

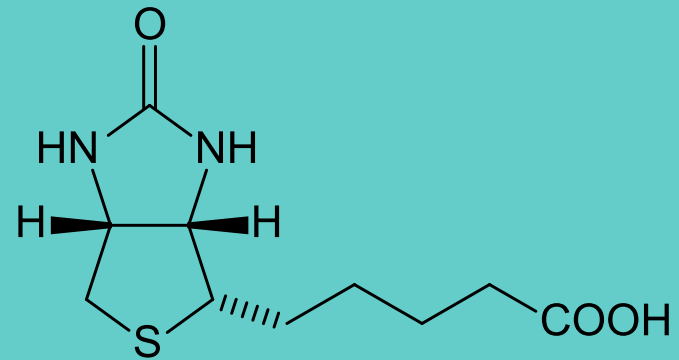
Biotin and Immunoassays: The Good, The Bad and The Ugly

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Agenda

- 1 What is Biotin – Clinical Utility**
- 2 Use of Biotin/Streptavidin in Immunoassay Design**
- 3 Impact of Plasma Biotin on Immunoassays**
- 4 Does it matter? Clinical Impact of Megadosing and Test Results**

What is Biotin



What is Biotin

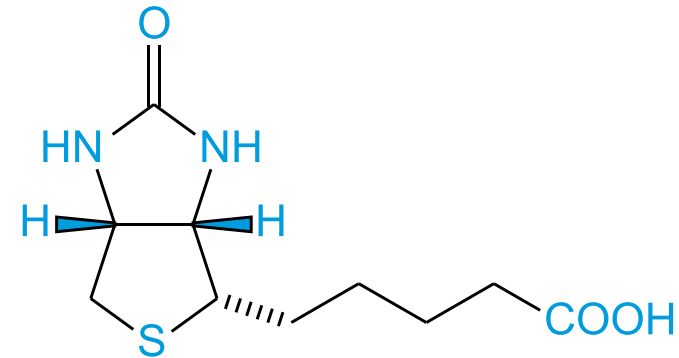
Biotin is a water-soluble molecule usually classified as a B-complex vitamin.

Bios IIB, protective factor X, vitamin H, coenzyme R, W factor, and vitamin B7.

Coenzyme for five mammalian carboxylases involved in the metabolism of carbohydrates (gluconeogenesis), amino acids, and fatty acids

Biotin is present in many foods: organ meats (like liver and kidney), egg yolk, some vegetables, and cow's milk

USDA DRI = 30 µg/day for an adult.



Category	Biotin: Adequate Intake
0-6 months	5 mcg/day
7-12 months	6 mcg/day
1-3 years	8 mcg/day
4-8 years	12 mcg/day
9-13 years	20 mcg/day
14-18 years	25 mcg/day
19 years and up	30 mcg/day
Pregnant women	30 mcg/day
Breastfeeding women	35 mcg/day

Biotin – Fact Sheet for Health Professionals". Office of Dietary Supplements, US National Institutes of Health. 8 December 2017. <https://ods.od.nih.gov/factsheets/Biotin-HealthProfessional/#h2> Retrieved 7 March 2018

Biotin Deficiencies

Spontaneous deficiency has been observed in some individuals who have consumed raw eggs over long periods.

Biotin deficiency was documented in parenteral nutrition without biotin supplementation in patients with short-gut syndrome and other causes of malabsorption.

Signs and symptoms of biotin insufficiency include:

- dermatitis, thinning of hair with loss of color, atrophic glossitis, hyperesthesia, muscle pain, lassitude, anorexia, slight anemia, and change in the electrocardiogram.[

Inborn errors causing biotinidase deficiency and biotin transporter deficiency also result in biotin deficiency.

Patients with severe biotinidase deficiency may suffer from:

- seizures, psychomotor delay, deafness, ataxia, visual pathology, conjunctivitis, and alopecia.

Medical Conditions for Use of Biotin

Prophylaxis and treatment of biotin deficiency, and in the treatment of alopecia (in France)

- Treatment: 0.1 mg to 20 mg/day.[53]

Biotinidase deficiency (inborn error of biotin metabolism)

- Unable to recycle biotin, Autosomal recessive
- 1:61,000 births
- Treatment: 5 – 10 mg/day

‘Biotin-responsive basal ganglia disease’ (BBGD)

- Disorder of energy metabolism
- Treatment: 5 – 10 mg/day

Holocarboxylase synthetase (HCS)/biotin transporter deficiency (BTD) deficiency

- Unable to use biotin effectively, autosomal recessive
- 1:87,000 births
- Treatment: 10 – 100 mg/day

Chronic progressive multiple sclerosis

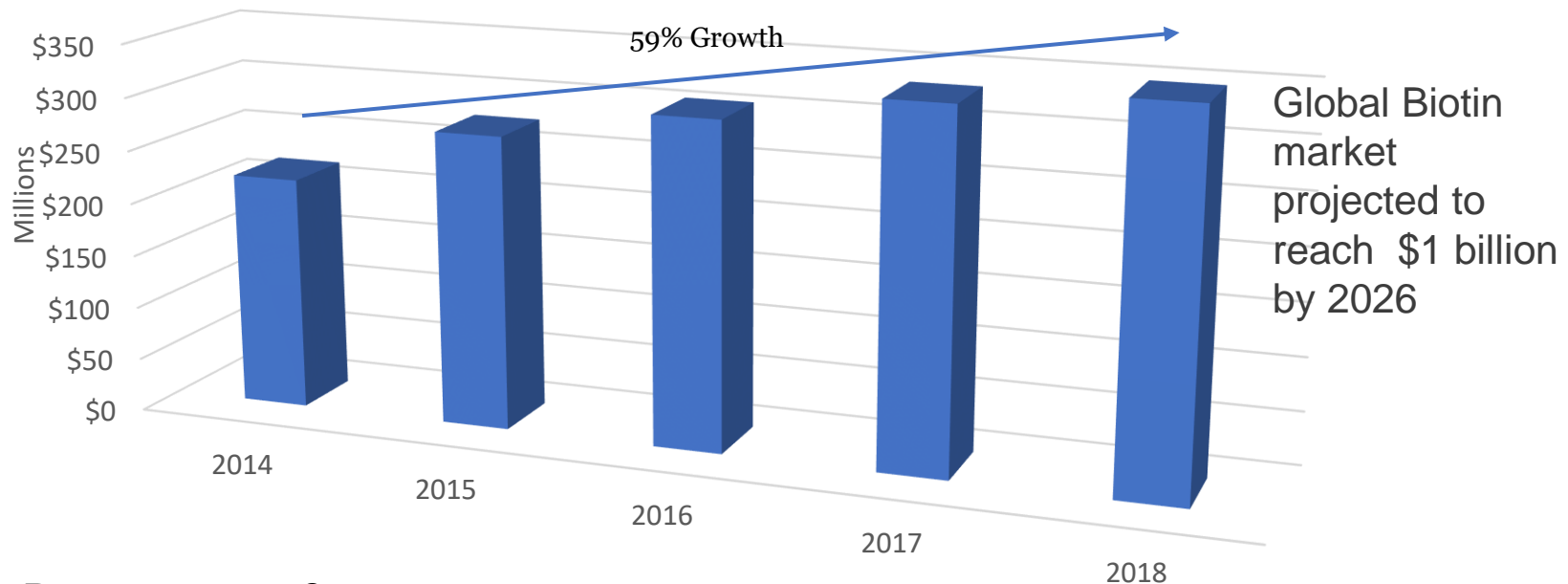
- Randomized, double-blind, placebo-controlled study in France
- Treatment; 100-300 mg/day

Consumer Trends in Biotin Use

Concern is nutraceutical use growth

LUSTROUS HAIR, RADIANT
SKIN, STRONG NAILS –
marketing, no strong evidence

In-Store Sales Biotin Supplements



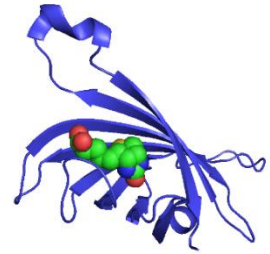
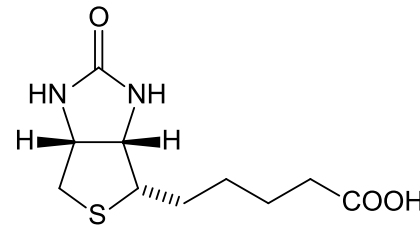
Nielsen Data, 2014-2018

Does not include on-line retailers where biotin dosage of 5,000 mcg is #1 supplement.

OTC supplements - 5,000 – 10,000 ug – more than 125x DRI

Biotin/Streptavidin in Immunoassay Design

Why Used in Immunoassays



Used in conjunction with Streptavidin

- 52.8 kDa protein from bacterium *Streptomyces avidinii*.


Extraordinary high affinity for biotin

- disassociation constant $K_d \sim 10^{-14}$ mmole/L
- one of strongest non-covalent interaction, and binds under a wide array of condition.

Widely used in Western blotting and immunoassays conjugated to some reporter molecule, such as horseradish peroxidase.

Streptavidin has also been used in the developing field of Nanobiotechnology, the use of biological molecules such as proteins or lipids to create nanoscale devices/structures.

How is Biotin Used in Immunoassays



Soluble	<ul style="list-style-type: none">• Free biotinylated analogues/antibodies and streptavidin-coated particle
Pre-complexed reagents	<ul style="list-style-type: none">• Pre-formed biotin-streptavidin complexes
Not used	<ul style="list-style-type: none">• Anti-animal antibodies may be used instead

1-Step (Competitive) Immunoassay

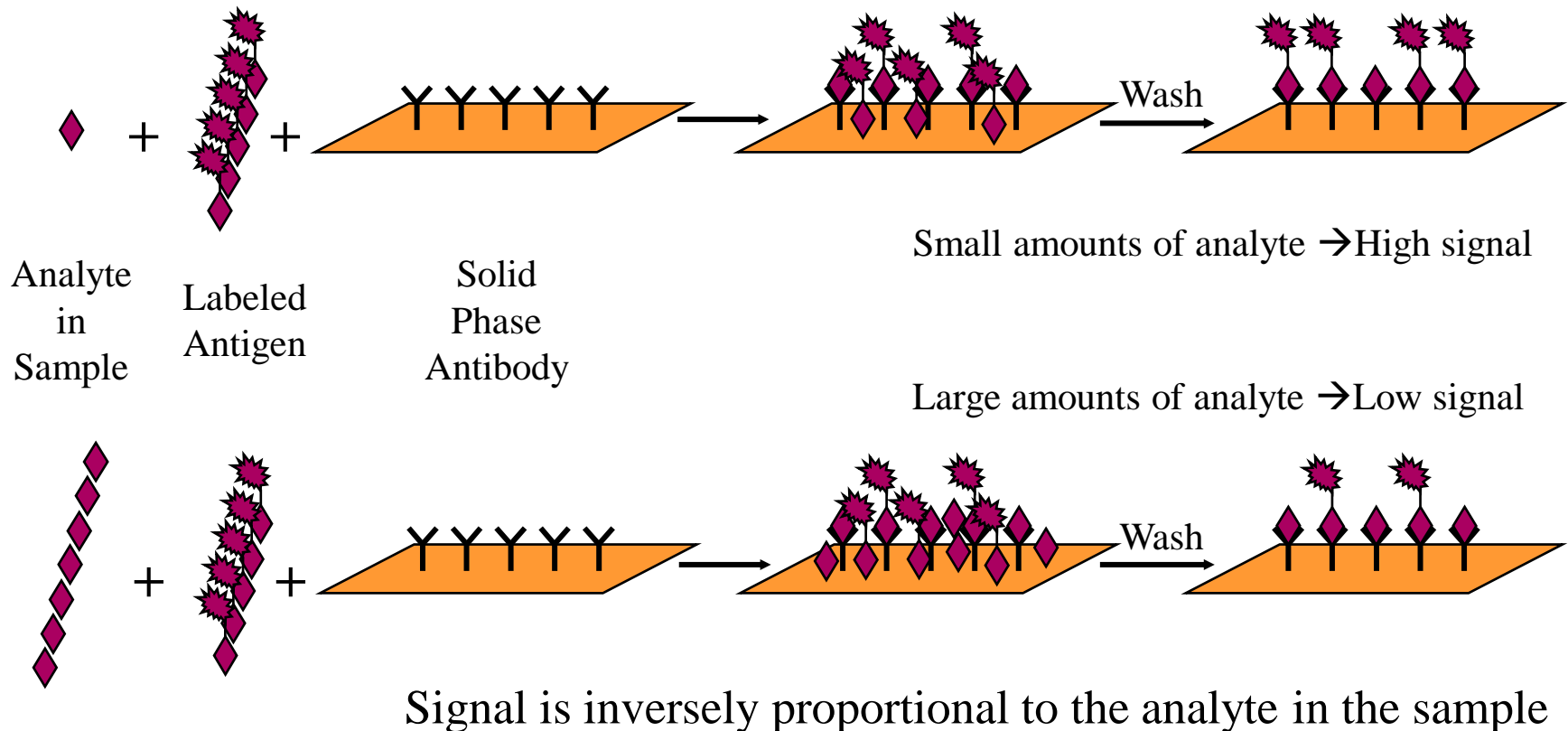
Immunoassay in which the analyte competes with a labeled antigen (that is identical to or similar to the analyte) for binding sites on a solid phase (capture) antibody.

Example – Assay for cocaine in urine

- Solid phase is a plastic surface affixed with antibody that binds cocaine.
- Signal generator is pharmaceutical grade cocaine with colloidal gold attached to it (visible color).
- Add urine sample & signal generator to solid phase
- Cocaine in urine competes with colloidal gold labeled cocaine for capture by solid phase antibody.
- Separate bound from unbound and read.

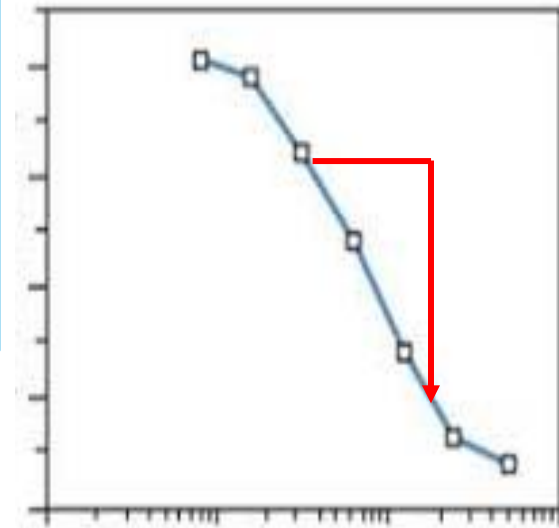
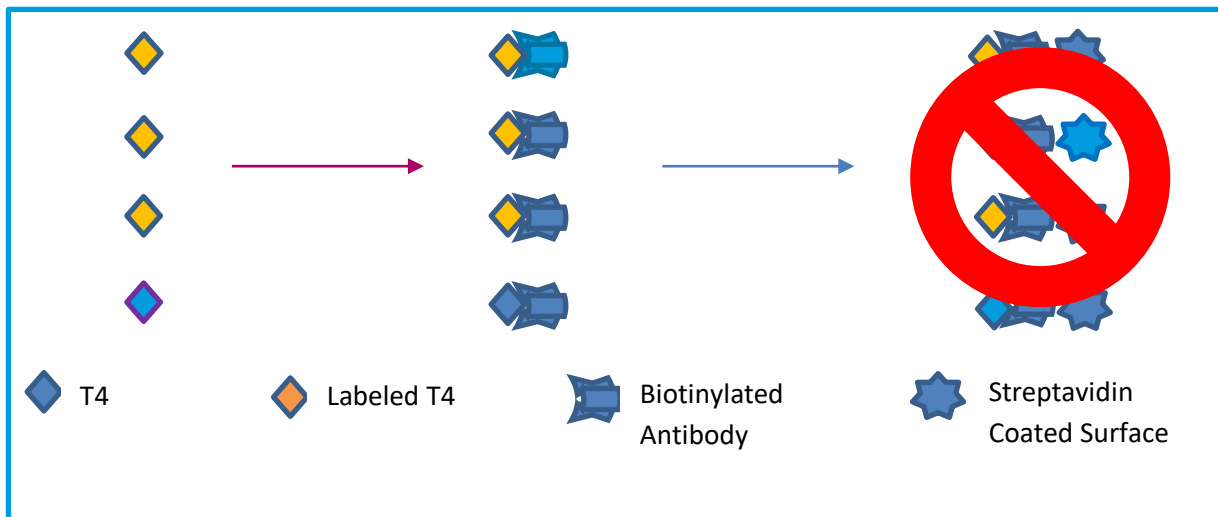
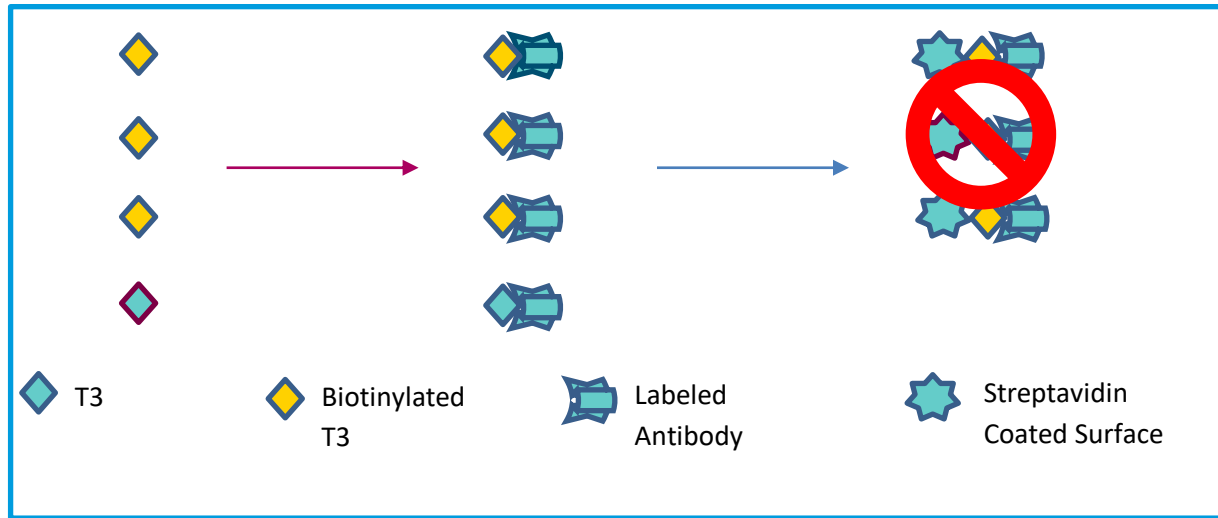
1-Step Immunoassay

Typically used for small analytes (*e.g.*, DOA, FT4, FT3, testosterone, etc) that only have one antibody binding site (epitope).



Competitive Immunoassays

Biotin suppresses signal, falsely elevating result



2-Step (Sandwich) Immunoassay

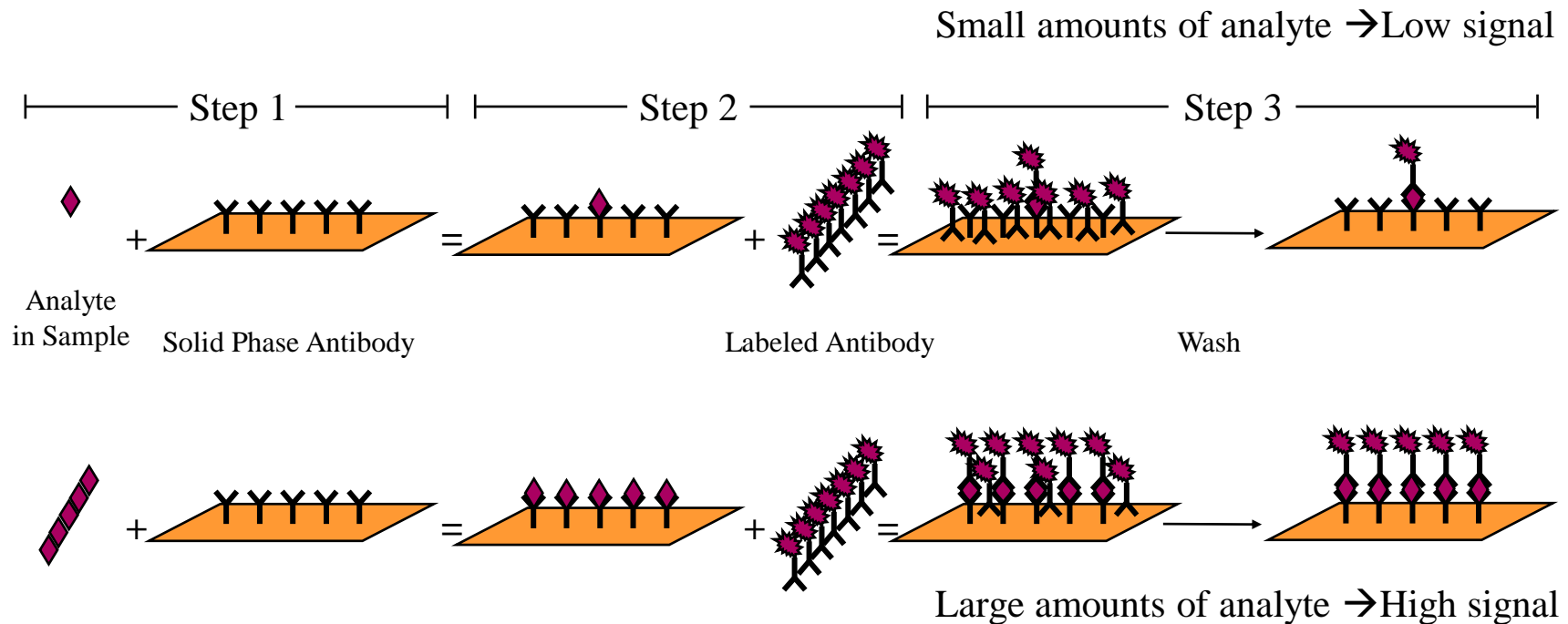
Immunoassay where the analyte is “sandwiched” between a solid phase antibody and a liquid phase antibody conjugate.

Example – Assay for troponin I in blood

- Solid phase is a plastic surface affixed with antibody that binds troponin I.
- Signal generator is a second antibody with a fluorescent dye attached to it that binds troponin I.
- Add blood sample to solid phase for analyte capture.
- Add fluorescent dye labeled antibody to detect capture analyte.
- Separate bound from unbound and read in a fluorometer.

“Sandwich” Immunoassay

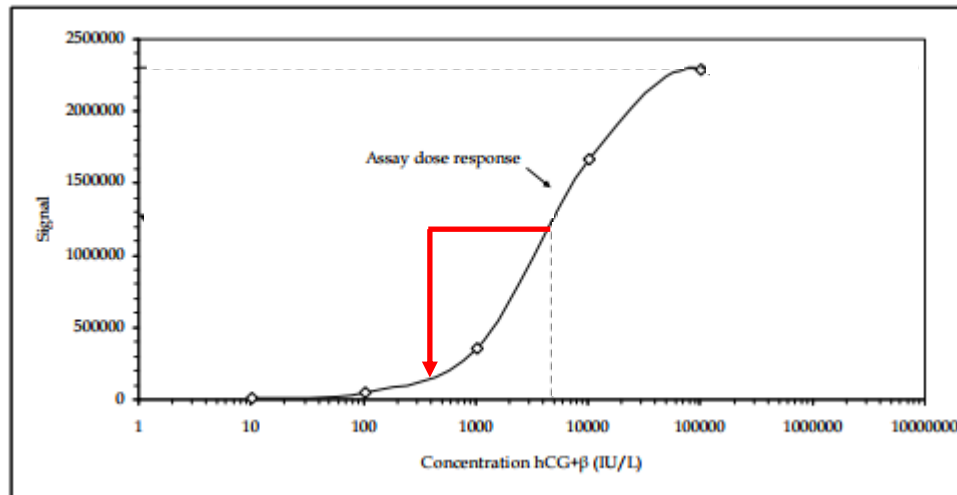
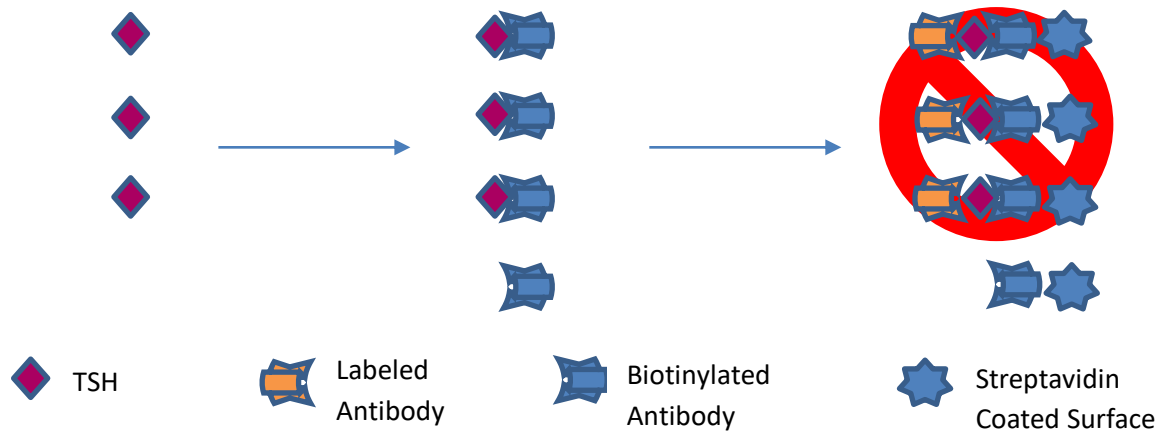
Typically used for analytes with multiple epitopes (Cardiac Markers, Microbiology, TSH, FSH, LH, etc.).



Signal is proportional to the analyte in the sample

Non-Competitive (Sandwich) Immunoassays

Biotin suppresses signal, falsely depressing result



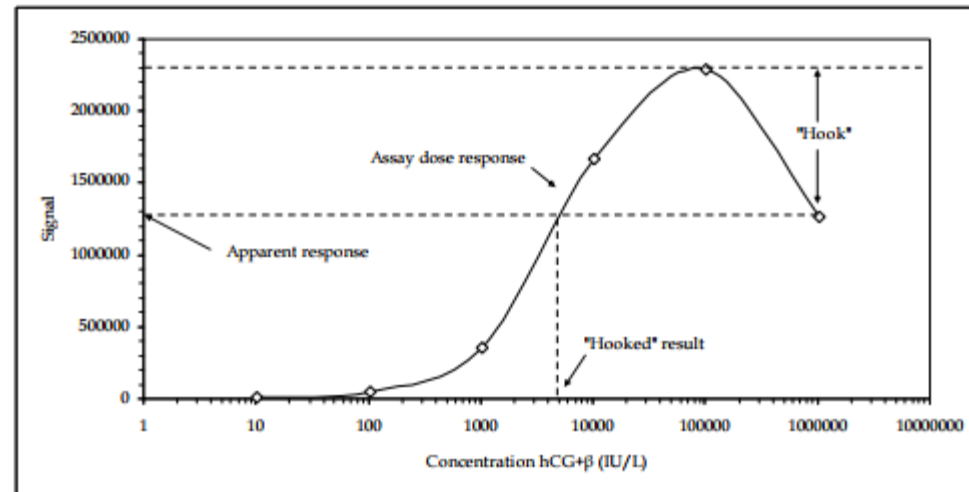
Possible Immunoassay Interference

Heterophile Antibodies - FP or FN

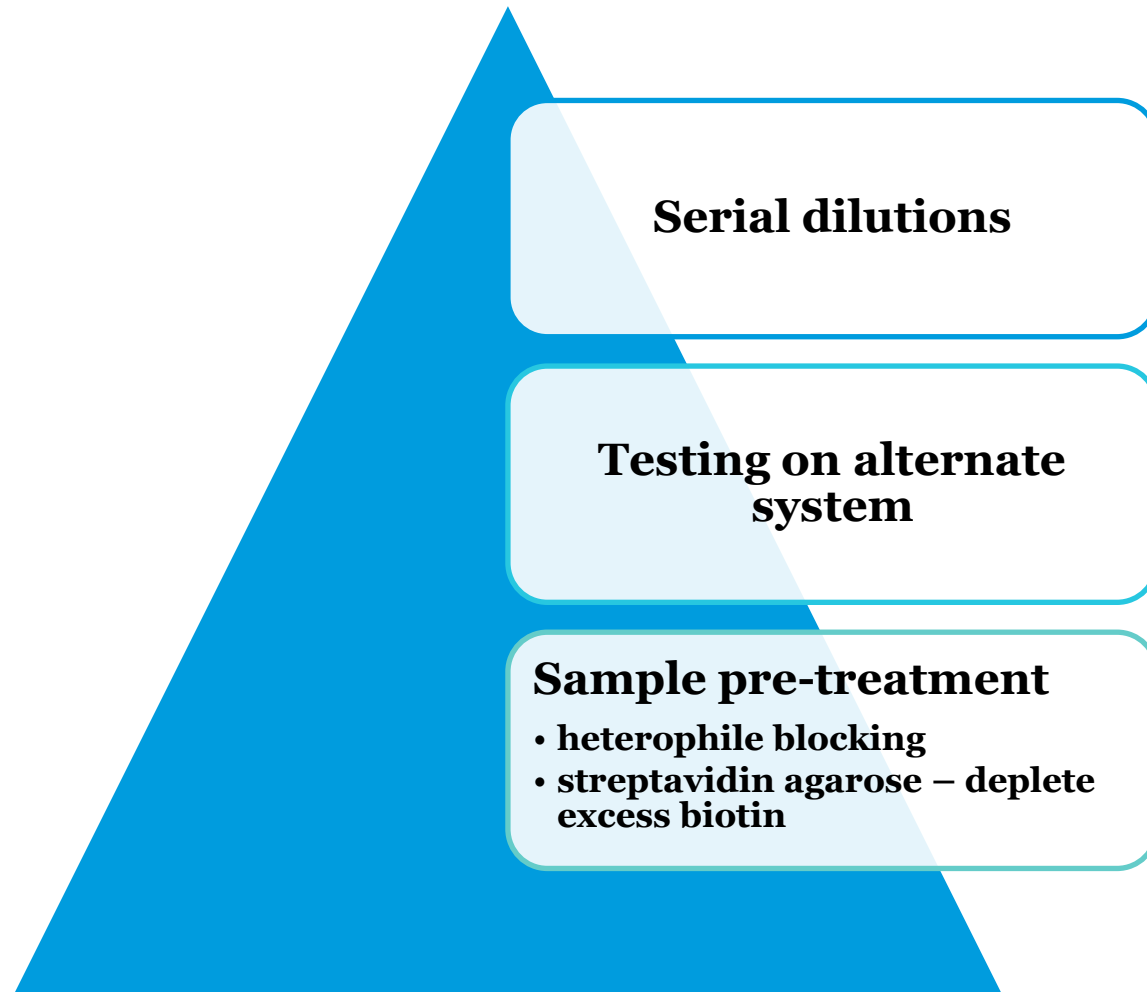
Hook Effect - FN

Immunoassay

- Anti-alk phosp Ab
- Anti-streptavidin Ab
- Anti-ruthenium Ab

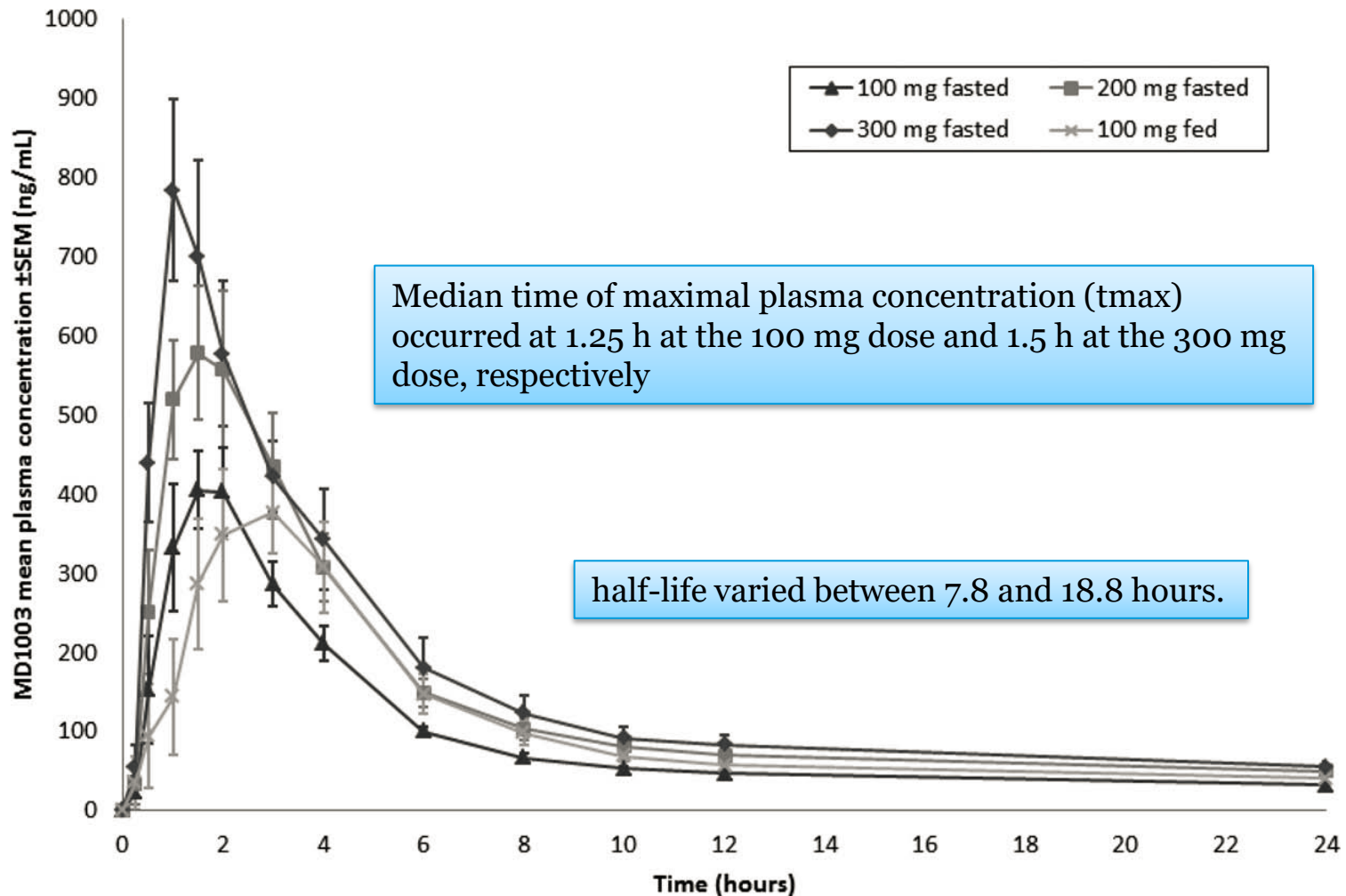


Trouble shooting Immunoassay



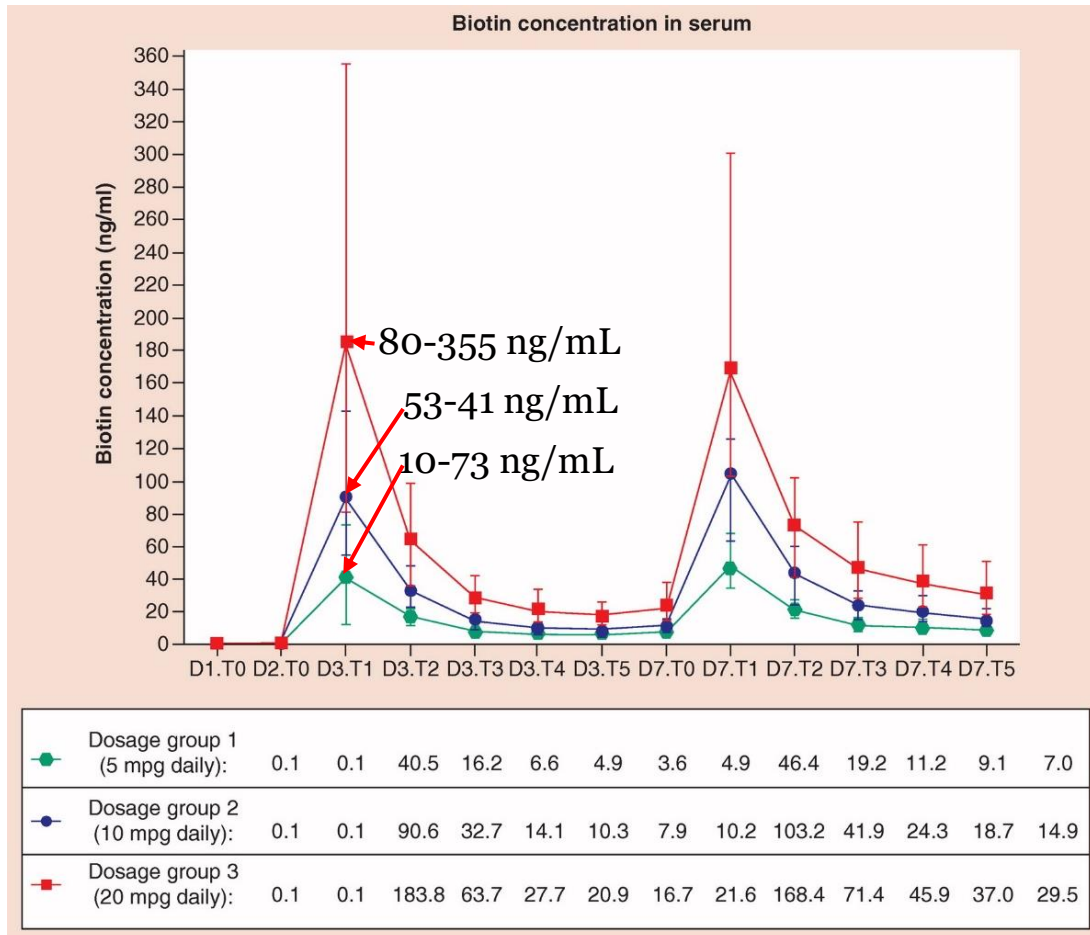
Biotin Pharmacokinetics

Mean plasma biotin levels) after single oral administration of 100, 200, and 300 mg



Saint Paul LP, Debruyne D, Bernard D, et al., Expert Opinion on Drug Metabolism & Toxicology, 2016, 12, 3, 327–344

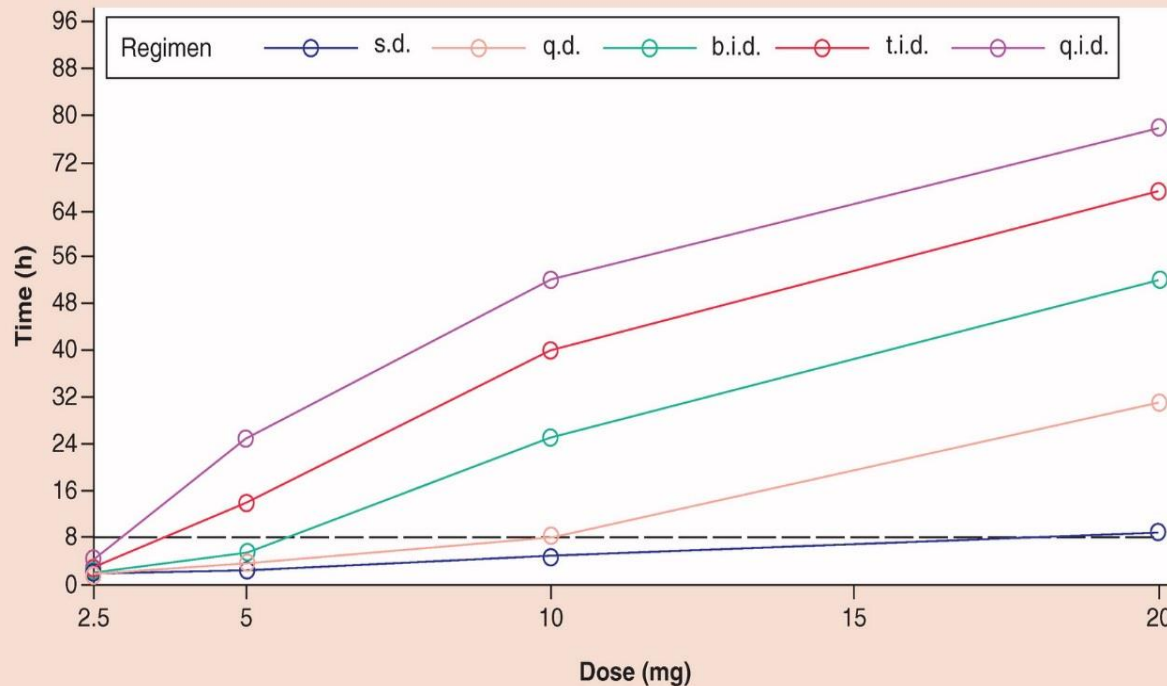
Biotin concentration in serum for 5, 10, & 20 mg/day dosing groups



D: Day;
To: Prebiotin intake
T1: 1-h postdose
T2: 3-h postdose
T3: 6-h postdose
T4: 8-h postdose
T5: 12-h postdose.

Grimsey P, Frey N, Bendig G, et al, Int J Pharmacokinet, 2017 2:4, 247-56

Time required for biotin serum concentration to fall below 30 ng/ml following biotin intake



Following 5, 10 or 20 mg biotin q.d. for 5 days, the 30 ng/ml interference threshold was reached within 3.5, 8 or 31 h, respectively.

s.d.: Single dose
b.d.: Twice a day
q.d.: Once daily
t.i.d.: Three-times daily
q.i.d.: Four-times a day

Grimsey P, Frey N, Bendig G, et al, Int J Pharmacokinet, 2017 2:4, 247-56

Biotin Pharmacokinetics

In humans, single biotin doses (600 and 900 µg) were rapidly eliminated from plasma to urine with an elimination half-life calculated to be approximately 1.8 h,

- indicating a credible total disappearance of biotin in plasma within 12 h

Population PK analysis showed that biotin has linear PK over 5 -20 mg dosing

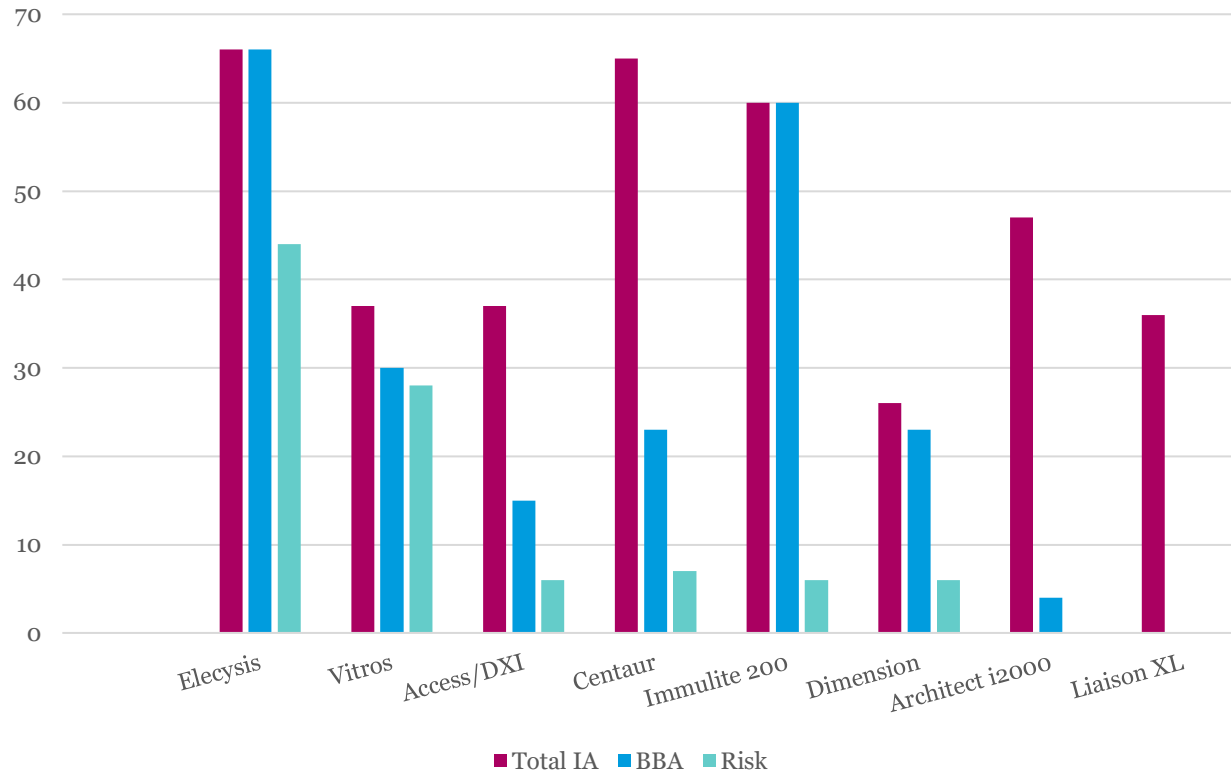
- rapidly absorbed and has an effective serum half-life of 15 h.
- Single dose study half-life ranged 7.8 – 18.8 hrs

Reference Range

- Pediatric (< 12 yrs) : 57.0 – 2460.2 pg/mL
- Adult (≥ 12 yrs) : 221.0 – 3004.0 pg/mL

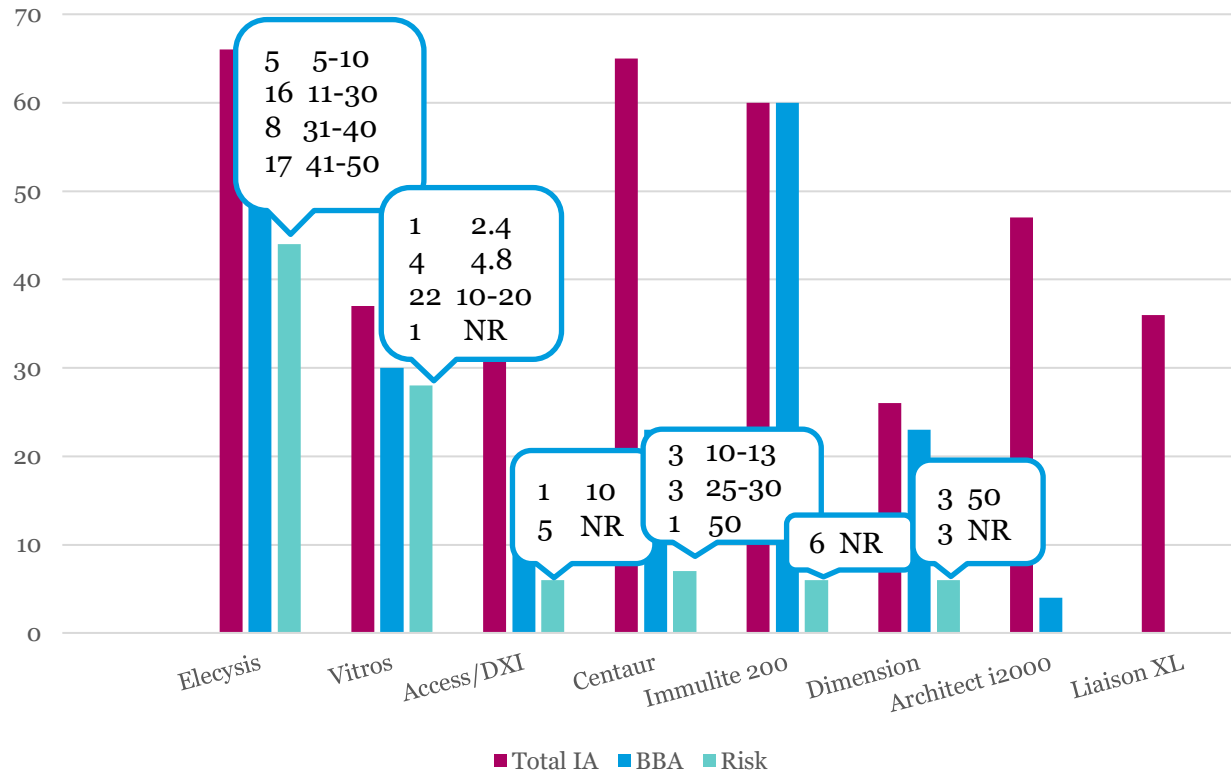
Saint Paul LP, Debruyne D, Bernard D, et al., Expert Opinion on Drug Metabolism & Toxicology, 2016, 12, 3, 327–344
Grimsey P, Frey N, Bendig G, et al, International Journal of Pharmacokinetics, 2017 2:4, 247-56

Biotin-Based Immunoassays at High Risk



Holmes EW, Samarasinghe S, Emanuele MA, Meah F., Arch Path Lab Med, 2017 141, 1459-60.

Biotin-Based Immunoassays at High Risk



Holmes EW, Samarasinghe S, Emanuele MA, Meah F., Arch Path Lab Med, 2017 141, 1459-60.

Biotin-Based Immunoassays at High Risk

Biotin-Based Immunoassays at High Risk for Analytic Interference by Biotin Supplements				
Multitest Assay System	No. of Methods			Vulnerable Immunometric and Competitive Methods With IFTs of <51 ng/mL or no IFTs Reported in the Product Labeling Method(s) [IFT in ng/mL] or Method(s) [NR] ^b
	Total	BBAs	High Risk ^a	
Elecsys ^c	66	66	44	Folate [5]; anti-HBsAg [8]; anti-TPO, anti-TSHR, and total T3 [10]; free T4, progesterone, and TnT [20]; TSH [25]; anti-CCP, anti-HBc, procaltitonon, CK-MB, cortisol, DHEAS, free PSA, GH, BNP, TSTN, thyroglobulin, and Tnl [30]; CA 125 [35]; estradiol [36]; calcitonin, HBeAg, HBsAg, hCG, prolactin, and T uptake [40]; anti-HCV [42]; iPTH, anti-HAV, anti-HAV IgM, B12, Cyfra 21-1, ferritin, HE4, HSV-1 IgG, HSV-2 IgG, LH, myoglobin, osteocalcin, rubella IgG, and rubella IgM [50]
Vitros ^d	37	30	28	Tnl [2.4]; estradiol, iPTH, LH, and TSH [4.8]; cortisol, hCG, AFP, anti-HAV, anti-HAV IgM, anti-HBe, CA 125, CA 15-3, CEA, CK-MB, ferritin, folate, FSH, HBeAg, prolactin, TSTN, and total PSA [10]; 25 OHD [15]; B12, myoglobin, BNP, and progesterone [20]; anti-HBc IgM [NR]
Access/DXI ^e	37	15	6	Free T3 [10]; CA 19-9, free T4, myoglobin, thyroglobulin, and total T3 [NR]
Centaur ^f	65	23	7	HBsAG and Tnl [10]; folate [13]; HAV total [25]; TSTN and anti-HBc IgM [30]; anti-HAV IgM [50]
Immulite 2000 ^f	60	60	6	IgE allergy, gastrin, CA 15-3, CRP, free T3, and thyroglobulin [NR]
Dimension ^f	26	23	6	Free T3, free T4, and digoxin [50]; hCG, myoglobin, and Tnl [NR]
Architect i2000 ^g	47	4	0	None
Liaison XL ^h	36	0	0	None

Holmes EW, Samarasinghe S, Emanuele MA, Meah F., Arch Path Lab Med, 2017 141, 1459-60.

Potential Clinical Misinterpretations due to Biotin

Thyroid Testing

- **FT4, FT3 falsely elevated, (positive TSH receptor antibodies)**
- **TSH falsely suppressed**
- **“Biochemical” presentation of hyperthyroidism (Graves thyrotoxicosis)**

Steroids

- **High steroid hormone concentration with suppressed LH or FSH**
- **suggestive of tumors**

Cardiac

- **Troponin I – suppressed value**

Virology

- **HIV, Hepatitis C – suppression of results**

<https://www.fda.gov/medical-devices/vitro-diagnostics/biotin-interference-troponin-lab-tests-assays-subject-biotin-interference>

Clinical Impact of Biotin on Critical Care Assays

Troponin I/T

- If a patient has taken high dose of biotin prior to on-set of chest pain, true elevation of TnT/I will be seen if following guidelines for serial measurements

BNP/ NT-proBNP

- Even a 20% reduction due to biotin consumption is not likely to impact clinical decision making due to high intra-personal daily variation in results

Procalcitonin

- Clinical decisions are made based on a change in PCT over 4 days, making the influence of biotin unlikely to have clinical significance

Clinical Impact of Biotin on Thyroid Testing

Thyroid disease is the only instance in which a set of erroneous results mimics a disease, e.g. Graves' disease.

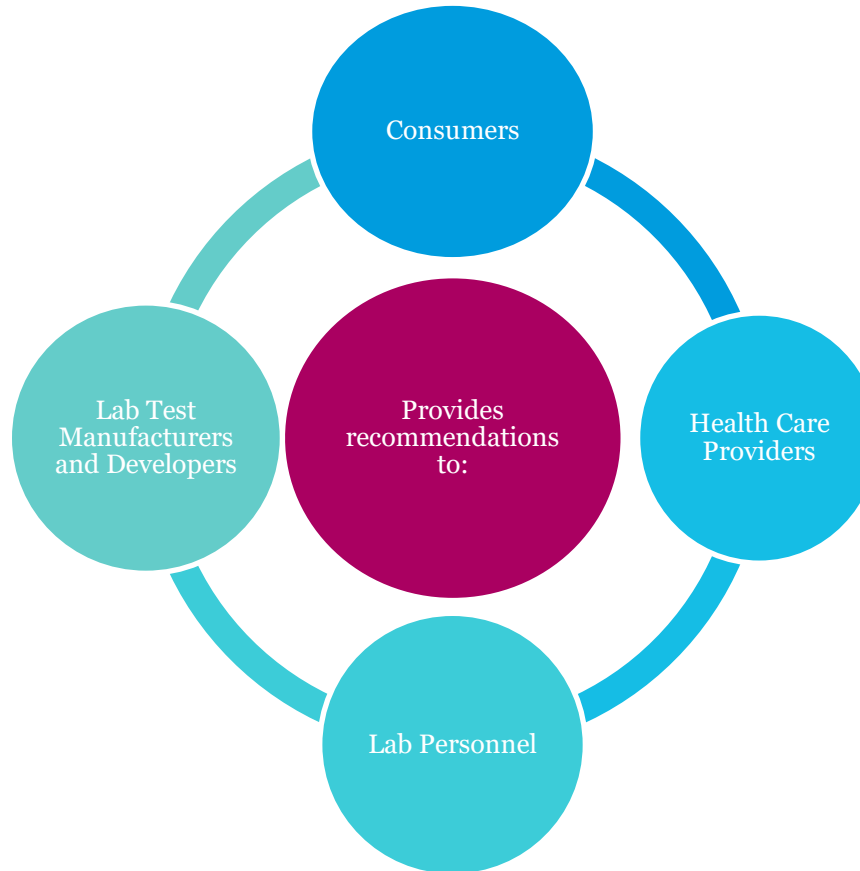
Two common blood tests ordered to aid in the diagnosis of Grave disease are TSH and FT4-

- Biotin *falsely suppresses* TSH results (sandwich assay)
- Biotin *falsely elevates* FT4 results (competitive assay)
- The combination of a low TSH and a high FT4 result will often prompt an investigation of the possibility of Graves' disease.

Since thyroid testing is a planned event, patients should be told to avoid high dose biotin intake for at least 8 hours prior to phlebotomy.

The FDA Warns that Biotin May Interfere with Lab Tests: FDA Safety Communication

Date Issued: November 28, 2017



<https://www.fda.gov/MedicalDevices/Safety/AlertsandNotices/ucm586505.htm>

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<https://www.fda.gov/medical-devices/safety-communications/update-fda-warns-biotin-may-interfere-lab-tests-fda-safety-communication>

Biotin Concentrations in ED Population

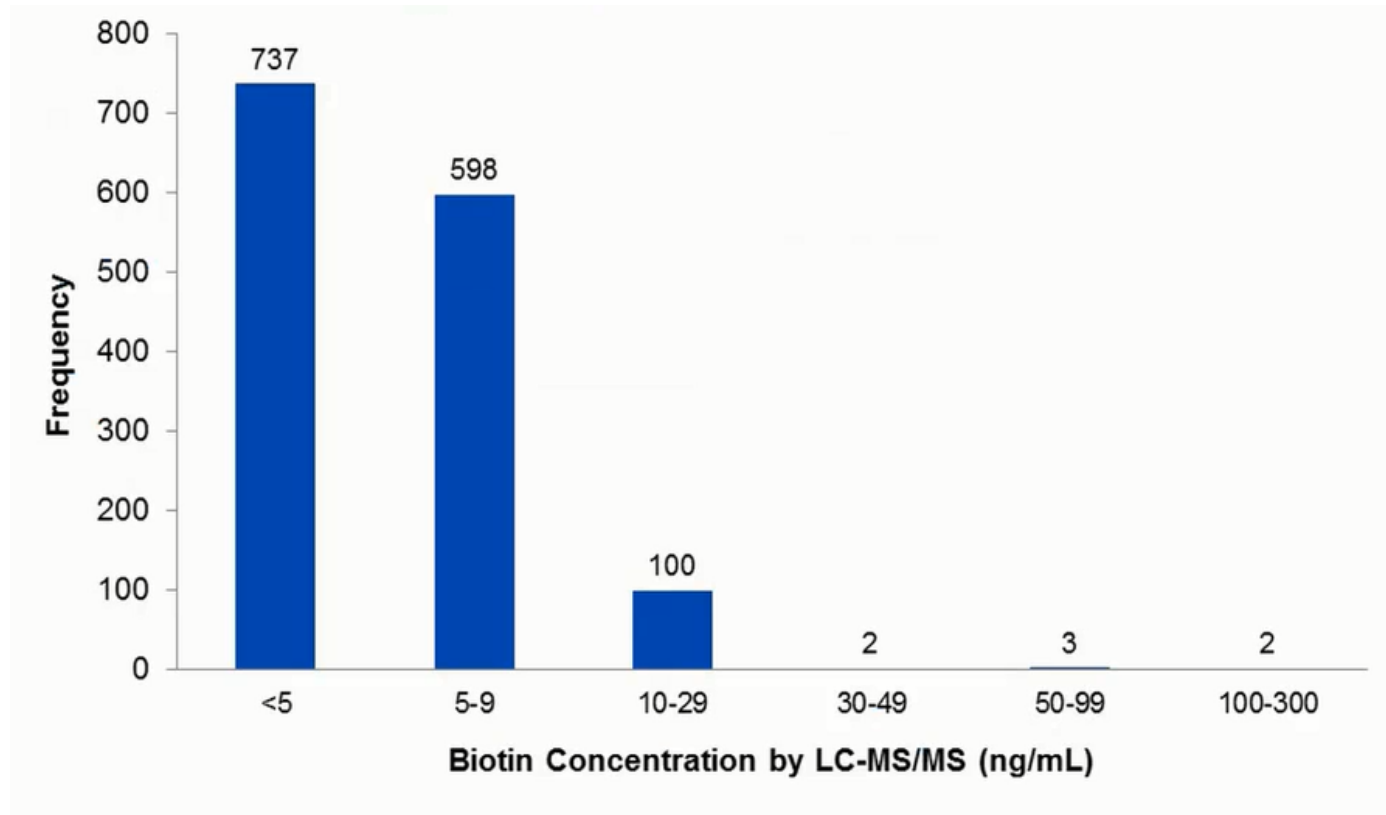
Biotin Concentrations in ED Patients – Mayo Clinic Study

Collected 1442 unique residual samples from ED patients with orders for electrolyte panel

- 734 females, 708 males
- Median age (interquartile range): 58 (16-91) years

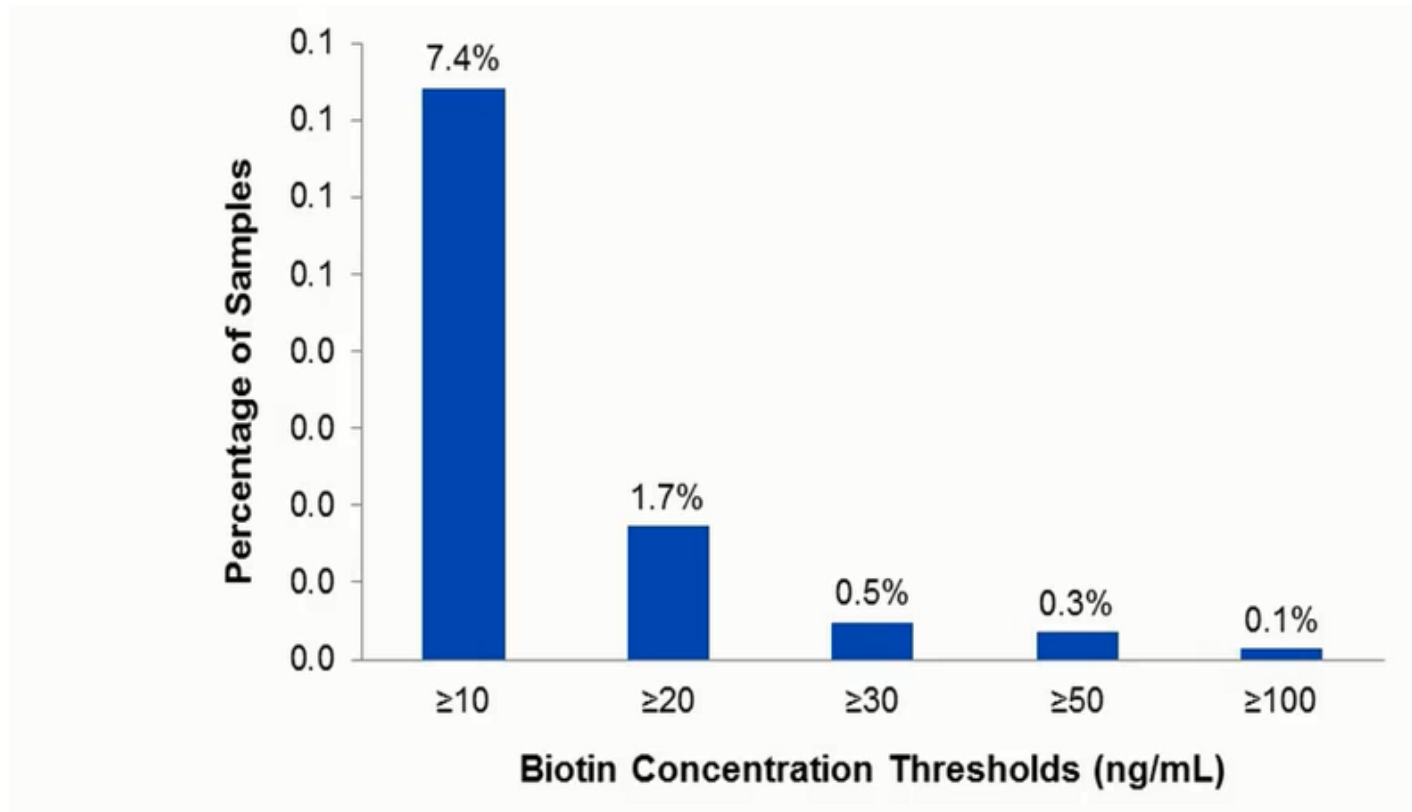
Biotin quantitated by in-house developed LC-MS/MS method

Distribution of Biotin Concentrations in ED Patient Samples



Katzman, B., Lueke, A. Donato, L., et al., Clin Biochem, 2018, 60,pp. 11-6

Percentage of ED specimens with Biotin Above Interference Thresholds



Katzman, B., Lueke, A. Donato, L., et al., Clin Biochem, 2018, 60,pp. 11-6

Biotin Study Conclusions

Biotin supplements were used in 8% of the patient population

Biotin can cause significant interference in some immunoassays when taken as at high doses.

- Clearance is rapid in healthy subjects (12 hours is sufficient).
- Decreased clearance with renal impairment.

Risk mitigation can occur at multiple steps in total testing process

- Awareness is most important

Katzman, B., Lueke, A. Donato, L., et al., Clin Biochem, 2018, 60,pp. 11-6

Summary

Biotin in human serum is a potential interfering factor for all streptavidin–biotin-based assay designs

Use of high doses of biotin as an over-the-counter lifestyle supplement is increasing, therefore the potential risk of erroneous immunoassay results due to biotin interference is growing.

Biotin had linear pharmacokinetic over the range of doses studied (5–20 mg), was rapidly absorbed and had an effective serum half-life of 15 h (steady-state reached in 3 days).

The time taken for biotin doses to drop below thresholds of 10–100 ng/ml was simulated for dosing regimens ranging from 1 mg s.d. to 300 mg q.i.d.

For biotin regimens of ≤ 10 mg q.d. (10 mg is >300 -times the adequate daily intake), serum biotin levels were below an *in vitro* interference threshold of ≥ 30 ng/ml after 8 h.

If the *in vitro* interference threshold of an immunoassay is <30 ng/ml, or in the extreme cases of patients taking a daily dose of >10 mg, extended washout periods are recommended.

Strategies to Mitigate Biotin Interference

Increase clinician and patient awareness of biotin's effects on tests.
Ensure proper patient preparation before specimen collection.

- Will never completely eliminate the risk, there will always be misunderstandings, miscommunication and failures to comply.

Biotin removal using immobilized streptavidin or paramagnetic beads

- Expensive, slow and not practical for routine use
- Need to perform extensive re-validation of all assays

Selection of assays and equipment that is not prone to interference.

- 100% method to eliminate the risk.
- There will always be some assays that are only available in the biotin/streptavidin format.

Conclusions

Use of Biotin may continue to be a major interfering factor in certain immunoassays on some platforms.

The risk of incorrect lab test results may still be present despite the warnings and additional mitigations in place.

Misdiagnosis and incorrect treatment decisions may result from incorrect lab test results.

Not all assays and not all platforms are vulnerable to biotin interference.

- Laboratories should be aware of the available options and consider selecting assays/instrumentation that are unaffected.
- For the few tests only available with biotin/streptavidin format, ensure compliance with proper patient preparation.

*Thank
You*

Questions?

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