Biotin Interference: Scientific Updates, Assessment of On-Market Assays, FDA Recommendations, and FAQ.
**Background:** Recent changes in clinical practice and consumer use of biotin-based supplements have contributed to an increased prevalence of biotin use and shifted the risk profile of lab test results produced by streptavidin-based immunoassays. As result, biotin interference in immunoassays from several manufacturers have been described in the literature, with examples of clinically misleading test results including one associated death\(^1\)\(^2\).

**Medical and Supplementation Use:** Biotin (Vitamin B7) is a hydrophilic compound that acts as a coenzyme in carboxylase reactions and is therefore an essential nutrient for supporting normal biochemical functions. Although readily available in a balanced diet with a recommended daily intake (~30 \(\mu\)g per day), higher doses of biotin (5mg – 20mg) have been used for the purported health benefits it may provide to hair, skin, and nails; while prescription doses (50 – 300mg) have been used to treat inherited enzyme deficiencies, basal ganglia disease, and more recently included in a clinical trial for secondary progressive multiple sclerosis \(^3\)-\(^5\).

**Assay Interference:** The mechanism of biotin interference will differ depending on the immunoassay format used but has been associated specifically with “free capture” methodologies.\(^8\)\(^\text{-}\)\(^10\) When biotin-streptavidin binding is used as part of a “sandwich” method, excess biotin in the sample can displace biotinylated antibodies resulting in falsely low results. In contrast, for “competitive” immunoassays, excess biotin in the specimen can compete with the biotinylated analog for the binding sites on streptavidin, resulting in falsely high results. Immunoassays using free capture streptavidin-biotin mechanisms are used by many reagent manufacturers and have the potential to show interference from biotin by one of the mechanisms described above and should therefore be assessed for risk profiling to understand the clinical risk. With such a wide range of biotin doses, longer clearance times may be needed to reach concentrations in blood below which interference occurs but has been reported to be as long as 7 days \(^3\),\(^6\),\(^7\).

**Scientific & Regulatory Awareness:** While an increase in case reports linking biotin use and laboratory test interferences has increased in recent years \(^9\),\(^11\), review articles and mechanistic studies have helped further describe the risk to patients \(^8\)-\(^12\). More recently, the FDA issued a Safety Communication to alert the public, health care providers, lab personnel, and lab test developers that biotin can significantly interfere with certain assays and cause incorrect test results. These results, if undetected, could lead to diagnostic errors or misdiagnoses\(^1\)\(^2\). The FDA recommendations, summarized in supplement A, provide guidance to help raise awareness, reduce risk and prompt action by manufacturers to assess and communicate to their customers regarding biotin interference and the assays they develop and market.

**Prevalence:** One of the questions that remains largely unanswered is the prevalence of both biotin usage and blood levels of biotin in patients at the time of lab testing or when presenting to acute care settings such as the Emergency Department (ED) where treatment decisions frequently rely on laboratory tests in patients with critical conditions. In a recent publication, the Mayo Clinic report\(^14\) on their findings of both outpatient patient surveys regarding biotin usage and biotin quantification of plasma samples collected from ED patients.

Results suggest from completed surveys, 7.7% indicated biotin use, 7.4% of 1442 ED patient samples had biotin concentrations at or above 10 ng/mL which is the lowest known reported threshold for biotin interference by Roche Diagnostics, and biotin concentrations of \(\geq\)30 ng/mL, \(\geq\)50ng/mL and \(\geq\)100ng/mL were reported in seven, five and two patients, respectively, while
nearly 50% of emergency department patients had measurable levels of biotin over 5ng/mL (Figure 1).

The authors conclude that reported use of biotin was common, the range of biotin concentrations in ED patients highlights the magnitude of the biotin interference and identifies a population at risk for potential harm, and the findings should guide laboratorians and clinicians in developing effective strategies to mitigate safety risks and in assessing biotin usage trends within their own patient populations.

Interference Assessment of Abbott Immunoassays: Assessment of each assay design and format was performed through a comprehensive review of all ARCHITECT and Alinity assay formats (Figure 2). Results confirmed that no on-market ARCHITECT or Alinity assay formulations use a free capture streptavidin/biotin assay format referenced in recent articles and associated with interference from biotin. Although not in the free capture streptavidin/biotin format, 5 ARCHITECT and 1 Alinity on market assay(s) utilize preformed biotin in the assay design. To confirm these assays are not impacted, samples spiked with biotin at concentrations up to 4,250ng/mL were evaluated. The highest level of biotin tested (4,250 ng/mL) was selected to exceed the Clinical and Laboratory Standards Institute (CLSI) guidelines (EP-37) of 3,510 ng/mL. Results show the concentration and % difference with 95% confidence; table 1.

![Graph showing biotin concentration and frequency.]

- 7.4% of Emergency Medicine patient samples were above 10ng/mL.
- >50% of the samples tested were greater than 5ng/mL.

1 Adapted from publication.
Table 1. Biotin Interference Testing. ARCHITECT i and Alinity i Systems

<table>
<thead>
<tr>
<th>SYSTEM</th>
<th>ASSAY</th>
<th>ANALYTE CONCENTRATION</th>
<th>BIOTIN TEST LEVELS (ng/mL)</th>
<th>1,500</th>
<th>2,000</th>
<th>2,500</th>
<th>3,500</th>
<th>4,250</th>
</tr>
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<tbody>
<tr>
<td>Active-B12</td>
<td>(3P24)</td>
<td>51.3 pmol/L</td>
<td>2.29%</td>
<td>0.42%</td>
<td>1.35%</td>
<td>1.65%</td>
<td>1.49%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(3P24)</td>
<td>87.3 pmol/L</td>
<td>2.27%</td>
<td>0.82%</td>
<td>2.36%</td>
<td>0.53%</td>
<td>1.05%</td>
<td></td>
</tr>
<tr>
<td>anti-CCP</td>
<td>(1P65)</td>
<td>4.30 U/mL</td>
<td>2.17%</td>
<td>1.19%</td>
<td>-0.23%</td>
<td>1.47%</td>
<td>1.20%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(1P65)</td>
<td>14.2 U/mL</td>
<td>0.64%</td>
<td>0.75%</td>
<td>-0.81%</td>
<td>2.34%</td>
<td>1.64%</td>
<td></td>
</tr>
<tr>
<td>Methotrexate</td>
<td>(2P49)</td>
<td>0.048 µmol/L</td>
<td>-0.77%</td>
<td>2.60%</td>
<td>-0.09%</td>
<td>2.50%</td>
<td>1.70%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(2P49)</td>
<td>0.902 µmol/L</td>
<td>4.45%</td>
<td>4.86%</td>
<td>5.44%</td>
<td>4.68%</td>
<td>8.30%</td>
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</tr>
<tr>
<td>NT-proBNP</td>
<td>(2R10)</td>
<td>9.36 pmol/L</td>
<td>0.74%</td>
<td>-0.96%</td>
<td>1.18%</td>
<td>-0.64%</td>
<td>0.55%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(2R10)</td>
<td>214.2 pmol/L</td>
<td>-2.05%</td>
<td>-1.01%</td>
<td>-0.57%</td>
<td>-2.76%</td>
<td>0.82%</td>
<td></td>
</tr>
<tr>
<td>2nd Gen Testosterone</td>
<td>(2P13)</td>
<td>8.48 µmol/L</td>
<td>-5.13%</td>
<td>-5.59%</td>
<td>-6.35%</td>
<td>-6.62%</td>
<td>-5.20%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(2P13)</td>
<td>32.4 µmol/L</td>
<td>-4.98%</td>
<td>-5.85%</td>
<td>-4.74%</td>
<td>-5.21%</td>
<td>-4.24%</td>
<td></td>
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<tr>
<td>Alinity</td>
<td>2nd Gen Testosterone</td>
<td>(07P68)</td>
<td>8.49 µmol/L</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-2.70%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>32.9 µmol/L</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-4.42%</td>
<td></td>
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</tbody>
</table>

Acceptability Criteria: +/- 10% (95% confidence) from target concentration.

KEY TAKEAWAYS:

- Biotin (Vitamin B7) is an essential nutrient for which an intake of 30 µg per day is recommended. This level is not associated with assay interference. Supplement and prescription dose levels (5 – 300mg) have been reported to interfere with susceptible assay formats and potentially lead to diagnostic errors.

- Biotin has been demonstrated to impact assays that use biotin–streptavidin free capture binding as part of the immunoassay format and is not limited to hormone and cardiovascular diagnostic assays. Consult lab leadership to understand which assays are at risk in your health care network, including testing that may be sent to reference labs.

- Some immunoassay formats are not affected by biotin and may be used to confirm unexpected results or be preferred if the patient is known to be receiving biotin supplementation.

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2 The Alinity i and ARCHITECT i systems utilize the same reagents and sample/reagent ratios in their assay design.
3 Alere NT-proBNP for ARCHITECT
4 Biotin Test Levels for NT-proBNP testing were 1,593ng/mL; 2,125ng/mL; 2,656ng/mL; 3,718ng/mL; 4,250ng/mL; respectively.


FDA RECOMMENDATIONS:1-2

For Health Care Providers:
- Talk to your patients about any biotin supplements they may be taking, including supplements marketed for hair, skin, and nail growth.
- Be aware that many lab tests, including but not limited to cardiovascular diagnostic tests and hormone tests, that use biotin technology are potentially affected, and incorrect test results may be generated if there is biotin in the patient’s specimen.
- Communicate to the lab conducting the testing if your patient is taking biotin.
- If a lab test result doesn’t match the clinical presentation of your patient, consider biotin interference as a possible source of error.
- Know that biotin is found in multivitamins, including prenatal multivitamins, biotin supplements, and dietary supplements for hair, skin, and nail growth in levels that may interfere with lab tests.
- Report to the lab test manufacturer and the FDA if you become aware of a patient experiencing an adverse event following potentially incorrect laboratory test results due to biotin interference.

For Lab Personnel:
- If you use assays with biotin technology, be aware that it is difficult to identify samples that contain biotin; therefore, it is important to communicate with health care providers and patients to prevent incorrect test results.
- If you are collecting samples in the lab, ask whether the patient is taking biotin.
- Educate health care providers about biotin interference with certain lab tests used in your lab.
- Consider that the daily recommended allowance for biotin is 0.03 mg and these biotin levels do not typically cause significant interference. However, supplements containing high biotin levels including those marketed for hair, skin, and nail benefits, may contain up to 20 mg of biotin, and physicians may recommend up to 300 mg per day for conditions such as multiple sclerosis. Biotin levels higher than the recommended daily allowance may cause significant interference with affected lab tests.
- Be aware that specimens collected from patients taking high levels of biotin may contain more than 100 ng/mL biotin. Concentrations of biotin up to 1200 ng/mL may be present in specimens collected from patients taking up to 300 mg per day.
- Currently available data is insufficient to support recommendations for safe testing using affected tests in patients taking high levels of biotin, including about the length of time for biotin clearance from the blood.
- Communicate with the lab test manufacturer if you have questions about biotin interference.

For Consumers:
- Talk to your doctor if you are currently taking biotin or are considering adding biotin, or a supplement containing biotin, to your diet.
- Know that biotin is found in multivitamins, including prenatal multivitamins, biotin supplements, and supplements for hair, skin, and nail growth in levels that may interfere with laboratory tests.
- Be aware that some supplements, particularly those labeled for hair, skin, and nail benefits, may have high levels of biotin, which may not be clear from the name of the supplement.
- If you have had a lab test done and are concerned about the results, talk to your health care provider about the possibility of biotin interference.
**FREQUENTLY ASKED QUESTIONS REGARDING BIOTIN**

1.) **WHY IS BIOTIN USED IN IMMUNOASSAYS?**

Biotin is a small molecule that can be attached covalently to a variety of targets from large proteins such as antibodies to tiny steroid hormones. Once attached to the analyte of interest, biotinylated complexes can be form strong, stable, and specific non-covalent bounds with streptavidin proteins, allowing for the capture and quantification of analytes of interest in blood.

2.) **WHAT EFFECT DOES BIOTIN INTERFERENCE HAVE?**

The type of interference (bias) depends on the design of the assay and can result in falsely increased or decreased results. Two of the most common immunoassay designs are the sandwich and competitive assay formats.

**Sandwich Assay**

*Direction of Interference:* Falsely Decreased

*How biotin interferes:* Free biotin binds to the streptavidin-coated capture surfaces, leaving fewer binding sites for the antibody complexes to bind. Subsequent washes falsely remove the analyte intended for measurement. The resulting signal produced is lower than it should be in the reaction without biotin interference. Sandwich assay results are proportional to signal generated and in the presence of excess free biotin can cause falsely decreased results.

**Competitive Assay**

*Direction of Interference:* Falsely Increased

*How biotin interferes:* Free biotin binds to the streptavidin-coated capture surfaces, leaving fewer binding sites for the antibody complex to bind. Subsequent washes falsely remove the analyte intended for measurement. The resulting signal produced is lower than it should be in the reaction without biotin present. Competitive assay results are inversely proportional to signal generated and in the presence of excess free biotin can cause falsely elevated results.

3.) **ARE ALL IMMUNOASSAYS SUSCEPTIBLE?**

No, some immunoassay platforms and assays are not susceptible because they do not use the biotin-streptavidin free capture methodology.

4.) **HOW LONG DOES IT TAKE FOR BIOTIN TO CLEAR THE BODY?**

More research is needed to better understand the impact chronic use and different doses may have on clearance to provide accurate recommendations regarding timing. Higher doses have been shown to take longer to clear while patients with poor renal function could take even longer. Because interference thresholds differ widely among assays and vendors the timing of safe blood collection may vary as well.
5.) ABBOTT HAS 5 IMMUNOASSAYS THAT USE PRECOMPLEXED BIOTIN IN THE ASSAY DESIGN, WHY ARE THEY NOT AFFECTED BY BIOTIN INTERFERENCE?

Precomplexing biotin during the assay design eliminates binding sites for free (exogenous) biotin that may be in a patient’s specimen following supplementation. To confirm these assays are not impacted by free biotin, internal studies were conducted according the FDA and CLSI recommendations. Interference testing up to 4,250ng/mL did not show any interference when compared to controls. Testing up to 4,250ng/mL exceeds the recommended threshold of 3,510ng/mL by CLSI.

6.) TO MY KNOWLEDGE I AM NOT AWARE OF ANY LAB RESULTS IMPACTED BY BIOTIN INTERFERENCE IN MY LAB, SHOULD I BE CONCERNED?

Unless your hospital and lab have a comprehensive risk mitigation plan in place for educating and detecting biotin interference it could go unnoticed and the impact not realized. Recommendations from the FDA\textsuperscript{1,2} describe what key stakeholders can do to help prevent diagnostic errors associated with biotin interference. If your assays are impacted by biotin there will always be some level of risk to your patient population.