

Biotin Interference in Allergy Testing

Edsel Sinson, Kelline Rodems, and Eric Whitters, Ph.D.

“Biotin can significantly interfere with certain lab tests and cause incorrect test results which may go undetected”

– FDA Safety Communication

A HYCOR® BIOMEDICAL
WHITE PAPER

Biotin Interference in Allergy Testing

Edsel Sinson, Kelline Rodems, and Eric Whitters, Ph.D.
HYCOR® Biomedical

EXECUTIVE SUMMARY

With the recent emergence of high dose biotin consumption from dietary and beauty supplements, the FDA and the European Competent Authorities, such as ANSM (France) and HPRA (Ireland), are making known the increasing prevalence of inaccurate lab tests caused by biotin interference. According to the safety communication released by the FDA, such inaccurate results have caused misdiagnosis and, in one instance, death.¹ More specifically, diagnostic assays which utilize the biotin-streptavidin interaction may be susceptible to elevated levels of biotin in patient serum, thereby causing false negative results.

“Biotin can significantly interfere with certain lab tests and cause incorrect test results which may go undetected”

– FDA Safety Communication

This paper provides a technical overview of the mechanism for biotin interference and is intended to help laboratories understand the potential for false negatives in *in vitro* allergy testing due to biotin interference. Three Specific IgE allergy assays were used for this study. IMMULITE 2000 3G Allergy and NOVEOS assays utilize biotin-streptavidin chemistry. ImmunoCAP assays do not use biotin-streptavidin and therefore is used as a comparative control. Internal data provided in this paper demonstrate that NOVEOS results were on par with results from ImmunoCAP Specific IgE. However, the IMMULITE 2000 3G Allergy methodology demonstrated significant biotin interference with all patient samples reporting false negative at all levels of biotin tested.

Biotin Consumption Facts

Biotin, a B vitamin, is an essential nutrient that is present in foods and dietary supplements. Approximately one-third of the US population currently supplement their diet with biotin.²

- Retail sales of biotin supplements grew more than 260% between 2013 and 2016 (Figure 1).³
- The recommended daily allowance is 30 to 60 µg for multivitamins, 5 to 10 mg for hair & nail supplements, and greater than 10 mg for therapeutic regimens, respectively.⁴
- Dietary biotin produces no adverse effects up to 50 mg/day; biotin supplements have no apparent impact up to 200 mg/day.⁵

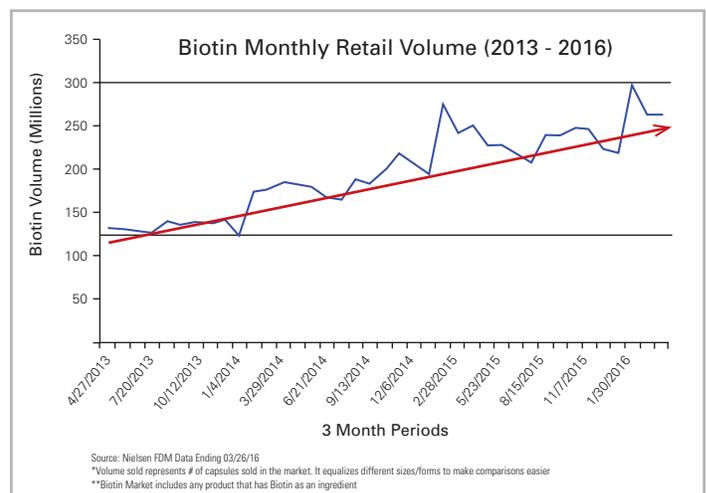


Figure 1.

INTRODUCTION

Biotin in Diagnostic Devices

Biotin-streptavidin is considered one of the strongest non-covalent bonds found in nature; the interaction is biologically/chemically inert since it is highly specific, stable and resistant to changes in temperature and/or pH. In addition, the conjugation of biotin to antibodies and other proteins does not alter their biological properties and provides an efficient coupling to a streptavidin surface. For these reasons, it has been used by diagnostic manufacturers for over a decade.

Biotin in human consumption

Biotin, also known as vitamin B7, can be found in a vitamin and mineral supplements and functions as a co-enzyme in the metabolism of fatty acids, amino acids, and glucose.⁶ The use of supplements is typically not necessary, as sufficient dietary biotin is present in foods. However, for the past several years, biotin has been marketed as a beauty supplement, driving an increase in biotin consumption and costing U.S. consumers over \$30 billion annually.⁷ As there are no adverse effects with consumption of large doses of biotin, many consumers ingest biotin supplements that can contain up to 650 times the recommended Dietary Reference Intake.

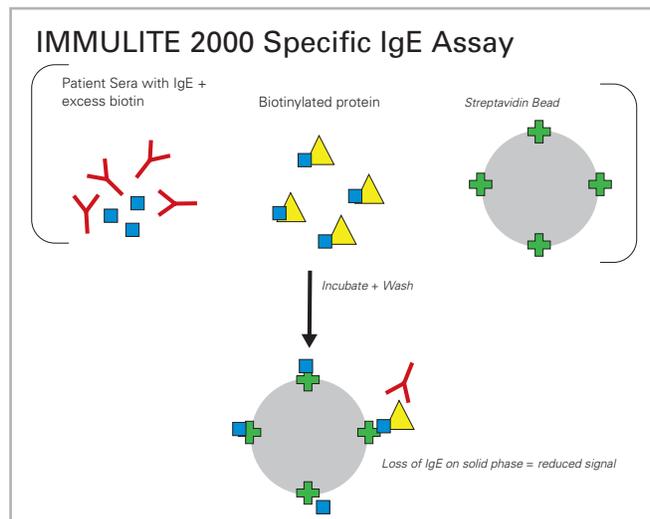


Figure 2.

Mechanism of Biotin Interference in Specific IgE Assays

In many immunoassays, proteins or analytes of interest are biotinylated then added to a streptavidin-coated solid phase. Biotin interference is seen when endogenous biotin in the patient sample competes with the biotinylated protein of interest for binding sites on the streptavidin surface. This occurs in a competitive assay when the patient sample and the biotinylated analyte are co-incubated with the solid phase as it does in the IMMULITE 2000 3G Allergy assay (Figure 2). In non-competitive indirect assays like the NOVEOS assay (Figure 3), the biotinylated-protein is initially bound to the streptavidin-coated solid phase, followed by incubation with patient sample. Any biotin in the sample, therefore, does not compete for binding.

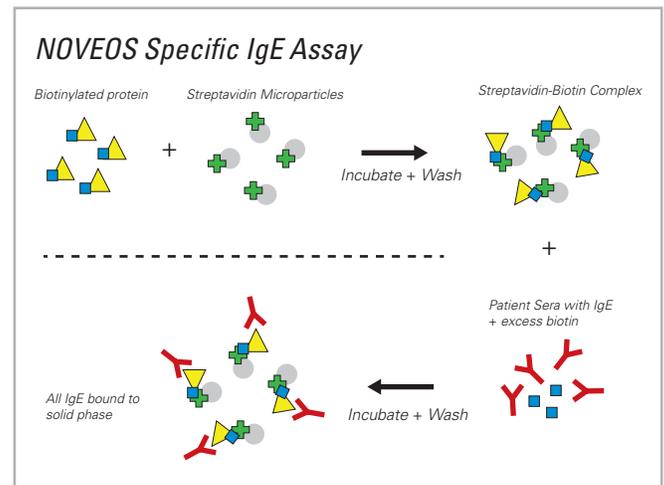


Figure 3.

Interference of excess biotin in patient serum on IMMULITE 2000 3G Allergy assay and NOVEOS systems.

IMMULITE 2000 Specific IgE Assay combines patient sera, biotinylated-protein, and a streptavidin bead in a single incubation step. If a patient's sera contains excess biotin, this allows for the biotin to compete with the biotinylated protein and IgE for the limited surface available on the bead, which may

result in a false negative or falsely diminished signal. With the NOVEOS Specific IgE Assay, the streptavidin microparticles and biotinylated protein are coupled prior to the introduction of sample, removing any risk of biotin interfering with the assay.

INTERNAL DATA:

Biotin Interference on NOVEOS, ImmunoCAP Specific IgE, & IMMULITE 2000 3G Allergy

A panel of patients were selected based on their reactivity to allergens (D001) House Dust Mite, (E001) Cat Dander, (G006) Timothy Grass, (M006) Alternaria

alternata, and (T007) Oak. Reconstituted D-biotin was spiked into each sample at 5 levels from 0 ng/mL to 2,000 ng/mL. The recommended level of biotin testing according to the FDA is 1,200 ng/mL. Samples were then tested on NOVEOS, IMMULITE 2000 3G Allergy, and ImmunoCAP Specific IgE assays.⁸ Results were compared against their respective controls.

While other streptavidin-biotin assays may be susceptible to biotin interference due to the dramatic increase in biotin supplements in recent years, data confirm that NOVEOS Specific IgE assay does not experience biotin interference.

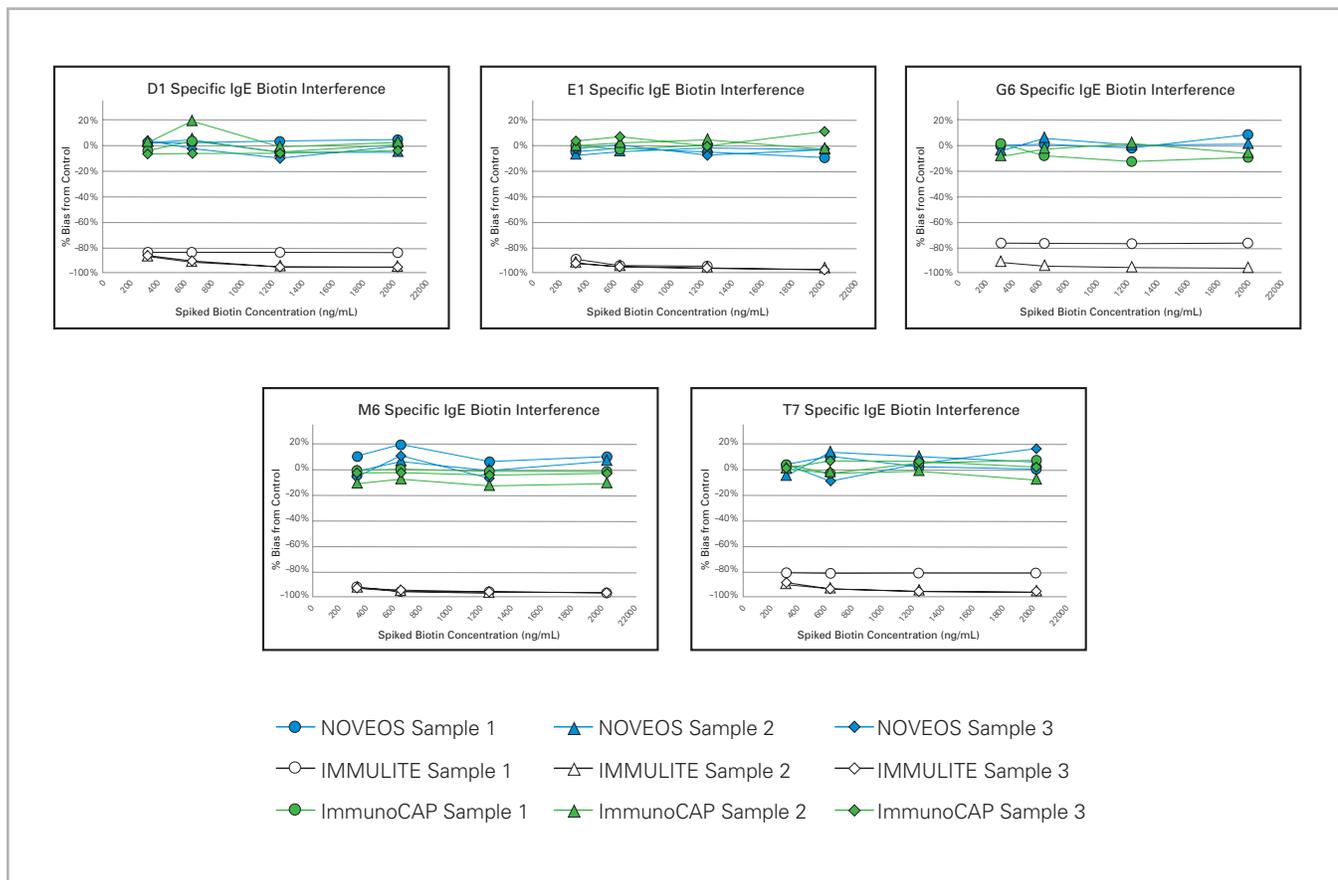


Figure 4. NOVEOS and ImmunoCAP specific IgE assays show minimal bias with all samples retaining their positive values (≥ 0.35 kU/L). IMMULITE 3G Allergy however showed significant bias and resulted in all samples reporting as negative (< 0.35 kU/L).

The NOVEOS™ Chemiluminescent Method

Cost-Effective, Accurate and Precise

- Highly-automated
- Superior walk-away time/ability
- Intuitive user interface for ease of training and operation
- Liquid, ready-to-use reagents

Uses only 4µL of Specimen per Test

- Improves lab workflow and operational costs
- Reduces Quantity Not Sufficient (QNS) errors
- Reduces patient resampling due to insufficient volume
- Reduces trauma for hard-to-draw patients

Reduction of variability

- Large reagent lot sizes
- Use of standardized extracts when available
- Every allergen receives extensive biochemical characterization to ensure performance
- Assay design is unaffected by biotin or cellulose-related cross-reactive carbohydrate determinants interferences

Trusted Analytical Performance

- Cutting-edge immunochemistry technology
- Paramagnetic microparticles
- High sensitivity and excellent low-end precision

About HYCOR® Biomedical

With over 40 years of experience, HYCOR Biomedical is a global manufacturer and marketer of in vitro diagnostic products.

Since its founding in 1981, HYCOR has supported clinical laboratories, hospitals and doctors' offices worldwide with allergy and autoimmune instrumentation and reagents. Among its products, HYCOR markets the HYTEC™ and AUTOSTAT® instruments and most recently the NOVEOS™ Immunoassay System. Each has received CE Mark for the European Union and FDA clearance in the United States.

The company is focused on delivering innovative technology products and comprehensive services that provide the highest value to clinicians and laboratories.

REFERENCES

- 1) <https://www.fda.gov/medicaldevices/safety/alertsandnotices/ucm586505.htm>. Accessed October 10, 2018.
- 2) Bailey RL, Gahche JJ, Lentino CV, et al. Dietary supplement use in the United States: 2003 – 2006. *J Nutr*, 2011;141(2):261-266.
- 3) <https://www.mlo-online.com/closer-look-recent-fda-safety-communication-biotin-interference>. Accessed on October 10, 2018.
- 4) Biotin facts <https://biotinfacts.roche.com/webinar/>. Accessed on August 28, 2019.
- 5) <https://ods.od.nih.gov/factsheets/Biotin-HealthProfessional>. Accessed on October 10, 2018.
- 6) Elston MS, Sehgal S, DuToit S, Yarndley T, Conaglen JV. Factitious Graves' disease due to biotin immunoassay interference — a case and review of the literature. *J Clin Endocrinol Metab*. 2016;101:3251–5.
- 7) Chun K. Biotin Interference in Diagnostic Tests. *Clin Chem*. 2017; 63:619-620.
- 8) NOVEOS™ Immunoassay Analyzer and Capture Reagents for D001, E001, G006 & T007 are CE marked, M006 is for Performance Evaluation Only, CE mark for M006 is pending. In the U.S. the NOVEOS Capture Reagents for allergens E001, G006, M006 & T007 are for "Investigational Use Only," pending submission and clearance by the United States Food and Drug Administration. The performance characteristics of these products has not been established.

HYCOR Biomedical

7272 Chapman Avenue
Garden Grove, California 92841
United States of America
+1 714 933 3000



www.hycorbiomedical.com
www.NOVEOSdx.com

HYCOR Europe B.V.

Waldauer Weg 84
34253 Kassel - Lohfelden
Germany
+49 561 816 7000 - 0