Validation of a Commercial Reagent for the Depletion of Biotin from Serum/Plasma: A Rapid and Simple Tool to Detect Biotin Interference with Immunoassay Testing

Article:
Brooke M. Katzman, Erin A. Hain, Leslie J. Donato, and Nikola A. Baumann.
Validation of a Commercial Reagent for the Depletion of Biotin from Serum/Plasma: A Rapid and Simple Tool to Detect Biotin Interference with Immunoassay Testing.
J Appl Lab Med 2021;6:4 992-97. https://doi.org/10.1093/jalm/jfab022

Guest: Dr. Brooke Katzman is an assistant professor in the Department of Laboratory Medicine and Pathology and co-director of the Hospital Clinical Laboratory and Point-of-Care at the Mayo Clinic in Rochester, Minnesota.

Randye Kaye: Hello and welcome to this edition of JALM Talk from the *Journal of Applied Laboratory Medicine*, a publication of the American Association for Clinical Chemistry. I'm your host, Randye Kaye. In recent years, reports from the literature Diagnostics Companies and the U.S. Food and Drug Administration have brought awareness to the issue of biotin interference in immunoassays used in clinical laboratories. Despite increased awareness, it remains challenging for laboratories to know if and when their patients' results are affected by biotin interference. Typically, laboratories would have to develop and validate their own techniques to detect and mitigate biotin interference. A focused report in the July 2021 issue of JALM describes the evaluation of a new commercial product designed to bind excess biotin in serum or plasma samples. In this study, the authors sought to determine whether the product could sufficiently deplete biotin from biotin-containing serum and plasma samples and whether the product would impact immunoassay analyte recovery. On today's podcast, we are joined by the article's first author, Dr. Brooke Katzman. Dr. Katzman is an assistant professor in the Department of Laboratory Medicine and Pathology and co-director of the Hospital Clinical Laboratory and Point-of-Care at the Mayo Clinic in Rochester, Minnesota. Welcome Dr. Katzman. Can you first of all remind our audience what is biotin and what's the concern with its use as it relates to clinical laboratory testing?

Brooke Katzman: Yes, so biotin is also known as vitamin B7 or vitamin H and it's one of the water-soluble B complex vitamins, and it really plays a key role in the body as it's an essential co-factor for several carboxylases that are responsible for the synthesis of fatty acids, the catabolism of branched-chain amino acids, and also involved in gluconeogenesis. And so really, the issue comes over the last several years, there have been a number of reports in the literature regarding biotin interference with some laboratory tests and particularly immunoassays where thyroid function was actually being evaluated. And this seems to correlate with an increased usage of over-the-counter biotin-containing supplements. And so when a patient sample contains excess biotin from these supplements, depending on the analyte that's measured,

those results could be falsely increased or falsely decreased. And so because of the risk of misdiagnosis or mistreatment, the FDA has released several safety statements warning consumers, health care providers, lab personnel and lab test manufacturers about the potential for biotin interference.

Randye Kaye: All right. Thank you. Tell me more about the mechanism by which it can interfere with laboratory testing?

Brooke Katzman: So the mechanism of interference with biotinylated immunoassays can really be explained in the context of how biotin and streptavidin binding is used in these biotinylated assays. So immunoassays are generally categorized as either competitive or non-competitive, also known as sandwich immunoassay. And so, in general, streptavidin biotin binding is used during the assay incubation to couple biotinylated antibodies in the sandwich immunoassays or biotinylated antigens in the competitive immunoassays to the streptavidin-coated magnetic surfaces, and usually these are magnetic beads. So when a biological specimen contains excess biotin, the biotin competes with the biotinylated antibodies or antigens for binding to the streptavidin-coated magnetic surfaces. And so this results in a reduced capture of the biotinylated antibodies or antigens. And so excess biotin produces false to low results in sandwich immunoassays because the assay signal is directly proportional to the analyte concentration. And this is in contrast to competitive immunoassays where excess biotin causes falsely elevated results because the assay signal is inversely proportional to the analyte concentration.

Randye Kaye: All right. Thank you. Now, how long after ingestion could it be present in patient's blood or interfere with the lab results?

Brooke Katzman: So a normal circulating concentration of biotin is in the range between 0.1 to 0.8 ng/mL and this would be in individuals that are consuming that recommended daily dose of only 30 mcg. So, what ends up happening is when you're taking biotin, it's readily absorbed after ingestion and a peak plasma concentration would occur within about one to two hours. Some of the studies have shown that oral doses of 10 mg, this is equivalent to some of the highest doses in the over-the-counter products, will result in peak plasma concentrations ranging in anywhere between 55 to 140 ng/mL. And so obviously higher oral doses would result in peak plasma concentrations much higher. So, when looking at these subjects, you know, in general, low concentrations of biotin are cleared fairly quickly from circulation with an elimination half-life of approximately two hours. Whereas, the experiments with these high doses showed that the half-life can go up to about 19 hours.

And so, in a recent pharmacokinetic study, these were apparently healthy individuals. The authors actually determined that blood concentrations are expected to drop below about 20 ng/mL approximately 20 hours after an oral biotin dose of 10 mg. The important thing to note here though is that these were studies performed in healthy individuals and so delayed clearance and/or higher circulating concentrations of biotin could be observed in those with impaired renal function.

Randye Kaye: Okay. Thanks. Now, if laboratories are trying to mitigate the risk of biotin interference, what tools are available for them?

Brooke Katzman: Yeah, so there are a number of different options to try to investigate. The first that we think of is serial dilutions of a specimen to determine if that specimen has a linear recovery. The next is some of these reagents that can be used to actually deplete biotin from a specimen, so whether it's a lab-developed test that uses Streptavidin Agarose to remove that biotin, or in our study, we evaluated a commercial reagent called VeraPrep Biotin used to deplete that patient specimen. And then the next available I would say, although is not as widely used, would be to actually quantitate biotin specifically in a specimen. We do this with our lab-developed liquid chromatography mass spec assay.

Randye Kaye: Okay. Now, in your article, you described the evaluation of that product VeraPrep Biotin and it's designed to deplete biotin from patient samples prior to testing, so what were the major findings of this study?

Brooke Katzman: Yeah, so we really had two goals in this. The one was we want to show that VeraPrep Biotin does not affect analyte recovery in biotin negative samples or control samples – so it won't impact samples that don't have biotin. And the next was does it effectively deplete biotin from samples that either were spiked with biotin or endogenous biotin from patients or subjects that were actually taking or consuming biotin. So, to answer that first question, in those control samples that contain no biotin, we found that 90% to 110% analyte recovery was observed post VeraPrep treatment in over 95% of samples, suggesting that it truly does not affect recovery and samples that don't contain biotin. We also then did find that VeraPrep Biotin was also effective at removing biotin from serum samples that were spiked with biotin or from those donor samples were VeraPrep Biotin reduced the biotin concentrations to below physiologic biotin concentrations. So, in all of these samples, we were able to reduce the biotin concentration to below 2.5 ng/mL.

Randye Kaye: Thank you and finally do you think that biotin interference will continue to be a major patient safety concern?

Brooke Katzman: So fortunately, over the last several years, the awareness of potential biotin interference has really significantly increased among our clinical colleagues, laboratorians and patients. And so the efforts that are spearheaded by manufacturers, clinical labs, and the FDA I would say have successfully reached our target audience. And so we do hope that the incidence of biotin interference decreases as those efforts continue, but until manufacturers reformulate their assays and eliminate the possibility of biotin interference, I do feel that laboratorians and clinicians will be challenged with biotin and its impact on clinical testing. Fortunately, some manufacturers have taken those steps to address their assays. In the meantime, tools like VeraPrep Biotin will offer laboratories a very simple self-contained kit for detecting biotin interference and mitigating the risk of patient harm.

Randye Kaye: Wonderful. Thank you so much for your time today.

Brooke Katzman: Thank you.

Randye Kaye: That was Dr. Brooke Katzman discussing the JALM article, "Validation of a Commercial Reagent for the Depletion of Biotin from Serum Plasma: a Rapid and Simple Tool to Detect Biotin Interference with Immunoassay Testing." Thanks for tuning in to this episode of JALM Talk. See you next time and don't forget to submit something for us to talk about.