE	-
<i>HCG-B</i>	ROCHE; ORTHO	-
Hep B Ag	ROCHE	-
<b>Hep B Core AB</b>	ROCHE	+
Hep B Surface Ag	ROCHE	-
<b>HGH</b>	ROCHE	-
IGF-1	ROCHE	-
<i>IgE</i>	ROCHE	-
Insulin	ROCHE; ORTHO	-
<b>LH</b>	ROCHE; SIEMENS; ORTHO	-
Myoglobin	ROCHE; ORTHO	-
Osteocalcin	ROCHE	-
PTH	ROCHE	-
<b>NT-proBNP</b>	ROCHE; SIEMENS; ORTHO	-
<b>Procalcitonin</b>	ROCHE; SIEMENS	-
<b>Progesterone</b>	ROCHE; SIEMENS; ORTHO	+
Prolactin	ROCHE; SIEMENS; ORTHO	-
PSA	ROCHE; ORTHO	-
SHBG	ROCHE; SIEMENS	-
<b>Testosterone</b>	ROCHE; SIEMENS; ORTHO	+
<i>TSH</i>	ROCHE; SIEMENS; BECKMAN COULTER	-
<b>Troponin I</b>	ROCHE; SIEMENS; ORTHO	-
Troponin T	ROCHE	-
Vitamin B12	ROCHE; SIEMENS; ORTHO	+
25-OH VitD	ROCHE; ORTHO	+
<b>VitD Total</b>	ROCHE; SIEMENS	+

Parameters in bold are easily influenced (concentrations < 40 ng/mL), others are somewhat more resistant (concentrations from > 40 to 100 ng/mL) and the parameters in italic are those that will only be affected at concentrations of more than 100 ng/mL of biotin. The exact concentrations at which interference occurs is platform dependent. This table was made using the Roche platform as standard to which others were compared. Some known very susceptible parameters (for example FT3 and FT4) are not represented in bold because the reagent has been adapted and newer generation reagents are already widely used.

misleading lab results from patients taking high doses biotin. They stated that significant interference can be expected and incorrect results can be generated. This publication was a result of an increasing number of case reports concerning adverse events due to biotin intake. Recently, the FDA has supplemented this with a specific

warning concerning Troponin [21]. In Europe also, warnings were formulated. In January 2019, the European Pharmacovigilance Risk Assessment Committee (PRAC) published recommendations concerning the intake of biotin. Based on literature and Eudravigilance, they concluded that every oral dose of over 150 µg can interfere in immunoassays. They make a call for extra vigilance, certainly in cases where the lab results are incoherent with the patient's clinical presentation [22].

### How to recognize and counteract the interference?

The major manufacturers of diagnostic assays have realized the severity of the problem and are trying to make their assays less vulnerable for biotin interference. Roche Diagnostics is investing in the renewing of its reagents in order to let it perform better in the presence of biotin. In June 2019 the analysis kits of TSH, anti-TSHR and Troponin T have been adapted in wave 1. For 2020 biotin wave 2 and 3 are scheduled. Wave 2 is planned for September 2020 and will consist of adapting the reagent for PSA, βHCG, proBNP, anti-HBs and HBsAg. In November 2020, the reagent of AMH, HIV and PTH will be redesigned. An example of an assay that has already been adapted in February 2018 is the Roche Diagnostics Elecsys FT4 III assay in follow-up of the Elecsys FT4 II assay. Studies have already been performed to compare the impact of interference on FT4 between both assay types. Therefore, biotin-spiked samples were analyzed in duplicate with the two different reagent kits and the relative bias was calculated. Results of the samples analyzed with the Elecsys FT4 II assay showed the possibility of a positive bias at biotin concentrations of ≥50 ng/mL. The package insert mentions an interference threshold of ≤25 ng/mL, which seems like a safe range. Analyzed with the Elecsys FT4 III assay, positive interference was observed in samples containing concentrations of 200 ng/mL biotin and higher, whereas the insert mentions 100 ng/mL as threshold. In conclusion these results clearly indicate a reduced sensitivity of the third generation of the assay to biotin interference. However this does not mean a complete absence of possible falsely elevated results and a critical evaluation of generated data remains necessary [23].

As can be deduced from the explanations above, the wide variety of assays, devices and manufacturers can make it challenging to distinguish who/which parameters are at risk. Especially since patients are not always aware of the fact that OTC medications or supplements are also worth mentioning when consulting a clinician. Because of this, the possible use of biotin often remains unknown. Clinicians must be aware of this and, when doubts about possible interferences, specifically ask if the patient uses biotin or vitamin supplements. This is particularly true for endocrinologists. Moreover, all neurologists treating MS patients should always keep in mind the possibility of extremely distorted lab results. In general, most laboratory specialists are well informed and aware of this problem. Careful inspection of the results, certainly if these don't correlate with the clinical presentation, is definitely recommended to prevent misdiagnosis and – treatment.

When there are doubts about the possibility of biotin intake and interference, it is important to know that there are a number of options for identifying, as well as dealing with the problem. A standard method to identify interferences is a serial dilution. In the case of biotin, the degree of interference is dependent on the concentration. Hence, this method will not be very useful. Here, we suggest two useful options for tracking the possible presence of biotin as interferent. The first consists of determining testosterone for female and estrogen for male patients. Both will be elevated when interference of biotin is present. Secondly, one can also use the VeraTest Biotin<sup>TM</sup>. This is a lateral flow assay in which biotin, if present, will compete with immobilized biotin for binding to a dye labeled protein. When using this test stick, a pink color band will appear in the absence of biotin. The system, which has a threshold of 15 ng/mL, will thereby provide you of a yes or no answer to the question of the possible presence of biotin [24].

If there is certainty about the presence of biotin, the next important step is to ensure correct results when analyzing the sample on a susceptible platform. A first possible solution is to dilute the specimen with assay diluent, however this may not be possible for all parameters [25]. Alternatively, Piketty et al. developed a method to overcome the interference. They absorbed the excess of biotin using streptavidin-coated beads which are still present in used reagents kits from immunoassays. The procedure is relatively simple, fast and very economic since it utilizes empty kits that would otherwise be thrown away [26]. Results generated post treatment can be reported without any issue. A new approach has recently been published. Researchers studied the possibility of altering the reagent of Roche C-peptide, making it robust against biotin interference. In this method, reagent M (containing streptavidin coated beads) and R1 (containing biotinylated antibody) were premixed and incubated. Next, the beads were replaced in container M and the supernatant in R1. Experiments on biotin spiked samples learned that the original reagent could only tolerate 44 ng/mL biotin, while the modified reagent could analyze correctly until 1055 ng/mL. This possibility of altering the reagent instead of the sample provides an interesting new insight [27]. In line with the test device proposed to detect the presence of biotin, a similar system exists to remove it from the sample. VeraPrep Biotin<sup>TM</sup> contains magnetic nanoparticles covalently conjugated to streptavidin. After incubating the sample with this reagent, the VeraMag<sup>TM</sup> (a magnet) will separate the bound biotin, thereby removing it from the sample. Another commercial device is the BioT-Filter. Here, the sample is forced through a biotin-removing filter [24]. Last, it was already mentioned that not all devices are equally vulnerable. Devices using a magnetic bead-based capture system are completely free of this type of interference. Forwarding the sample to a lab that uses this alternative assays is an easy solution to the problem.

The previous mentioned options overcome the need for biotin discontinuation, which is the easiest solution, but not recommendable in MS patients. If biotin

supplementation is reported and is not a therapeutic necessity, this should be discontinued and blood should be drawn after it has been cleared. The recommended time interval after discontinuation is dependent on the concentration used.

## Conclusion

The problem concerning biotin and its interference in immunoassays is real and should not be minimized. It is expected that the magnitude of the issue will only increase since the OTC and mainly pharmacologic usage will continue to rise. Alertness and awareness are the key to the solution. When the problem is noticed in time, there are a number of strategies to overcome this interference: informing the patient prior to analysis (avoiding peak biotin values at the moment of sampling), choice of appropriate immunoassays, and (in case of doubtful result), use of biotin removing steps prior to analysis).

## Disclosure statement

No potential conflict of interest was reported by the authors.

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