AACC Better health through laboratory medicine.

PEARLS OF LABORATORY MEDICINE

Pearl Title: **The Enigma of Biotin Interference**

Name of Presenter: Dr Saswati Das

Affiliation:Central Government Health Service, New Delhi India

DOI: 10.15428/CCTC.2020.317768

CCTC Clinical Chemistry Trainee Council

What is Biotin?

- Biotin, also known as vitamin B7, is a water-soluble vitamin
- Found in:
  - Diet: Egg yolk, soybeans, yeast, liver and kidney, nuts and cereals
  - Multi-vitamins
  - **Supplements marketed for hair, skin and nail growth**
- Prescribed:
  - Multiple sclerosis (experimental)

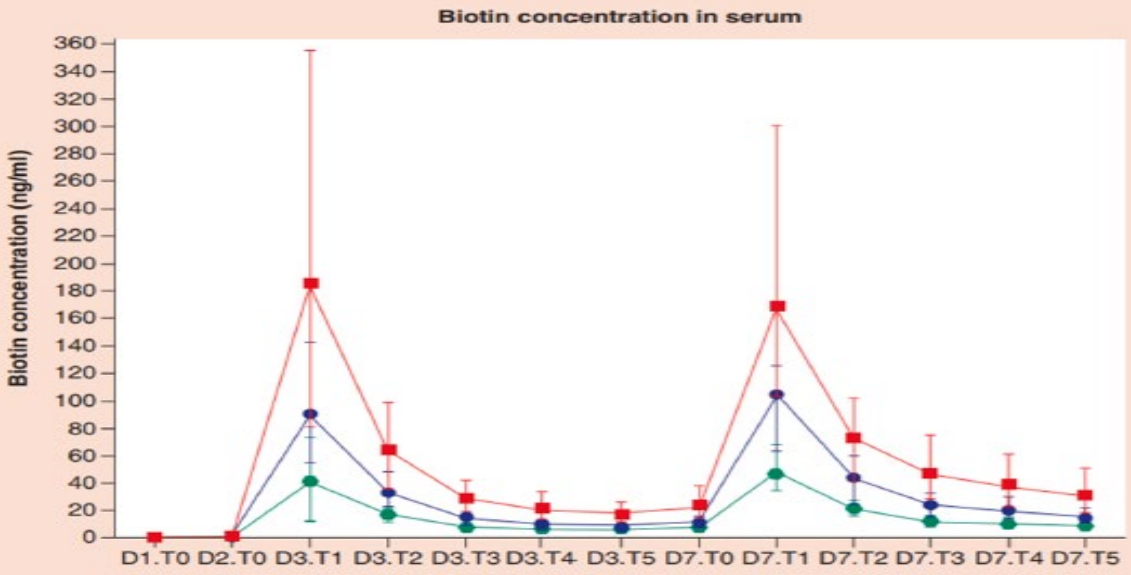
The "Biotin" problem

- Biotin in human serum is a potential interfering factor for streptavidin-biotin-based assays
- Biotin in patient samples can cause falsely high or falsely low results
- Immunoassay-based laboratory tests
- The interaction of streptavidin and biotin has been utilized for the development of robust and highly sensitive immunoassays by many manufacturers

Biotin Supplemention

- In Western populations, average dietary biotin intake is estimated to be 35 to 70 µg daily
- Common multivitamin pills contain about 30 µg of biotin.
- High-dose supplementation (doses greater than 1 mg/d) have a role in therapy for several diseases.
- Doses up to 10 mg a day in nutritional supplements taken to improve hair, skin, and nail health

Serum Biotin Concentration following 5.10. 20 mg dosing



Dosage group 1 (5 mpg daily):	0.1	0.1	40.5	16.2	6.6	4.9	3.6	4.9	46.4	19.2	11.2	9.1	7.0
Dosage group 2 (10 mpg daily):	0.1	0.1	90.6	32.7	14.1	10.3	7.9	10.2	103.2	41.9	24.3	18.7	14.9
Dosage group 3 (20 mpg daily):	0.1	0.1	183.8	63.7	27.7	20.9	16.7	21.6	168.4	71.4	45.9	37.0	29.5

Role of biotin in immunoassays

- Biotin is a small molecule that can be attached by covalent bond to a variety of targets without effecting their biological activity.

- Biotin thus makes the target easy to capture because it forms a strong, stable, and specific non-covalent bond with avidin, **streptavidin**, or NeutrAvidin proteins.

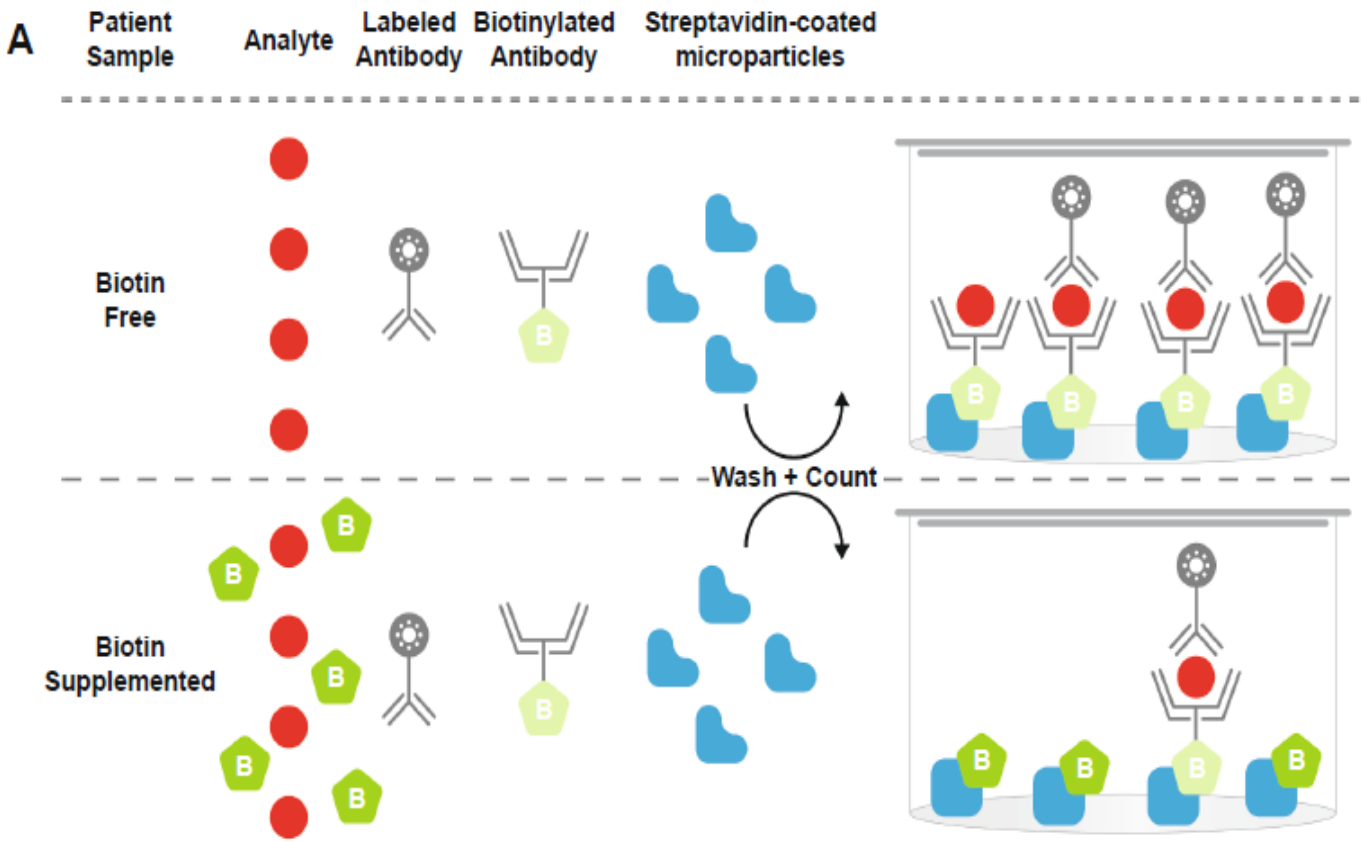
What effect does biotin interference have?

- The direction of interference depends on the design of the assay.
- Some results are falsely elevated, some falsely lowered.
- Two of the most common immunoassay designs are the sandwich assay and the competitive assay.

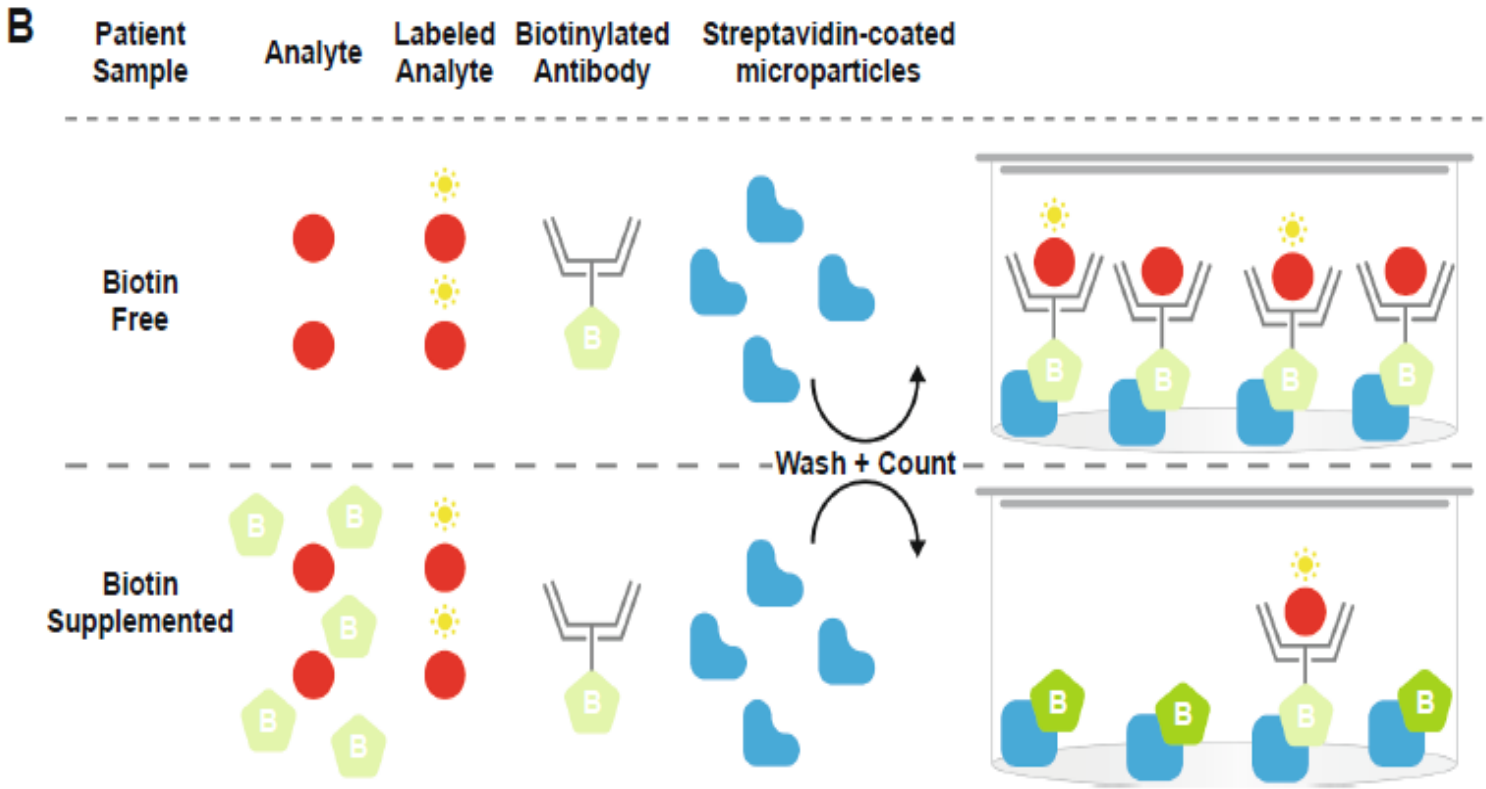
Platforms using Biotin based detection

- Roche Elecsys
- Ortho Clinical Diagnostics Vitros;
- Beckman Coulter Access
- Beckman Coulter DXI
- Siemens Centaur
- Siemens Immulite
- Siemens Dimension.

How Biotin Interferes (Sandwich Assays)



How Biotin Interferes (Competitive Assays)



Examples

IMMUNOASSAYS (Roche)	Method	Antibodies used
TSH	Sandwich	Biotinylated monoclonal anti-TSH Ab (mouse) and monoclonal anti-TSH Ab (mouse/human) labeled with ruthenium complex
FT4	Competitive	Polyclonal anti-T4-Ab (sheep) labeled with ruthenium complex competes with biotinylated T4.
FT3	Competitive	Monoclonal anti-T3-Ab (sheep) labeled with ruthenium complex competes with biotinylated T3.

Assays commonly affected by Biotin interference

- ACTH
- AFP
- Anti TPO
- Anti-TG
- Ca125
- Ca15-3
- Ca19-9
- CEA
- Cortisol
- C-peptide
- DHEAS
- Digoxin
- Estradiol
- Folate
- Free PSA
- FSH
- FT3
- FT4
- Beta HCG
- Hs TnT
- IgE
- Insulin
- LH
- Progesterone
- prolactin
- PSA
- PTH
- SHBG
- Testosterone
- Total B12
- TSH

Detecting & Mitigating Biotin Interference

- Serial Dilution Study
- Repeat the test using an alternate method unaffected by biotin
- Confirmation of the presence of biotin using depletion protocols
- Direct measurement of biotin concentrations.
- Using streptavidin-agarose beads to remove the biotin before the sample is run on the affected analyzer

Precautions

- Withhold biotin for at least 3 days before test
  - At higher doses, might need to wait for few more days

- Rule out other interferences in immunoassays
  - Heterophilic antibodies
  - Human anti-animal antibodies
  - High-dose hook effect (HCG)
  - Other causes

Best Practice Recommendations (R. Bowen et al)

Stakeholders	Recommendations
Manufacturers	1.Determine biotin interference thresholds and include in product inserts. 2 Increase awareness of biotin interference and provide guidance via bulletins or online resources.
Laboratory Staff	1.Provide key contact information with assay results/request forms. 2. Advise healthcare providers on how to minimize the risk of inaccurate test results 3. Develop internal algorithms to investigate inaccurate test results.
Healthcare Providers	1.Ask patients about supplement/biotin use, including dosages. 2. Provide patient instructions to prepare for blood tests several days prior to appointment. 3 Contact laboratory if biotin interference suspected
Patients	1.Check supplement labels for biotin. 2. Report all supplement intake to healthcare provider. 3. Do not take biotin prior to undergoing blood tests.

In Conclusion

- Always think about assay interference if
  - Discrepancy between test result and clinical picture
- Assay interference is not common but can happen
- If patient is on biotin supplement, stop the supplement before blood tests done by immunoassay
- If in doubt a dialogue with the clinician is recommended.

References

1. Tourbah A, Lebrun-Frenay C, Edan G, Clanet M, Papeix C, Vukusic S, et al. MD1003 (high-dose biotin) for the treatment of progressive multiple sclerosis: a randomised, double-blind, placebo controlled study. *Mult Scler 2016;22(13):1719-31. Epub 2016 Sep 1.*
2. Piketty ML, Prie D, Sedel F, Bernard D, Hercend C, Chanson, et al. High-dose biotin therapy leading to false biochemical endocrine profiles: validation of a simple method to overcome biotin interference. *Clin Chem Lab Med 2017;55(6):817-25.*
3. S. Samarasinghe, F. Meah, V. Singh, A. Basit, N. Emanuele, M.A. Emanuele,A. Mazhari, et al Biotin interference with routine clinical immunoassays:understand the causes and mitigate the risks, Endocr. Pract. 23 (8) (2017)989–998.
4. P. Grimsey, N. Frey, G. Bendig, J. Zitzler, O. Lorenz, D. Kasapic, et al Population pharmacokinetics of exogenous biotin and the relationship between biotin serum levels and in vitro immunoassay interference, Int. J. Pharm. 2 (2017)247–256.
5. Bowen R, Benavides R, Colon-Franco JM, Katzman BM, Muthukumar A, Sadrzadeh H et al Best practices in mitigating the risk of biotin interference with laboratory testing (https://doi.org/10.1016/j.clinbiochem.2019.08.012)

Disclosures/Potential Conflicts of Interest

Upon Pearl submission, the presenter completed the Clinical Chemistry disclosure form. Disclosures and/or potential conflicts of interest:

- **Employment or Leadership**: No disclosures
- **Consultant or Advisory Role:** No disclosures
- **Stock Ownership:** No disclosures
- **Honoraria:** No disclosures
- **Research Funding:** No disclosures
- **Expert Testimony:** No disclosures
- **Patents:** No disclosures

Thank you for participating in this *Clinical Chemistry* Trainee Council Pearl of Laboratory Medicine.

Find our upcoming Pearls and other Trainee Council information at www.traineecouncil.org

Download the free *Clinical Chemistry* app on iTunes today for additional content!

Follow us: