Letter to the Editor

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Evaluation of biotin interference on immunoassays: new data for troponin I, digoxin, NT-Pro-BNP, and progesterone

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To the Editor,

Biotin, or vitamin H, is a water-soluble vitamin involved as a metabolic coenzyme in the synthesis of fatty acids and in gluconeogenesis. Biotin has been and still is a complementary therapy for alopecia diffusa. Since 2014 in *Neurology*, high-dose biotin treatment (daily doses 100–300 mg) has been evaluated for multiple sclerosis [1]. Vitamin H is also used in several inherited metabolic diseases and could be a complementary treatment for patients with disorders of mitochondrial energy metabolism [2]. Biotin is commonly found in over-the-counter dietary supplements, in smaller amount (up to 10 mg per tablet). Biotin is found in injectable form or tablet. Interferences have been reported on laboratory assays and especially for function thyroid tests. Laboratory results often mimic Graves' disease [3, 4], among others. Biotin interferences on hormonal immunoassays have also been reported [4]. Siemens (Munich, Germany) Dimension Vista® 1500 LOCI (Luminescent Oxygen Channeling Assay) method is also based on biotin-streptavidin interaction to quantify hormones, drugs, or proteins.

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The aim of this study was to investigate the biotin effect on 16 routine immunoassays on Siemens Dimension Vista® 1500 analyzer using LOCI detection: human chorionic gonadotropin and β-subunit (βhCG), luteinizing hormone (LH), progesterone, estradiol, ferritin, troponin I, myoglobin, N-terminal prohormone of brain natriuretic peptide (NT-Pro-BNP), digoxin, thyrotropin (TSH), free T_3 (FT_3), free T_4 (FT_4), CA15.3, CA19.9, total PSA (TPSA), and free PSA (FPSA).

Spiked-in experiments with increasing concentrations of biotin were performed on Siemens Dimension® Vista 1500 analyzers. Six different pools of plasma and serum were prepared from daily routine patient samples to replicate each assay matrix on different days. Therefore, we had six replicas of the experiment. Samples also had been selected for having subnormal or pathologic laboratory results and normal lipemic and hemolysis indices. Experiments were carried out on different days, on three Dimension® Vista 1500 analyzers, and in the same laboratory. Injectable biotin 0.5% (5 mg/mL) Bayer (Leverkusen, Germany) diluted in ultrapure water has been used. Physiological biotin plasmatic levels are between 0.12 and 0.8 µg/L. Peyro Saint Paul et al. observed plasmatic biotin concentrations between 700 and 900 µg/L 2 h after an isolated 300 + mg oral intake and around 75 µg/L 24 h after the intake [5]. Therefore, biotin concentrations were tested from 50 to 1000 µg/L.

Results for spiked-in samples were then compared to the baseline results from the sample without biotin. Interference was then calculated as a percentage of variation between the baseline value and the value in presence of the interferent molecule: interference (%) = 100 × (measured value – baseline value)/baseline value. Significant interference was defined when the calculated value was above 10% including the 95% confidence interval. Clinically significant interference or reference change value (RCV) is defined as a result that in the presence of the interferent molecule differs from the baseline result more than $1.96 \times \sqrt{(CV^2_{analytical} + CV^2_{within\ subject\ biological\ variation})}$ at 95% significance. $CV_{analytical}$ have been selected from daily

Table 1: Immunoassays affected at different concentrations of biotin on Siemens Dimension Vista 1500.

Assay	Progesterone	Estradiol	Troponin	NT-Pro-BNP	Digoxin	TSH	FT_3	FT_4
LOCI Method	Competitive	Competitive	Sandwich	Sandwich	Competitive	Sandwich	Competitive	Competitive
CV_a, %	5.11	9.04	5.51	2.37	1.99	2.7	5.34	2.62
RCV, %	39.7	47.5	73.5	20.1	–	57.7	18.7	14.8
Biotin concentration, µg/L	Mean interference (%) and 95% confidence interval (n = 6)							
50	– 2.07	1.91	– 2.53	– 0.98	0.73	0.51	1.44	0.2
	(– 10.5 to – 6.43)	(– 4.6 to 8.5)	(– 4.1 to – 1.0)	(– 2.70 to 0.73)	(– 2.29 to 3.74)	(– 1.20 to 2.22)	(– 2.98 to 5.86)	(– 1.05 to 1.44)
100	– 6.7	– 1.25	– 1.98	– 0.72	2.01	– 0.77	– 1.4	0.68
	(– 12.88 to – 0.52)	(– 4.26 to 1.75)	(– 4.00 to 0.05)	(– 2.55 to 1.11)	(– 1.77 to 5.78)	(– 2.45 to 0.91)	(– 6.12 to 3.32)	(– 0.71 to 2.07)
150	– 8.9	1.58	– 3.02	– 2.47	– 1.35	– 0.25	– 0.36	1.64
	(– 16.08 to – 1.72)	(0.79–3.69)	(– 4.61 to – 1.43)	(– 4.06 to – 0.88)	(– 6.58 to 3.88)	(– 1.48 to 0.98)	(– 6.28 to 5.57)	(0.65–2.62)
200	– 1.35	– 0.43	– 2.42	– 3.07	– 2.16	– 0.26	– 1.08	1.31
	(– 6.87 to 4.16)	(– 6.68 to 5.81)	(– 3.86 to – 0.98)	(– 5.29 to – 0.85)	(– 5.46 to 1.15)	(– 2.42 to 1.90)	(– 4.07 to 1.92)	(– 0.05 to 2.67)
300	54.04	20.59	– 37.38	– 9.35	11.2	– 12.01	**380.59**	12.93
	(– 10.26 to 118.34)	(– 3.97 to 45.14)	(– 53.92 to – 20.85)	(– 12.12 to – 6.57)	(3.79–18.61)	(– 18.54 to – 5.48)	(42.66–718.53)	(7.09–18.77)
400	**225.51**	**52.3**	– 66.66	**– 15.85**	**21.59**	– 20.21	**865.79**	**25.86**
	(94.78–356.24)	(19.19–85.41)	(– 91.24 to – 42.09)	(– 20.58 to – 11.11)	(14.18–29.00)	(– 27.18 to – 13.25)	(383.30–1348.28)	(17.38–34.34)
500	**432.53**	**99.92**	**– 79.24**	**– 23.98**	**39.71**	– 33.87	**1216.98**	**60.07**
	(181.51–683.55)	(36.05–163.79)	(– 106.34 to – 52.14)	(– 34.10 to – 13.85)	(26.47–52.94)	(– 54.55 to – 13.18)	(696.11–1737.85)	(13.95–106.20)
700	**2343.22**	**235.38**	**– 91.93**	**– 49.38**	**84.16**	**– 55.94**	**1458.09**	**177.33**
	(637.53–4048.9)	(77.0–393.76)	(– 103.92 to – 79.94)	(– 74.86 to – 23.90)	(47.36–120.97)	(– 84.57 to – 27.30)	(954.75–1961.43)	(13.71–340.95)
1000	**7786.76**	**1809.02**	**– 98.44**	**– 57.2**	**305.57**	**– 64.74**	**1716.83**	**220.63**
	(2361.9–13211.6)	(180.5–3437.5)	(– 98.92 to – 97.96)	(– 79.35 to – 35.04)	(108.33–502.81)	(– 82.93 to – 46.55)	(1522.0–1911.7)	(69.21–372.05)

Values highlighted in gray indicate more than 10% difference from baseline value, including the 95% confidence interval. Values in bold indicate clinically significant interference. CV_a, coefficient variation analytical; RCV, reference change value.

routine internal quality control (ICQ) database. CV within-subject biological variation have been selected from the RICOS database.

From 50 to 200 µg/L of biotin, no interference has been observed for any parameters. Interferences were observed at 300 µg/L for troponin and FT_3 (Table 1). Above 400 µg/L, we observed interferences for troponin, digoxin, NT-pro-BNP, FT_3, FT_4, TSH, progesterone, and estradiol assays with the most significant effect for FT_3 and progesterone. Indeed, extent of interference is variable and can be massive (Table 2). However, no interference has been detected for βhCG, LH, ferritin, myoglobin, CA15.3, CA19.9, FPSA, and TPSA assays, even with high concentrations of biotin (Supplemental Data, Table S1). Biotin concentration of 400 µg/L seems to be the threshold concentration that triggers the interference and the effect is increasing with its concentration. We can note that biotin concentration of 300 µg/L is enough to trigger a significant interference for FT_3 assay. As expected, we observed, on the one hand, positive interference for competitive methods and, on the other hand, negative interference for sandwich methods.

On Siemens Dimension Vista® 1500 methods, sandwich methods tend to reduce laboratory results and lead to false-negative results, whereas competitive methods seem to increase them and produce false-positive results. It is consistent with previous reports of interference [4]. Data from high-dose biotin pharmacokinetics study [5] showed that around 4 h after per os biotin intake, plasmatic concentrations were ~400 µg/L. This concentration represents the threshold value of interference for troponin, NT-pro-BNP, T_3, T_4, TSH, progesterone, and estradiol.

Biomarkers with a very low diagnostic threshold, such as troponin I which is considered positive above 50 ng/L [6], have to be monitored closely to avoid those interferences. Interestingly, it is the first time that a troponin I negative interference is reported due to biotin. This interference can lead to wrongly exclude an acute myocardial infarction. It is also the first time that a positive interference on digoxin plasmatic levels is reported due to biotin. Around 2 h after the high-dose biotin intake, digoxin concentration could be overestimated of ~300%. Misinterpreting therapeutic drug monitoring could lead to unjustified dose adjustment and ineffective therapy. Fertility checkups could also be affected. Indeed, progesterone and estradiol are used to assess chances of successful in vitro fertilization (IVF). Premature progesterone rise in stimulated IVF cycles seems to have a negative impact on the IVF outcome [7]. Erroneous increased levels of progesterone could therefore lead to incorrectly lower estimates of a successful IVF.

Table 2: Concentrations with maximum interference from replicas observed on Siemens Dimension Vista 1500.

Assay	Progesterone		Estradiol		Troponin		NT-Pro-BNP		Digoxin		TSH		FT_3		FT_4
Normal range	[a]5–25 ng/mL		[a]60–230 ng/L		< 500 ng/L		0–450 ng/L		0.6–1.2 µg/L		0.358–3.7 mUI/L		3.3–6.1 pmol/L		9.8–18.8 pmol/L
Biotin concentration, µg/L	Concentrations with maximum interference and baseline concentration														
	Baseline	w/biotin	Baseline	w/biotin	Baseline	w/biotin	Baseline	w/biotin	Baseline	w/biotin	Baseline	w/biotin	Baseline	w/biotin	Baseline	w/biotin
1000	0.26	**>40**	27.72	**1375**	3329	**<20**	2114	205	0.30	**1.59**	1.27	**0.017**	3.22	**68.15**	15.7	**>102.9**
700	0.45	25.5	24.1	145	3329	**<20**	2114	287	0.30	**0.69**	1.92	**0.021**	3.22	**64.7**	17.2	**102.3**
500	0.27	2.44	24.1	73.0	3329	**<20**	586	364	0.13	0.21	1.92	0.491	3.39	**64.15**	17.2	**44.8**
400	0.27	1.29	24.1	48.6	855	**90**	2306	1821	0.13	0.17	1.92	1.36	3.22	**53.03**	15.7	**21.8**
300	0.45	1.27	24.1	39.7	1016	373	2306	1998	0.13	0.16	1.92	1.47	3.39	**42.13**	15.7	**19.3**

Some values were below or above linearity range and could not be diluted. Values in bold could potentially lead to misdiagnosis. [a]Normal ranges during luteal phase.

Therefore, after a biotin intake, blood sample collection should be delayed after the timeframe during which assays could be disrupted. Patients should be advised to skip their daily morning biotin intake before blood samples are planned and to take their medicine after blood collection. However, we have to be prudent in the message delivered to patients. There data are in vitro data which do not allow the assessment of effects of metabolites or accumulation of biotin. Biotin has two main metabolites, bisnorbiotin and biotin sulfoxide, and other biotinylated compounds [5]. It would be interesting to gather in vivo data in order to set a recommended period during which blood tests are disrupted. In an emergency context such as a suspicion of acute myocardial infraction, biotin intake should be checked when troponin I results, unexpectedly negative, are incoherent with patient condition or electrocardiogram.

We confirm that biotin therapeutic concentration in patient has an effect, which we expected, on thyroid function tests (FT_3, FT_4, and TSH) and estradiol assay. This is the first time that this interference is also observed on progesterone, NT-pro-BNP, and on biomarkers with a very low diagnostic threshold such as troponin and digoxin on Siemens Dimension Vista® 1500 instrument assays. For βhCG, LH, ferritin, myoglobin, CA19.9, CA15.3, FPSA, and TPSA assays, no interference was observed, even with high concentrations of biotin.

All assays based on biotin-streptavidin interaction could be impacted by biotin intake. Although, our results concern Siemens Dimension Vista 1500 analyzers and cannot be extrapolated to other streptavidin-biotin assays. Further investigations on different biochemistry instruments are needed. However, those results have to be compared with in vivo data. Health authorities should require from the in vitro diagnostics industry rigorous studies of biotin effect on their instruments. This interference should also be mentioned in biotin information leaflets for patient awareness. Clinical chemists need to take into account biotin interference on their assays and it is now critical to emphasize biotin analytical interferences for patients, clinicians, and clinical chemists.

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