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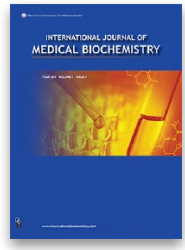

Review

Understanding and managing biotin interference in immunoassays

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Abstract

Objectives: Biotin is a water-soluble vitamin that is an essential coenzyme in the transfer of carboxyl groups by several carboxylases involved in the synthesis of fatty acids, gluconeogenesis, amino acid metabolism, and the citric acid cycle. In the last decade or so, several reports have highlighted biotin interference causing clinically incompatible immunoassay test results. The underlying reason for this relatively new issue of biotin interference is thought to be related to the widespread use of high-dose biotin, which has led to the potential for biotin interference in routine immunoassays that use a biotin-streptavidin interaction. In November 2017 and November 2019, the US Food and Drug Administration warned the public, healthcare providers, laboratory personnel, and kit manufacturers about biotin interference and its importance. Awareness of biotin interference among laboratory staff and clinicians can prevent misdiagnosis and inappropriate treatment. The vulnerability of an immunoassay test to biotin interference is not constant; the test results may be incorrectly high or low, depending on the assay technology. Biotin can be a challenging source of interference and analytic error in terms of detection and prevention. Laboratory staff should be informed about this potential interference in immunoassays using biotin-streptavidin linkage. To reduce the likelihood of erroneous results, particular attention must be given to the test results of certain patients (e.g., patients with multiple sclerosis) or those that are not compatible with the clinical findings. To mitigate this patient safety risk, laboratories using methods vulnerable to biotin interference should develop appropriate solutions to detect and overcome biotin interference.

Keywords: Biotin, immunoassay, interference, patient safety, streptavidin

Biotin, aka vitamin B7 or H, is a water-soluble vitamin that is an essential coenzyme in the transfer of carboxyl groups by several carboxylases that are involved in fatty acid synthesis, gluconeogenesis, and citric acid cycle. Biotin is also required in the branched-chain amino acids (leucine, isoleucine, and valine) catabolism and utilization.

Since avidin was first purified and characterized in 1940 by biochemist Esmond Snell and colleagues, its affinity for biotin was already known [1]. Streptavidin, an analogous protein in Streptomyces Avidinii, binds to biotin with a particularly strong affinity higher than avidin itself [2]. The streptavidin-biotin interaction is one of the strongest non-covalent interactions in nature [3] and therefore this high durability interaction employed in several analytic methods, including western blotting, flow cytometry, immunohistochemistry, and immunoassays [2].

2000's to present, many papers and case reports pointed out the biotin interference due to clinically incompatible immunoassay test results. The underlying reasons for the discovery of this relatively new biotin interference issue depend on the fact that the widespread use of high dose biotin has led to the potential for biotin interference in routine immunoassays that employs a biotin–streptavidin interaction. Biotin interference is a challenging interference and a source of analytic error in terms of detection and prevention [4].

In 2017, the Food and Drug Administration (FDA) alerted the public, health care providers, laboratorians, and kit manufacturers that biotin interference by leading incorrect test results could significantly affect certain lab tests. The reason underlies the warning was a report regarding the death of a patient taking high levels biotin following falsely low troponin test re-

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sults that analyzed with an assay is known to have biotin interference [5]. In November 2019, FDA has released an updated warning to raise the awareness about the biotin interference and posted a webpage on "Biotin Interference with Troponin Lab Tests - Assays Subject to Biotin Interference" to notify the public on troponin assays where the biotin interference studies have not yet been mentioned (https://www.fda.gov/medical-devices/vitro-diagnostics/biotin-interference-troponin-lab-tests-assays-subject-biotin-interference)[6]. In light of those warnings, laboratorians and clinicians must be aware of biotin interference that might lead to medical errors in terms of diagnosis and treatment can be avoided. In the review, biotin interference on immunoassay tests will further discussed from the laboratorians' perspective.

Biotin metabolism and clinical aspects

Adequate biotin intake is 0.03 mg/day [7], and it is readily available when it is sufficient in diet and completely absorbed mostly from the plant- and animal-based foods with a small amount synthesized by microbiota [8]. Biotin excretes from the kidneys with a physiological half-life of 8 to 16 hours that depends on renal capacity. Although they have less affinity than the parent biotin molecule, it has two active metabolites, bisnorbiotin and biotin sulfoxide, that both also bind to streptavidin Serum biotin levels peak 1-3 hours after ingestion [9].

Biotin deficiency in healthy people is uncommon. Still, its deficiency may observe in some conditions including inherited metabolic diseases such as biotinidase deficiency, holocarboxylase synthetase deficiency, or biotin-thiamine-responsive basal ganglia disease, long-term parental nutrition, anticonvulsant or protein pump inhibitor medication, Crohn's disease, alcoholism, and the excessive consumption of raw egg (in which avidin binds biotin) [10]. Depending on the deficiency cause, it can give in doses from 5 to 300 mg per day. In the USA, in anticipation of FDA approval, patients with multiple sclerosis (MS) have prescribed 300 mg of biotin therapy which are 10.000 times the daily-recommended intake and this may result in biotin concentrations spanning 150–700 µg/L in those patients [11]. Even in overdoses, biotin is promptly absorbed and excreted with little bioaccumulation or toxicity except perhaps for pregnancy.

In addition to its therapeutic use, biotin is marketed as a "beauty pill" with a claim to improve the health of hair, skin, and nails in several multi-vitamins or formulas that are available over-the-counter. These supplements usually contain 5-10 mg biotin but there are multi-vitamins and supplements with higher biotin concentrations, up to 100 mg. Owing to the lack of awareness of the consumers on the content and quantity of those, biotin consumption is usually not reported in medical history [4]. Consequently, the prevalence of patients with significant biotin concentrations and hence the potential of biotin interference increase in recent years.

Mechanism of biotin interference

In clinical laboratories, several biomarkers, including hormones, tumor markers, micronutrients, and therapeutic drugs are measured by immunoassay platforms and in those using biotin-streptavidin technology are susceptible to biotin interference. Following conjugating a biotin molecule to an antibody or analyte with minimum effect on the reaction dynamics, the biotinylated molecule is captured on a streptavidin microparticle or other solid phase, as streptavidin has a strong affinity for biotin and a tightly bound streptavidin–biotin complex can be promptly formed. By using this process, the analytes or assay elements in the sample rapidly distinct in the assay [10]. An immunoassay that employs biotin only as a structural element of either the microparticle or conjugate to join assay components together is not affected when biotin is present in the sample. Immunoassays using anti-animal antibodies instead of biotinylated antibodies are free of biotin interference. Additionally, some immunoassay designs that utilize reagents where streptavidin and biotin linkage are already formed before the addition of the patient's sample is less susceptible to the interference. To summarize, immunoassays use streptavidin–biotin interaction to capture an analyte are subject to the biotin interference [4]. Thus, it is essential to know which immunoassay platforms and which test methodology biotin interference can occur. Fortunately, Holmes and colleagues surveyed 374 immunoassays in the USA in 2016 [12]. The study revealed that 59% percent of the surveyed immunochemical assays used biotin in the methodology and that 22% of those were at 'high risk' of biotin interference (where biotin interfered at a concentration below 51 ng/mL).

Even so, the susceptibility of a test to biotin interference is not certain in terms of magnitude and depends on some features, including sample volume (lowering biotin concentration by lowering the volume), sandwich or competitive assays (Fig. 1) (excess antibody reagent in two-site assays), one- or two-step design, and washing or no-washing [13]. In typical competitive immunoassays for small molecules such as free thyroxine (fT4), free triiodothyronine (fT3), testosterone, estradiol, and cortisol, the analyte concentration is inversely proportional to the signal intensity (Fig. 1a). Excess biotin in the sample can cause a falsely decreased signal and hence increased test result by binding to the solid phase instead of antibody–labeled analyte that is removed in the wash step because of unbounding to the solid phase (Fig. 1b). The two-site "sandwich" or noncompetitive immunoassays are preferred to analyze larger molecules such as thyroid-stimulating hormone (TSH), cardiac troponin, thyroglobulin, follicle-stimulating hormone (FSH), luteinizing hormone (LH), insulin, and for autoantibodies. The analyte concentration is directly proportional to the signal intensity (Fig. 1c). The excessive biotin in the sample can decrease the signal by saturating the streptavidin binding sites preventing adherence of the signal bound antibody–antigen complex and leads to an erroneously decreased result (Fig. 1d) [4, 10]. In the presence of excess biotin, while a TSH test using biotin–streptavidin technology can produce falsely lower, falsely increased fT3 and/or fT4 results can obtain and hence it can mimic hyperthyroidism as presented by Kwok and colleagues' report as well as the others [14-17].

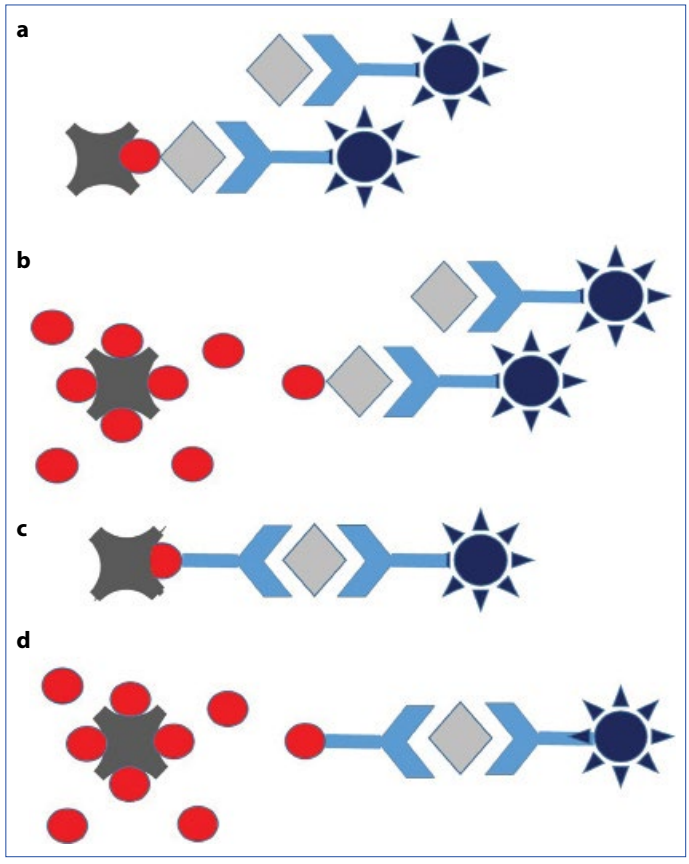

Figure 1. Schematic representations of typical competitive (1a) and sandwich (1c) immunoassays (1b). Excess free biotin in the sample by binding to the solid phase instead of antibody–labeled analyte that is removed in the wash step because of unbounding to the solid phase can lead to a falsely decreased signal in competitive immunoassay design (1d). The presence of excess biotin in the sample can decrease the signal by saturating the streptavidin binding sites preventing adherence of the signal bound antibody–antigen complex and leads to a falsely decreased test result in sandwich immunoassays.

Although any thyroid disorder specified by biomarker testing should be confirmed by medical examination and radiological techniques, any interference presence including biotin interference is an obstacle that leads to the patient undergoing unnecessary investigations even misdiagnosis. Besides, a critical and time-sensitive test such as high sensitive cardiac troponin T is prone to biotin interference and that might lead to a delay in diagnosis of acute myocardial infarction [18].

Detecting and limiting biotin interference

Before eliminate or limit the effect of the biotin interference, first laboratory specialists should be aware of the biotin interference susceptibility of immunoassay methodologies that implemented in their laboratories. Diagnostic companies often address the biotin cut-off value above that an interference might occur. However, these interference studies mostly perform in vitro and the assigned cut-off values may not represent in vivo conditions. Besides, biotin interference susceptibility and thresholds may not always be given in package inserts; still, laboratorians who implement those technologies in their lab must make the necessary efforts. Following these efforts, a warning regarding the possibility of biotin interference in these assay platforms can be shared with fellow physicians and other practitioners. By sharing the information, awareness might increase outside the laboratory. Thus, to inquire and to advise patients to abstain from biotin intake for a few days before blood collection would realize.

Although biotin has a half-life of about 2 hours, its interfering effects on lab tests may persist days according to the pharmacokinetic findings. Therefore, it is logical to avoid taking biotin for at least 2 days before blood sampling [19]. Still, it might keep in mind that there are differences among immunoassays in terms of "biotin intolerance", and the manufacturer's guidance would be valuable in this regard. In patients with MS taking biotin with overdoses, there is no further advice on the awaiting period. Several laboratories prefer to use awaiting time like 48-72 hours, even longer periods for biotin intolerant assays until a new recommendation is addressed. The awaiting time is also dependent on the dose, the duration of biotin intake, assay intolerance, and individual factors such as pediatric patients, renal capacity. It should be emphasized that withholding biotin might be harmful to some metabolic disorders e.g. biotinidase deficiency and in the acute setting [20].

The approach to educate and give a card describing the dose, the duration of treatment and assay interference warning for patients such as seen as patients with MS under high dose biotin treatment might be helpful to increase both patients' and healthcare providers' awareness [21]. Indeed, this patient-targeted approach might be useful to provide guidance to patients and to keep warned them to avoid taking any medications or supplements containing biotin before blood testing. To obtain information about the use of biotin or multivitamin from a patient during blood collection and to transfer this information to the hospital and/or laboratory information system might be an alternative strategy to mitigate the interference risk. It may also be helpful if a laboratory information system has the capacity to generate an alert whenever blood samples from a patient under biotin medication are received.

Some strategies for biotin interference can perform in clinical laboratories. In case of a clinically incompatible test result, among the possible interferences, the biotin interference should also be considered. Biotin levels can be measured in samples suspected of containing biotin. However, it is not feasible for most laboratories where serum biotin concentrations cannot routinely measure. To send a sample to a reference laboratory is both impractical and may inappropriately increase turnaround time that is not applicable for emergency room tests such as troponin and BNP. The best approach, in this case, is to test the sample on a platform that has a biotin tolerance. In this case, an alternative assay platform has to be reachable.

Recent studies have reported in vitro procedures employing surplus/salvaged streptavidin microparticles to remove excess in the sample before testing. This may provide a fast (in-

cubation up to a 1 h) and practical approach to evaluating the presence of biotin interference but still, a laboratory should validate the efficacy of the implemented techniques and decide to report the results [4].

Recently, the Clinical Laboratory Standards Institute (CLSI) (EP-37) has revised to the recommendation for biotin interference testing for manufacturers and has changed the threshold for the risk of interference from 7.5 ng/mL (30.7 nmol/L) up to 3510 ng/mL (14.356 nmol/L) [22]. This recommendation might result in the novel or altered immunoassay methodologies, which would be resistant to the biotin interference in the future.

Conclusion

To lessen this patient safety risk, laboratories using biotin-affected methods should develop their strategies to determine their assays' interference risk and hence take some measures to prevent and overcome the biotin interference in their laboratory settings. To include the education of fellow laboratorians, healthcare professionals, and patients about biotin's effects on laboratory test results into the strategies could be helpful in terms of prevention. Effective communications and consultations between clinicians and the laboratorians must maintain to minimize the rate and effect of assay interference on clinical management by deciding general and targeted procedures that the latter one is for the patients at maximum risk of clinical harm.

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