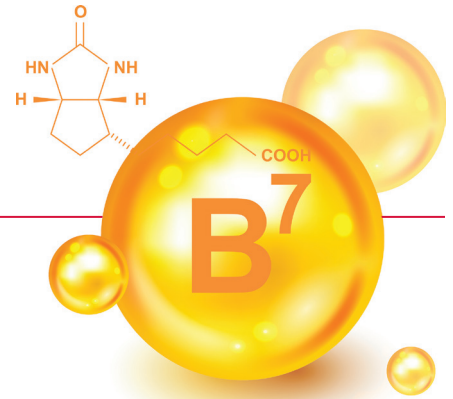


Managing Interferences in the Laboratory



At Ortho, we work together with laboratorians to minimize the impact of potential interferences. This collaboration helps ensure optimal patient outcomes for the clinicians and patients we serve.

ABOUT BIOTIN

Biotin is a water-soluble B complex vitamin that is necessary for the metabolism of fats and carbohydrates. It may also be called vitamin H or vitamin B7 and is found naturally occurring in foods such as milk, eggs, lean meat, nuts, and some fruits, vegetables and grains. There is no Recommended Daily Allowance of biotin, but the Food and Nutrition Board of the Institute of Medicine, National Academy of Sciences has set the Adequate Intake for biotin at 0.03 mg (30 µg) per day. This amount is easily obtained from a normal diet.¹

In recent years, biotin has been marketed to consumers in over-the-counter supplements. Although there is no scientific evidence, these products claim to enhance hair and nail health. These supplements contain biotin in very high doses, in some cases over 600 times adequate daily intake.² Use of these supplements with mega-doses of biotin has increased the probability of encountering a sample that could be impacted by biotin.



Interferences are a Common Challenge

In *Guidelines on Interference Testing in Clinical Chemistry*, the Clinical and Laboratory Standards Institute (CLSI) defined interferences as, “A cause of clinically significant bias in the measured analyte concentration due to the effect of another component or property of the sample.”⁶

Any time a sample is tested, there is a risk of interference.⁶ Because technologies vary between manufacturers, the degree of impact may vary widely. Common interferences include hemoglobin, bilirubin and lipids, paraproteins, medications, autoantibodies, supplements including biotin, and others.

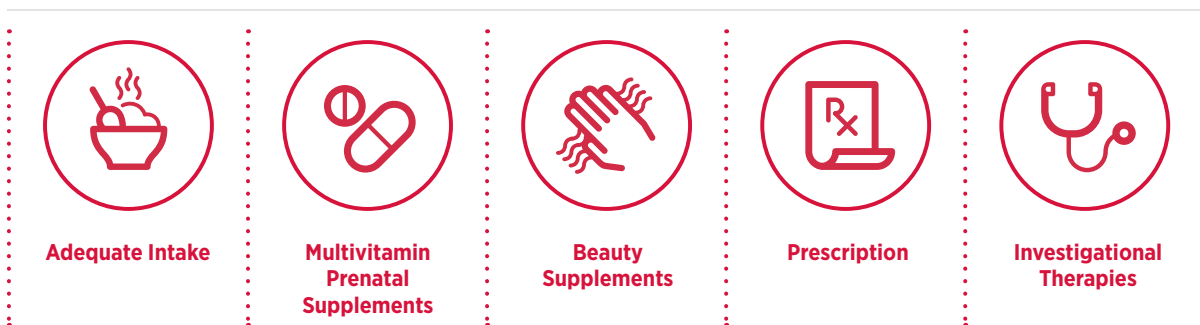
BIOTIN BY DOSAGE

- Normal dietary intake of biotin does not interfere with Ortho’s immunoassays.³
- The level of biotin in multivitamins—including prenatal supplements—does not interfere with Ortho’s immunoassays.^{3, 4, 5}
- Beauty supplements offering doses of biotin greater than 5 mg per day have the potential to interfere with some immunoassays.^{2, 3}

BIOTIN DOSES VS. ESTIMATED BIOTIN CONCENTRATIONS

The kinetics of biotin were assessed in studies performed in apparently healthy subjects.^{2,3,7,8} The table below describes the estimated relationship between biotin intake and serum blood concentration.

Table 1: Biotin doses vs. estimated biotin concentrations



Use	Normal Diet	Daily Vitamin	Hair & Nail Growth	Biotinase Deficiency (rare disease)	Experimental Treatments for Specific Diseases
Estimated Intake Amount	0.005–0.035 mg/day ¹	0.15–0.3 mg/day ^{4,5}	5–20 mg/day	10 mg/day ⁹	300 mg/day
Estimated Biotin Concentration (serum)	0.1–0.8 ng/mL ⁴	up to 2.4 ng/mL ^{4,7,8}	50–355 ng/mL ²	~100 ng/mL ^{2,4}	~1160 ng/mL ^{10,11}
Additional Information	Normal Diet (without vitamin supplementation)	No VITROS® assays are impacted by biotin interference at this concentration	150 to 600 times adequate intake Concentration varies with dose and time ^{2,7,8}	300 times adequate intake Concentration varies with dose and time ^{2,4}	10,00 times adequate intake

ORTHO: DESIGNING OUT THE RISK

Manufacturers use biotin in immunoassays because the streptavidin-biotin affinity is one of the strongest non-covalent interactions known in chemistry. For many in vitro diagnostic manufacturers, biotin has long been the foundation of immunoassay design, and proven to help ensure highly sensitive, specific, accurate and stable immunoassays.¹²

Ortho assays may use designs in one of two architectures: pre-bound (not susceptible to biotin interference) and non-pre-bound (may be susceptible to biotin interference). In a pre-bound assay architecture, the biotinylated antibody conjugate is immobilized to the well surface as part of the manufacturing process. Both of these architectures may utilize either sandwich or competitive immunoassay format.

Ortho Pre-Bound Assay Architecture Eliminates Biotin Challenge

Biotin in the assay formulation does not mean that the assay is susceptible to biotin interference. Ortho has redesigned some of our MicroWell assays with a biotin-antibody conjugate pre-bound to a streptavidin-coated well. This design retains the performance advantages of the streptavidin-biotin interaction while eliminating the risk of interference from biotin. Since 2015, all new VITROS® MicroWell assays have been designed to eliminate the challenge of biotin interference.

Pre-Bound Assay Architecture

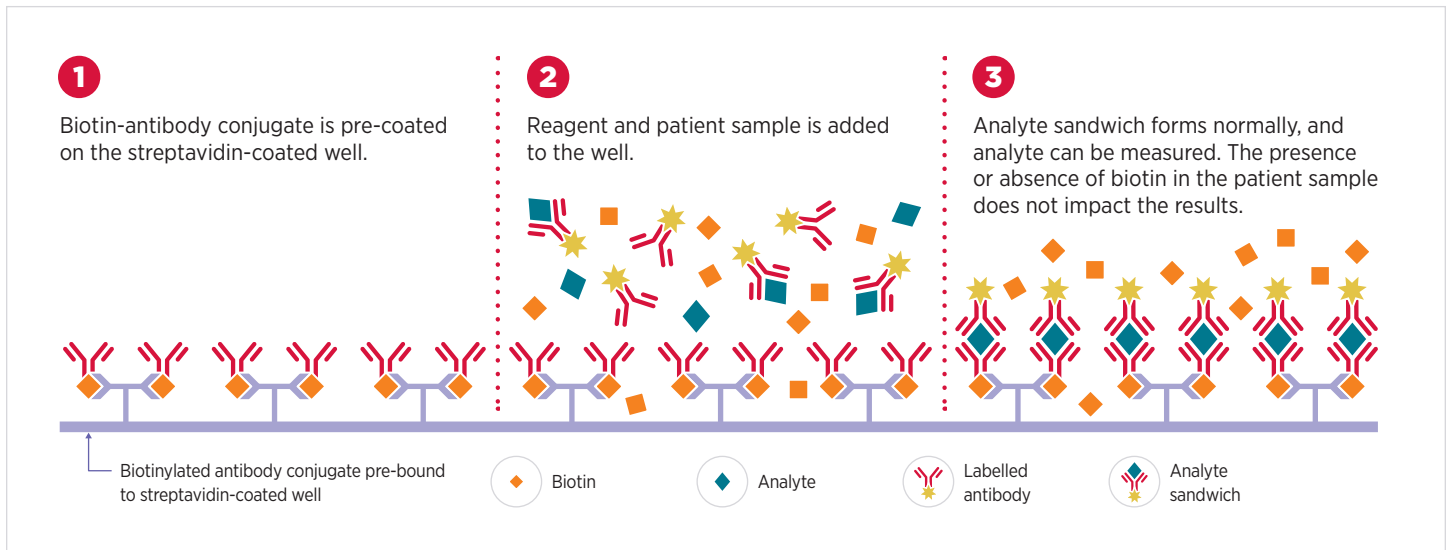
In a pre-bound assay, the immunocomplexes are pre-bound to the well surface in the factory. The pre-bound complexes are not displaced by free biotin in the sample, and no interference can occur. The presence or absence of biotin in the patient sample has no effect on the test.

Biotin-free formulations are not required to prevent biotin interference.¹²

The impact of biotin on assay performance depends on the assay format. Both VITROS sandwich and competitive assay formats rely on binding immunocomplexes to a streptavidin-coated well. (See Figure 1.)

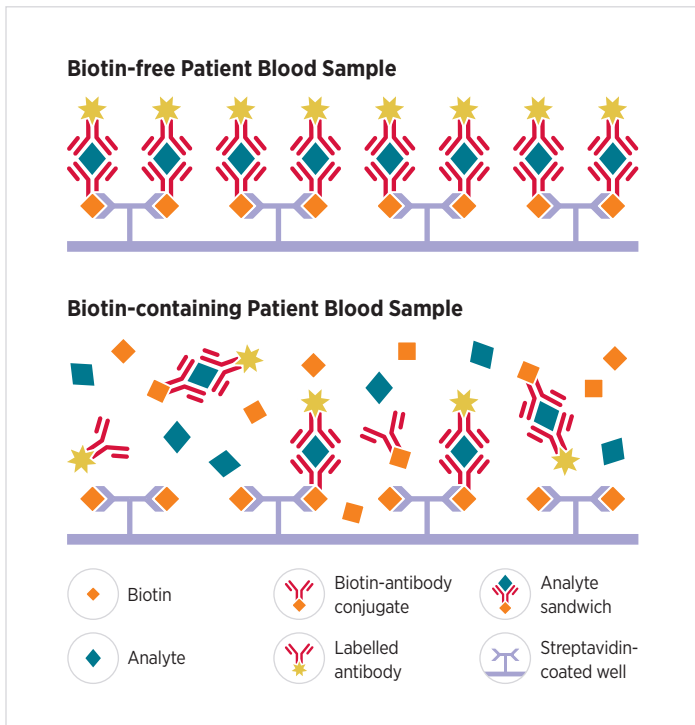
Non-pre-bound assays simultaneously react analyte present in the sample with a biotinylated antibody or an antibody/analyte complex (immunocomplex). If present, free biotin in the sample competes with the biotinylated immunocomplex for binding sites on the streptavidin-coated wells to falsely raise or lower results. Falsely lower or higher results can affect the clinical interpretation. (See Figures 2 and 3.)

Figure 1: VITROS Microwell Assays biotin-interference free design



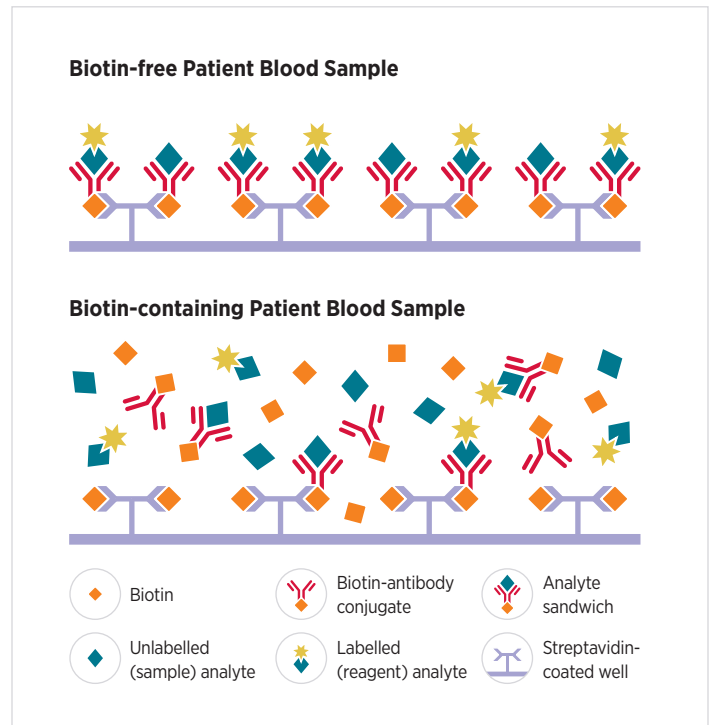
In assays using a sandwich format, a negative bias is typically observed.

Figure 2: Sandwich format assays



In assays using a competitive format, a positive bias is typically observed.

Figure 3: Competitive format assays

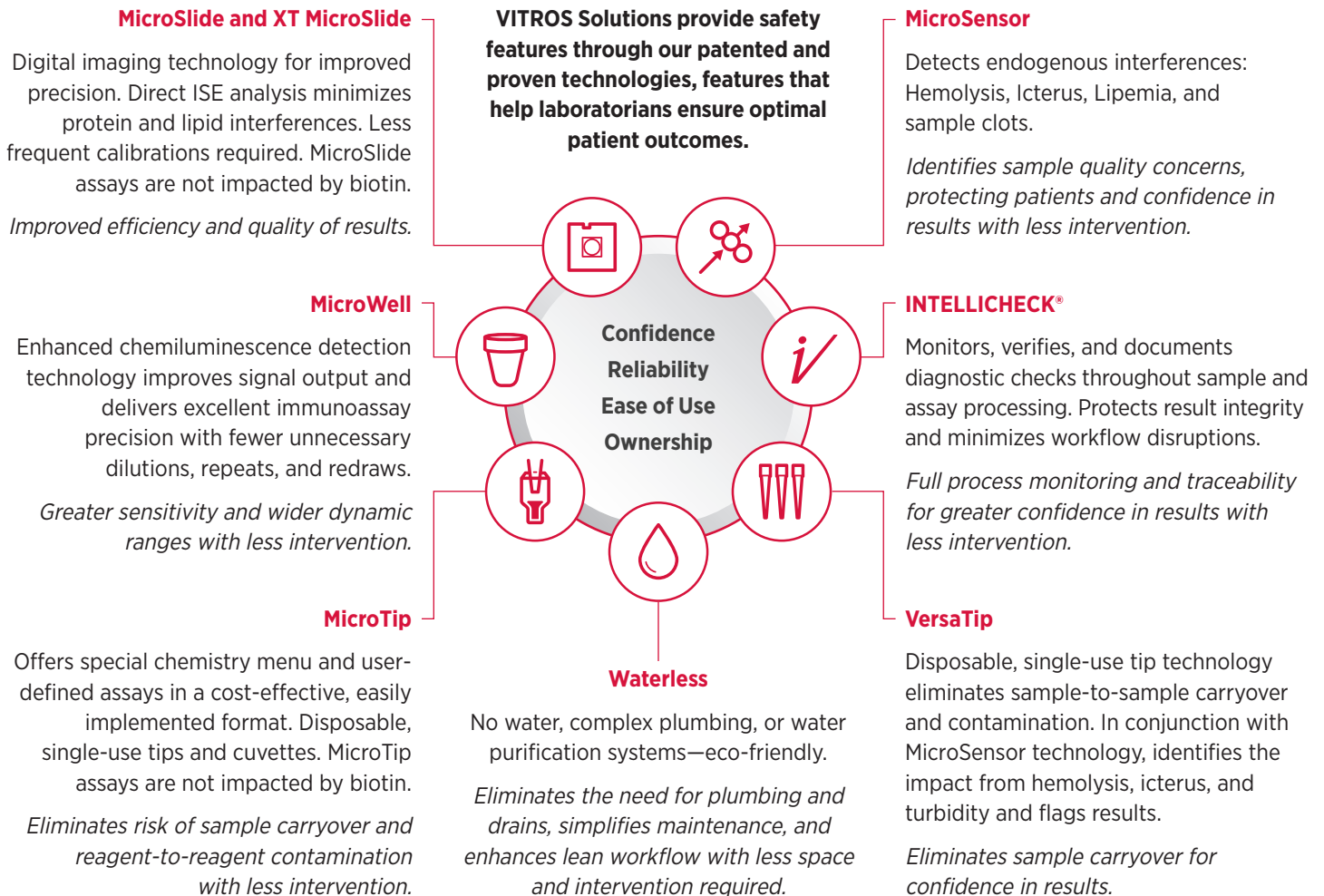


ACTIONS TO CONSIDER FOR YOUR LABORATORY

Interferences are not new to laboratory testing. The team in your laboratory is already knowledgeable about these challenges, as any type of assay or test may be susceptible to analytical interference. Actions you already take that improve awareness in laboratories, clinicians, and patients may be the best defense:

- ✓ **Educate.** Speak with clinicians about biotin interference with certain lab tests used in your facility. Know which assays are potentially affected by biotin interference. Refer to IFU for assay-specific information. Encourage clinicians to speak with patients about the risks associated with biotin supplements on diagnostic tests. Help providers educate patients by posting notices in patient collection areas.
- ✓ **Communicate.** Ortho supports FDA recommendation for laboratory, clinician and patient education about reporting all prescription medication and supplements, including biotin, prior to blood draws. Whenever practical, suggest clinicians specifically ask patients about biotin and other supplements when collecting information about medications.
- ✓ **Continue.** As the results from any diagnostic test should be used and interpreted only in the context of the overall clinical picture, remind clinicians to alert the laboratory about any clinically discordant results.

VITROS: DESIGNED TO RECOGNIZE, REDUCE, AND REMOVE THE IMPACT OF INTERFERENCES



OUR COMMITMENT TO YOU

We never stop working to deliver accurate test results, efficient and reliable instruments, and easy-to-use technology. Continuous collaboration ensures your lab is achieving the most important measure of success: exceptional patient care.

RESOURCES FOR FURTHER LEARNING

Listed below are some resources offering further information and guidance in managing the potential for interferences in the lab.

- [NIH Biotin Fact Sheet for Consumers](#)
- [NIH Biotin Fact Sheet for Health Professionals](#)
- [FDA Safety Communication](#)
- [AACC Webinar: Strategies for Reducing Analytical Interferences in the Clinical Chemistry Laboratory](#), Dr. David Grenache, PhD, FAACC DABCC, Chief Scientific officer of Tricore Reference Laboratories.
- [Biotin interference in immunoassays mimicking subclinical Graves' disease and hyperestrogenism: a case series](#), Marcelo C. Batista, MD, et al, Clinical Laboratory, Clin Chem Lab Med 2017; 55(6): e99-e103 DOI 10.1515/cclm-2016-0628.
- [Population pharmacokinetics of exogenous biotin and the relationship between biotin serum levels and in vitro immunoassay interference](#), Grimsey, Frey, Bendig et al., Int. J. Pharmacokinet. 14 Sep 2017 ISSN 2053-0846, 10.4155/ipk-2017-0013.
- [Understanding and managing interferences in clinical laboratory assays: the role of laboratory professionals](#), Martina Zaninotto et al., Clin Chem Lab Med. 2020 Feb 25;58(3):350-356. doi: 10.1515/cclm-2019-0898.
- [An overview on interference in clinical immunoassays: A cause for concern](#), Sequeira S., Hamdan Med J 2019;12:158-64.

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