Interference From High-Dose Biotin Intake in Immunoassays for Potentially Time-Critical Analytes by Roche

Evaluation of a Countermeasure for Worst-Case Scenarios

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• ***Context.***—**Immunoassays using the interaction between streptavidin and biotin are used for clinical chemical analytes on platforms by many different manufacturers. The design can be susceptible to interference from high-dose biotin intake in patients, which remains an often-overlooked confounder despite recently increased awareness.**

Objective.—**To evaluate an easily implementable method of in vitro biotin depletion for the removal of biotin interference in immunoassays for potentially time-critical analytes.**

Design.—**A biotin stock solution was made and deidentified patient samples were spiked to reach a biotin concentration of 1.126×10^6 pg/mL, the maximum reported biotin concentration 1 to 2 hours after a single oral dose of 300 mg biotin. Then, the resulting interference in Elecsys immunoassays for cortisol, cyclosporine A, tacrolimus, digitoxin, thyroid-stimulating hormone, free triiodothyronine, free thyroxine, C-peptide, insulin, N-terminal pro-B–type natriuretic peptide, troponin T high sensitive, human immunodeficiency virus, procalcitonin, β human chorionic gonadotropin, toxoplasma immunoglobulin M, and toxoplasma immunoglobulin G was evaluated before and after biotin depletion using streptavidin particles.**

Results.—**All tested immunoassays, with the exception of toxoplasma immunoglobulin M and toxoplasma immunoglobulin G, suffered from significant biotin interference. The depletion protocol removed assay interference due to biotin and produced results that were close or identical to initial prespike measurements.**

Conclusions.—**Despite an increase in turnaround times, biotin adsorption is a feasible countermeasure for biotin interference in Elecsys immunoassays. Until test kits with an increased resistance to the interference from high-dose biotin intake are distributed, the evaluated protocol can provide results properly reflecting the patient's clinical condition.**

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Laboratory analyses play a paramount role in about 70% of medical decisions and must be highly reliable to avoid false or missed diagnoses or unnecessary diagnostic procedures and treatments.[1] Currently, around 85% of the most common immunochemical analyzers are equipped with assays using the interaction between streptavidin and biotin (vitamin H/B_7; coenzyme R). The design offers advantages including signal amplification and increased sensitivity.[1–4] Unfortunately, this system can be vulnerable to interference from endogenous high biotin concentrations, causing false low results in sandwich immunoassays and false high results in competitive immunoassays. The skewed results can lead to misdiagnoses including suspected endocrine and autoimmune disorders, missed infectious or neoplastic diseases, delayed pregnancy detection, suspected drug intoxications, and missed myocardial infarctions.[1–8] In total, 163 of the available 265 assays (62%) across kits by Roche Elecsys (81 of 81), Ortho Vitros (29 of 43), Siemens Dimension (21 of 26), Siemens Centaur (18 of 67), and Beckmann Coulter Access/DXI (14 of 48) are potentially susceptible to biotin interference.[1,3] The impact of interference is dose dependent and specific to each platform and assay.[1–5] Assays on Abbott's ARCHITECT i series are supposed[6] to be unaffected up until biotin concentrations of 1.0×10^6 pg/mL.

Because the recommended daily intake of biotin (30–75 μg) does not influence current assays, biotin interference used to be an uncommon phenomenon that was mostly limited to biotin-dependent patients with rare inherited metabolic disorders receiving daily dosages between 10 and 200 mg.[1–5,7–11] But in recent years, long-term biotin supplementation in doses of 300 mg per day (4000–10 000

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times the recommended daily intake) has been adopted as a promising therapeutic strategy in progressive multiple sclerosis and other demyelinating disorders.[12–16] Additionally, supplementation with high-dose biotin could be beneficial in the treatment of dermatitis, diabetes mellitus, and depression.[17–22] In pregnancy, supplementation of up to 300 μg biotin per day might be required, which should not interfere with current immunoassays.[17,22] Biotin is also very popular as a self-medication to reduce hair loss or to improve the condition of skin and nails in supraphysiological doses of up to 30 mg daily, and supplements containing as much as 100 mg of biotin are available over-the-counter.[3,5,23] Patients often do not report these supplements in their medical history, which represents one of the greatest challenges in dealing with biotin interference.[3–5,7]

A study at the Mayo Clinic (Rochester, Minnesota) found that in 7.4% of patients presenting to the emergency department biotin was present at sufficient concentrations to cause interference in immunoassays using biotin-streptavidin technology.[24] These facts and several instances of actual mismanagement of patients taking high doses of biotin have raised concern about the reliability of biotin-susceptible immunoassays such that the US Food and Drug Administration,[25] the British ACB Scientific Committee,[26] the German Federal Institute for Drugs and Medical Devices,[27] and the European Medicines Agency[28] have found it necessary to issue public warnings.

The purpose of this study was to simulate the worst-case scenario, running samples with the upper limits of biotin, for potentially time-critical analytes and evaluate the effectiveness of a biotin-depletion protocol using streptavidin particles.

METHODS

Biotin Stock Solution and Sample Spiking

A stock solution containing 53.6×10^6 pg/mL biotin was made using 99.9% biotin lyophilized powder (catalog number B4501-500MG, lot number SLBS8478, Sigma-Aldrich Chemie GmbH, Schnelldorf, Germany) and 0.9% sodium chloride (B. Braun Melsungen AG, Melsungen, Germany). The solution's biotin concentration was confirmed via enzyme-linked immunosorbent assay (ELISA) measurements in triplicate (Biotin ELISA Kit, reference number K 8141, Immundiagnostik AG, Bensheim, Germany). This test is a competitive enzyme-linked assay with an upper quantification limit of 1100 pg/mL. The lower quantification limit is 48 pg/mL (evaluation according to Clinical and Laboratory Standards Institute guideline EP-17-A2).[41] Samples containing biotin levels above the upper quantification limit had to be (repeatedly) diluted and reassayed. After confirmation of the stock solution's biotin concentration, it was thoroughly vortexed again and aliquoted. Aliquots were frozen at –20°C until needed. For evaluation of the spiking, 21 μL of biotin stock solution was added to 979 μL serum of 10 de-identified samples from different patients not taking biotin to achieve a sample concentration of 1.126×10^6 pg/mL (to convert to nmol/L, multiply by 0.00409), the maximum reported biotin concentration 1 to 2 hours after a single oral dose of 300 mg biotin at a spiking volume of 2.1% of the final volume.[4,29,30] Then, achievement of the target concentration in the samples was confirmed via biotin ELISA. The acceptable deviation from the target value was ±2 SD. The 0.9% sodium chloride was assessed to ensure that it did not contribute to any biotin in the stock solution.

Evaluation of Adsorption Times

Using the 10 spiked serum samples, the efficiency of biotin adsorption during 15, 30, and 60 minutes was evaluated using pooled streptavidin-coated magnetic microparticles contained in all Roche Elecsys reagent packs at a concentration of 0.72 mg/mL (reagent M, Roche Deutschland Holding GmbH, Mannheim, Germany) (Figure 1). The binding capacity of streptavidin for free biotin is 0.327×10^6 pg/mL. With a target concentration of 1.126×10^6 pg/mL, 4 parts of streptavidin particles per 1 part serum should achieve (near) complete biotin depletion. However, a considerable safety margin seems prudent because of biological variability with a broad standard deviation.[4] Therefore, 1 part spiked serum (eg, 250 μL) was incubated with 5 parts streptavidin solution (eg, 1250 μL), leaving a safety margin of approximately 0.509×10^6 pg/mL for excess biotin. After adsorption, the biotin concentrations were reanalyzed via ELISA (Figure 2).

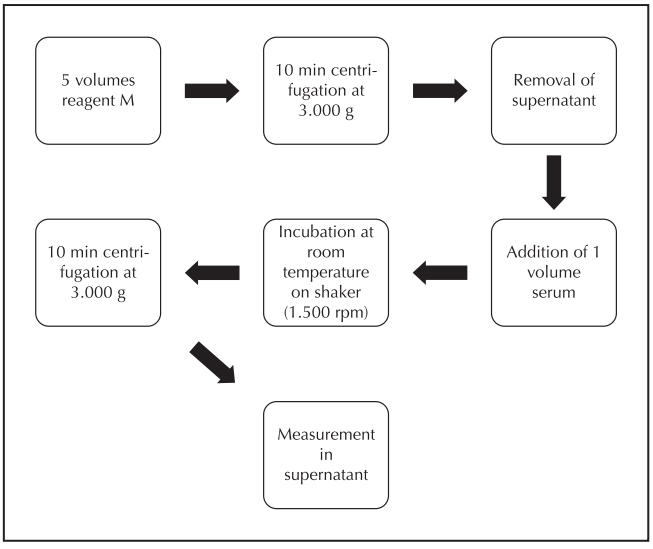

Figure 1. *Biotin adsorption protocol. Adsorption times (ie, incubation times on a multi-tube vortexer) of 15, 30, and 60 minutes were evaluated. In step 3, remaining preservative was removed via careful pipetting to minimize volume effects caused by the adsorption. A maximum of 5 μL preservative was accepted to remain in the tubes. Prepared 1.5-mL Eppendorf safe-lock tubes containing streptavidin particles were stored at −20°C until needed in order to maximize time efficiency.*

Interference of Biotin in Immunoassays

The interference of high-dose biotin in the assays for thyroid-stimulating hormone (TSH), free triiodothyronine (FT3), free thyroxine (FT4), digitoxin, tacrolimus, cyclosporine A, cortisol, C-peptide, insulin, N-terminal pro-B–type natriuretic peptide (NT-proBNP), troponin T high sensitive (hs), human immunodeficiency virus (HIV), procalcitonin, β human chorionic gonadotropin (β-HCG), toxoplasma immunoglobulin (Ig) M, and toxoplasma IgG (Table 1) was evaluated on e 801 modules of Roche's cobas 8000 modular analyzer series using a total of 160 different de-identified routine samples (10 different samples per assay) with varying analyte concentrations from patients without known biotin supplementation. After initial measurement, the samples were spiked with biotin stock solution as described above and then measurements were repeated. Then, after the 15-minute adsorption protocol described above, the samples were analyzed again. One measurement was performed from each specimen before biotin spiking, after biotin spiking, and after biotin adsorption. Initial results were compared versus results after spiking and after adsorption. The dilution caused by the addition of biotin stock solution was factored into the results by multiplying the measured analyte concentrations after spiking and after adsorption by a factor

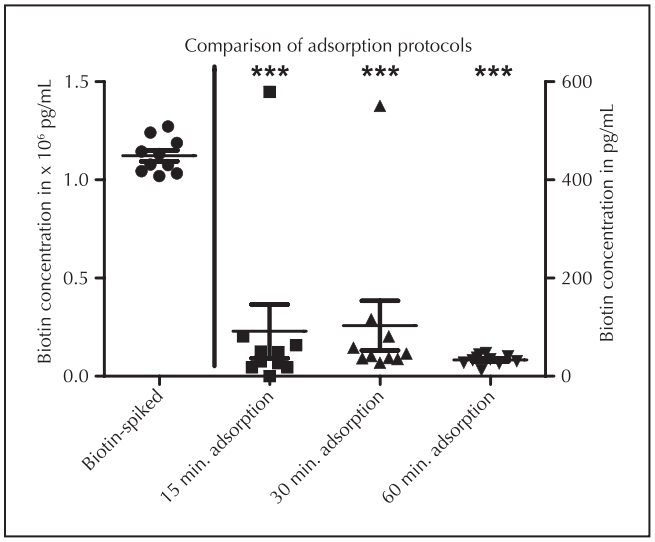

Figure 2. *Biotin concentration in spiked serum samples and after 15, 30, or 60 minutes of adsorption. Regardless of the incubation period, the adsorption protocol depleted most of the biotin from all tested samples. All results were statistically significant when compared with the spiked samples (***P <.001). Among the 3 adsorption methods, no statistically significant difference was detected. Please note that the biotin concentration of the spiked samples is plotted against the left y-axis and the biotin concentration after adsorption is plotted against the right y-axis. n = 10.*

of 1.0215. A difference of 10% or more between the means was considered to be significant.

Statistical Analysis

GraphPad Prism 8 (GraphPad Software, San Diego, California) was used for statistical analysis and graphical depiction. For better comparability, all results within a given set were normalized as percentages versus the initial measurement, which was set to the default of 100%. Graphical depiction was done as means ± standard error of the mean. Statistical analysis was performed by 1-way analysis of variance with Bonferroni multiple comparison posttest for paired observations. A threshold of $P < .05$ was set for statistical significance.

RESULTS

Sample Spiking

The 10 serum samples spiked with biotin stock solution reached biotin concentrations between 1.019×10^6 pg/mL and 1.272×10^6 pg/mL. The mean was 1.123×10^6 pg/mL (SD = 0.088×10^6 pg/mL. As confirmed via ELISA, 0.9% sodium chloride contained no relevant concentration of biotin (<48 pg/mL).

Evaluation of Adsorption Times

All 3 adsorption regimes led to significantly depleted biotin concentrations within the 10 spiked samples. The 60-minute adsorption protocol proved to be the most effective, with a mean remaining biotin concentration of 33.47 ± 9.95 pg/mL. The 30-minute regime resulted in a mean biotin concentration of 103.1 ± 159.9 pg/mL and the 15-minute regime led to a mean biotin concentration of 91.5 ± 172.9 pg/mL (Figure 2).

Biotin Interference in Competitive Immunoassays

High-dose biotin led to false high results in the competitive immunoassays for cortisol, cyclosporine A, digitoxin, tacrolimus, FT3, and FT4. The mean positive bias in spiked samples ranged from 15% (cortisol) to 71% (tacrolimus) and was overall independent of initial analyte concentrations within the samples. The 15-minute adsorption protocol returned all results to levels that were close or identical to the original prespike measurements. Cyclosporine A and

Table 1. Overview of Evaluated, Potentially Time-Critical Analytes on Roche's cobas 8000 Modular Analyzer Series, Their Thresholds for Biotin Interference, and the Range of Analyte Concentrations in This Study[a]

Parameter	Interference Threshold, × 10³ pg/mL	Analyte Range	Unit	Assay Type	Reference No.	Lot No.
β-HCG	80	13.7–2061	mU/mL	Sandwich	07251025190	385896
Cortisol II	70	5–22	µg/dL	Competitive	07027150190	353299
C-peptide	60	0.8–6.3	ng/mL	Sandwich	07027168190	359510
Cyclosporine A	30	45–234	ng/mL	Competitive	07251246190	372163
Digitoxin	50	8.8–38.3	ng/mL	Competitive	07027206190	361549
FT3 III	70	1.8–5.7	pg/mL	Competitive	07027362190	348359
FT4 III	100	0.7–4.1	ng/dL	Competitive	07976887190	380330
HIV Duo	28	3.1–756	COI	Sandwich	07229542190	385056
Insulin	60	4.1–28	mU/L	Sandwich	07027559190	378508
NT-proBNP II	30	24–4611	ng/L	Sandwich	07027664190	358699
PCT	30	0.14–19.5	µg/L	Sandwich	07301715200	358856
Tacrolimus	30	1.75–8.74	µg/L	Competitive	07251254190	375033
Toxoplasma IgM	60	1.0–5.3	COI	Sandwich	07028024190	387690
Toxoplasma IgG	60	154–516	IU/mL	Sandwich	07028008190	363409
Troponin T hs	20	59.6–8889	pg/mL	Sandwich	07028075190	370695
TSH	25	0.3–3.6	µU/mL	Sandwich	07028091190	386646

Abbreviations: β-HCG, β human chorionic gonadotropin; COI, cutoff index; FT3, free triiodothyronine; FT4, free thyroxine; HIV, human immunodeficiency virus; hs, high sensitive; Ig, immunoglobulin; NT-proBNP, N-terminal pro-B-type natriuretic peptide; PCT, procalcitonin; TSH, thyroid-stimulating hormone.

SI conversion factor: To convert FT4 to picomoles per liter, multiply by 12.871.

[a] All assays are manufactured by Roche Deutschland Holding GmbH, Mannheim, Germany. All thresholds for biotin interference are stated as provided by Roche's Elecsys method sheets.[38]

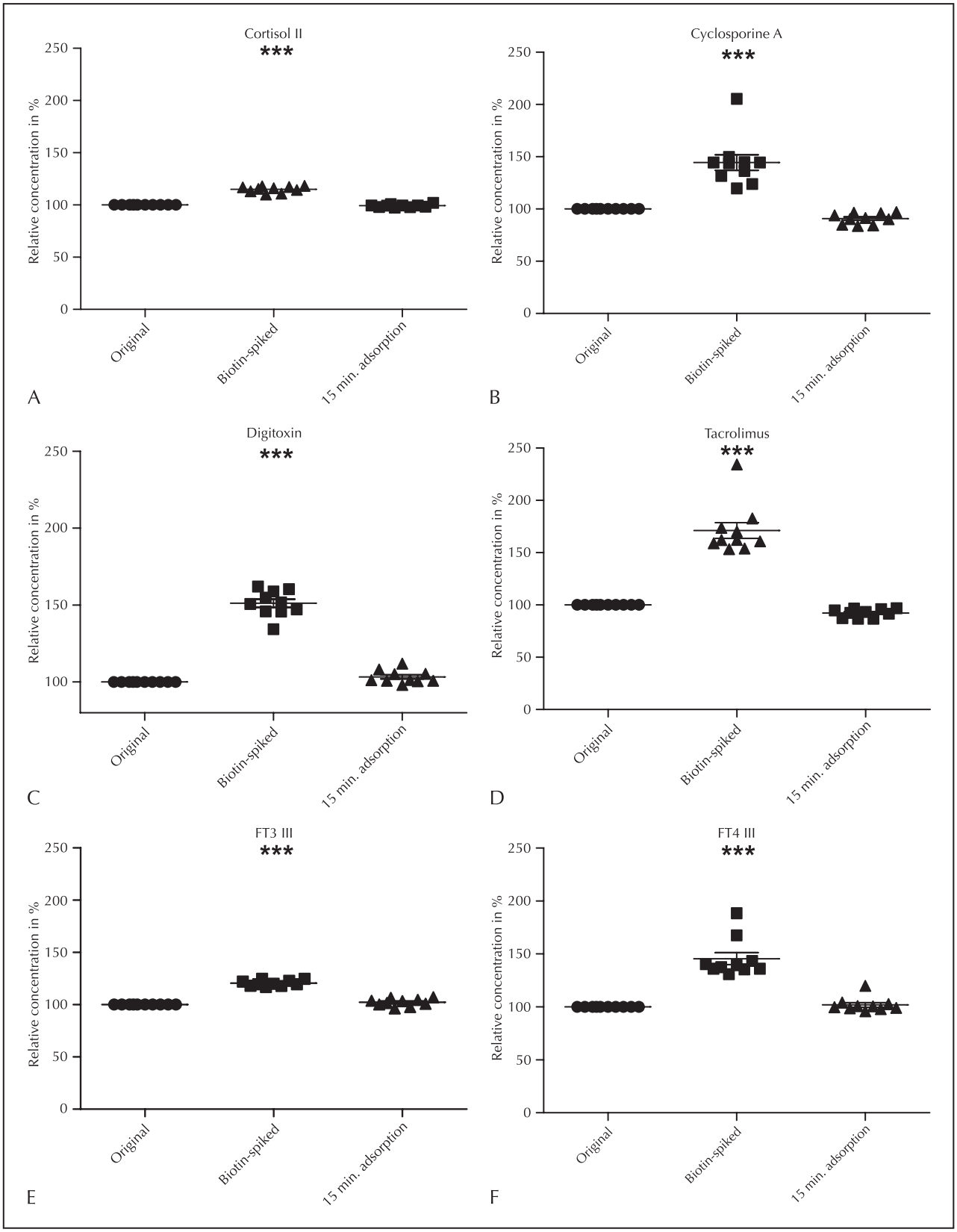

Figure 3. *Biotin interference in competitive immunoassays for cortisol, cyclosporine A, digitoxin, tacrolimus, free triiodothyronine (FT3), and free thyroxine (FT4). Regardless of initial analyte concentration, biotin caused statistically significant (***P < .001) interference in the immunoassays for cortisol (A), cyclosporine A (B), digitoxin (C), tacrolimus (D), FT3 (E), and FT4 (F). The adsorption procedure produced no statistically significant discrepancy when compared with the original prespike measurements (A through F). n = 10 per analyte.*

Table 2. Percentage Means, Standard Deviations, and Coefficients of Variation (CVs) Postspiking and After Biotin Depletion and 95% Confidence Intervals (CIs) Original Versus Biotin-Spiked and Original Versus 15-Minute Adsorption in Evaluated Competitive Immunoassays on Roche's cobas 8000 Modular Analyzer Series[a]

Assay	Biotin-Spiked, %			95% CI Original Versus Biotin-Spiked, %	After Depletion, %			95% CI Original Versus 15-min Adsorption, %	Manufacturer-Supplied CV for Comparable Analyte Ranges, % (Value)[38]
	Mean	SD	CV		Mean	SD	CV		
Cortisol II	115	2.9	2.5	112.8–117.0	99	1.4	1.4	98.2–100.2	1.1–1.6 (1.15–28.6 µg/dL)
Cyclosporine A	144	23.5	16.3	127.5–161.2	91	5.1	5.6	87.1–94.4	2.0–3.7 (36.2–213 ng/mL)
Digitoxin	151	8.4	5.6	145.2–157.2	103	4.3	4.2	100.3–106.3	1.8–2.6 (7.66–38.8 ng/mL)
FT3 III	121	2.8	2.3	118.5–122.6	102	3.5	3.4	99.6–104.6	2.1–7.6 (0.96–4.49 pg/mL)
FT4 III	146	18.1	12.4	132.6–158.5	102	6.7	6.6	97.1–106.7	1.5–2.4 (1.01–4.22 ng/dL)
Tacrolimus	171	23.9	14.0	154.0–188.3	92	4.0	4.3	89.4–95.1	2.2–3.6 (1.80–8.91 µg/L)

Abbreviations: FT3, free triiodothyronine; FT4, free thyroxine.

SI conversion factor: To convert FT4 to picomoles per liter, multiply by 12.871.

[a] All calculated CVs are compared with manufacturer-supplied CVs for analyte ranges most closely matching the analyte ranges evaluated in this study as stated in Table 1. Because of the study design, the calculated CVs include the imprecision from the adsorption protocol and the analytical imprecision of the given assay.

tacrolimus showed the worst recoveries following depletion treatment, with 91% and 92%, respectively (Figure 3, A through F). Means, standard deviations, coefficients of variation (CVs), and CIs are shown in Table 2.

Biotin Interference in Sandwich Immunoassays

High-dose biotin led to false low results in the sandwich immunoassays for C-peptide, insulin, TSH, NT-proBNP, troponin T hs, HIV, procalcitonin, and β-HCG. The mean negative bias in spiked samples ranged from 13% (β-HCG) to 98% (TSH) and was largely independent of initial analyte concentrations within the samples. The NT-proBNP assay was a notable exception. In this assay, 3 samples of different concentrations showed more than 90% signal loss, and the rest suffered from an average signal loss of 59%, amounting to a total mean negative bias of 69%. The postspike measurement was reduced to 5.9 ng/L in the sample containing 72.6 ng/L NT-proBNP (signal loss of 92%), to 30.5 ng/L in the sample containing 413 ng/L NT-proBN-P(signal loss of 93%), and to 138.9 ng/L in the sample containing 1888 ng/L NT-proBNP (signal loss of 93%). The sandwich immunoassays for toxoplasma IgM and toxoplasma IgG were largely unaffected by biotin. Mean postspike and recovery measurements for toxoplasma IgM equaled 104% and 102%, respectively. For toxoplasma IgG, the mean postspike and recovery measurements were 101% and 96%. The 15-minute adsorption protocol returned all results to levels that were close or identical to the original prespike measurements. Some of the measurements after adsorption showed results that were statistically significantly different from the original prespike measurement. However, the difference in means was always less than 10% and deemed insignificant with one exception: in the assay for insulin, only 85% of the original relative concentration could be recovered (Figures 4, A through F, and 5, A through D). Means, standard deviations, CVs, and CIs can be found in Table 3.

DISCUSSION

As demonstrated by our results, the interference caused by high-dose biotin can lead to dangerously false results in a wide variety of analytes in time-critical situations, thereby possibly resulting in misdiagnoses and the mistreatment of patients.[1–8] The often-recommended discontinuation of biotin intake for up to 7 (in one case 15) days seems unfeasible in emergency situations and has additional drawbacks: because of biological variability (eg, in renal clearance), the laboratory cannot be certain of the elimination of biotin interference until the biotin concentration has been determined or a dilution series has been tested, which costs additional time, manpower, and money.[1–5,7,8,31,32] Also, biotin can be included in intravenous nutrient solutions and might be impossible to withdraw in certain situations.[3] The second advocated work-around, the measurement of desired analytes on an alternative platform, seems equally impractical in time-critical situations.[1,32] To our knowledge, most laboratories use only one high-throughput clinical chemical platform.[4] Therefore, that work-around would often require shipping samples to another laboratory. The resulting delay is not tolerable in time-critical measurements, and results would suffer from decreased comparability with measurements in patient samples taken prior to the initiation of biotin supplementation. Active biotin depletion, on the other hand, can eliminate the need to withhold a (potentially) beneficial medication, the uncertainty of patient compliance (ie, cessation of supplementation), long analytical delays, and streptavidin antibodies as well as biotin and its metabolites within a given sample.[32] Nonetheless, close communication between clinicians and the laboratory is necessary to determine the best course of action in a given situation.

The evaluated adsorption protocol removed most of the biotin from spiked samples and the remaining biotin was well below any manufacturer provided threshold for biotin interference. The 2 outliers in the adsorption protocols with 15- and 30-minute incubation times were samples from 2 different patients. That 2 of 3 attempts depleted these same samples more completely suggests that the remaining incubation was not executed perfectly. Streptavidin particles have a tendency to sediment, and regular or continuous mixing is required for optimal interaction with biotin.

The 15-minute adsorption protocol and the necessary additional centrifugation increased turnaround times by 25 to 30 minutes. Previous studies often recommended incubation times of 45 to 60 minutes with regular mixing for adsorption, which can be impracticable with regard to some time-critical analytes.[32] Trambas et al[32] showed that incubation times between 5 and 30 minutes produced measured analyte concentrations similar to the values observed in unspiked samples for anti-thyroglobulin anti-

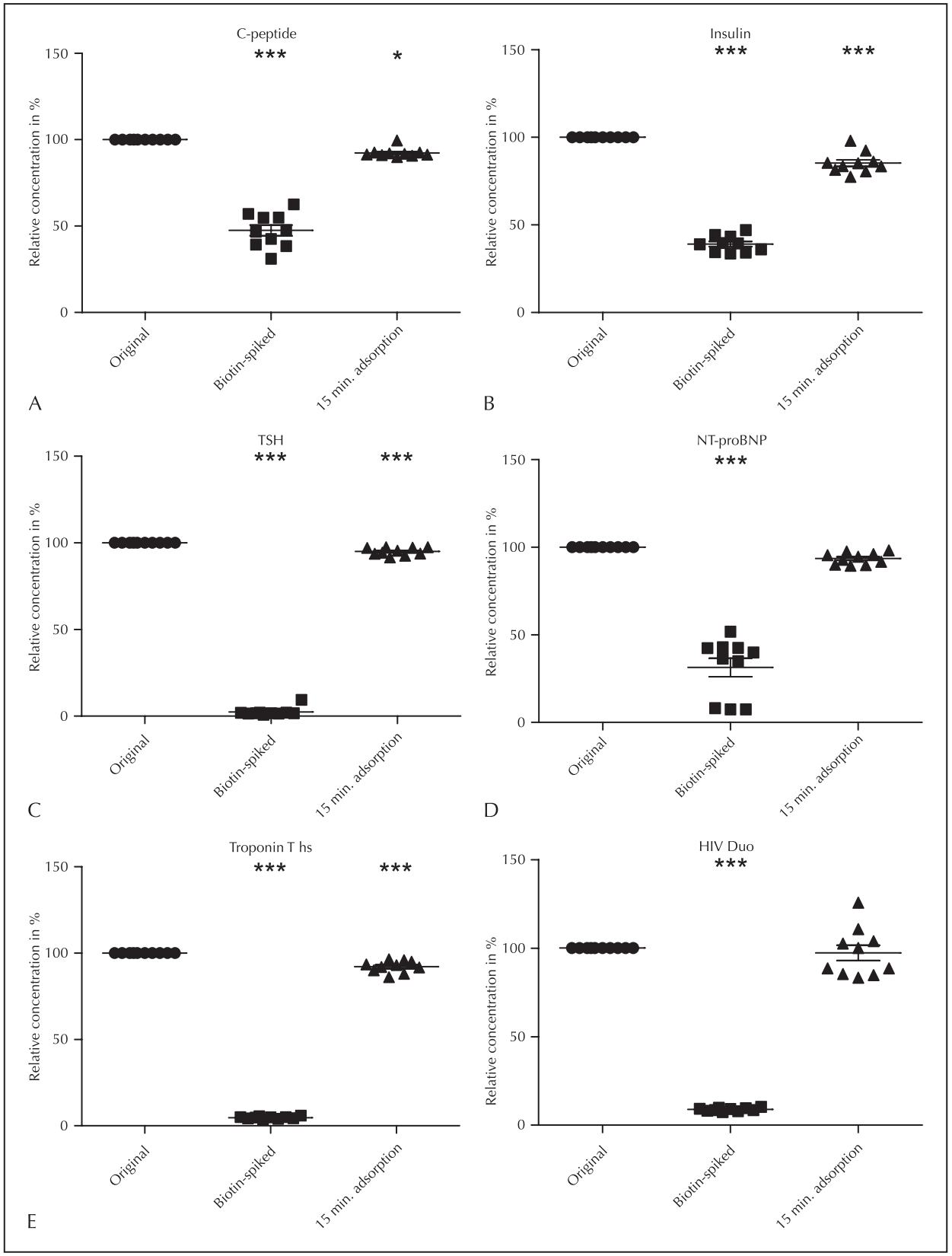

Figure 4. *Biotin interference in sandwich immunoassays for C-peptide, insulin, thyroid-stimulating hormone (TSH), N-terminal pro-B–type natriuretic peptide (NT-proBNP), troponin T high sensitive (hs), and human immunodeficiency virus (HIV). Biotin caused statistically significant (***P $< .001$) interference in the immunoassays for C-peptide (A), insulin (B), TSH (C), NT-proBNP (D), troponin T hs (E), and HIV (F). The depletion protocol produced statistically significant discrepancies, when compared with the original prespike measurements, in the assays for C-peptide (A) (*P $= .04$), insulin (B) (***P $< .001$), TSH (C) (***P $< .001$), and troponin T hs (***P $< .001$). n = 10 per analyte.*

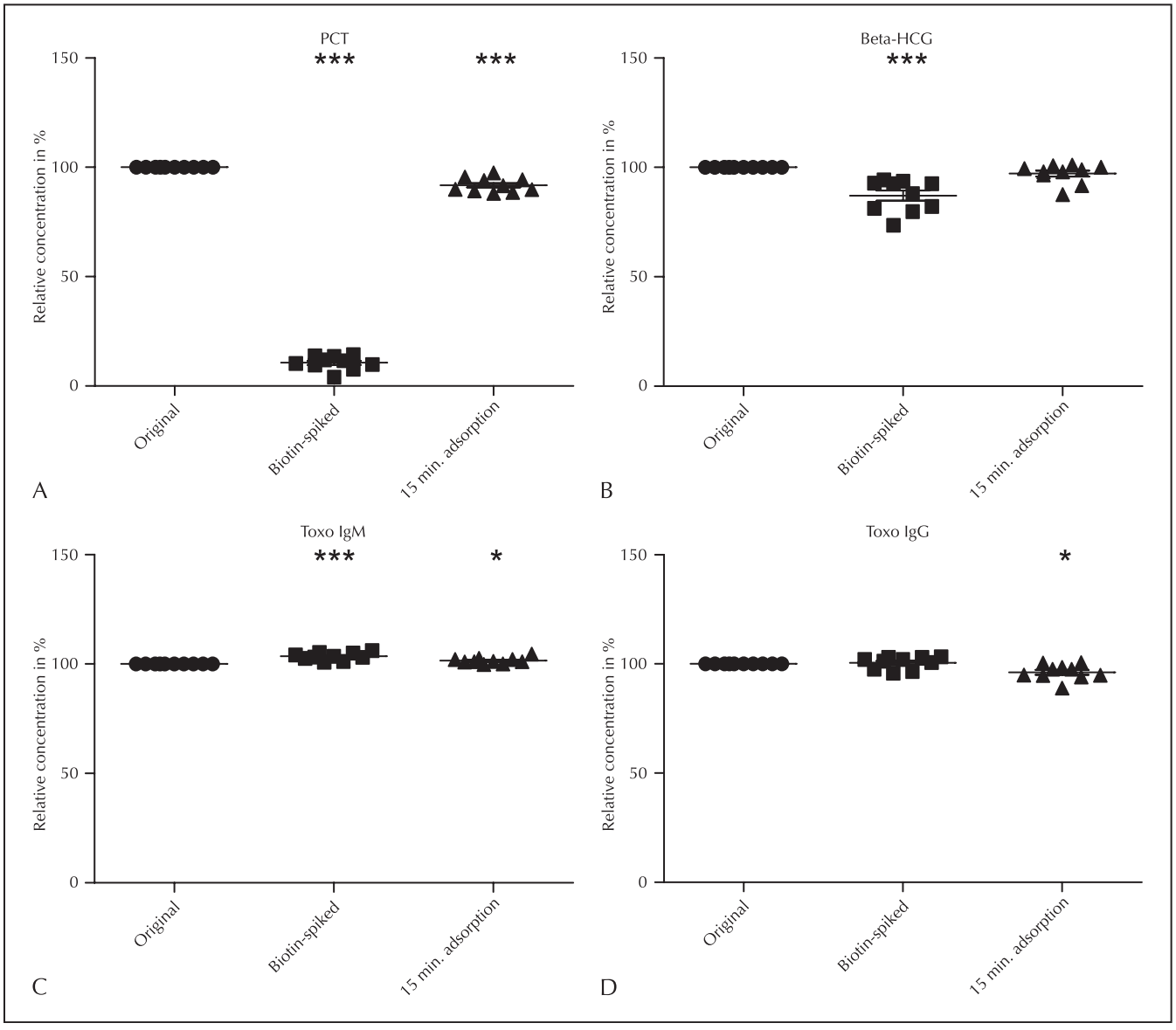

Figure 5. *Biotin interference in sandwich immunoassays for procalcitonin (PCT), β human chorionic gonadotropin (Beta-HCG), toxoplasma (Toxo) immunoglobulin (Ig) M, and Toxo IgG. Biotin caused statistically significant (***P $<$.001) interference in the immunoassays for PCT (A), Beta-HCG (B), and Toxo IgM (C). In Toxo IgG (D), no statistical significance was detected postspike. The depletion protocol produced statistically significant discrepancies, when compared with the original prespike measurements, in the assays for PCT (A) (***P $<$.001), Toxo IgM (C) (*P = .04), and Toxo IgG (D) (*P = .03). n = 10 per analyte.*

bodies, anti–thyroid peroxidase antibodies, TSH, FT3, and FT4. Another work group[33] evaluated incubation times of 0, 15, 30, 45, and 60 minutes in cardiac troponin T hs in samples spiked with 1.0×10^6 pg/mL biotin and found no difference in the effectiveness of incubation times. However, these analytes comprise only a fraction of the ones in this study, and shorter incubation times should be evaluated for all concerned assays before they are applied to minimize the increase in turnaround times even further.

Schrapp et al[33] described that the interference in troponin T hs is independent of initial troponin concentrations, an observation that we also made in most tested competitive and sandwich immunoassays, with the exception of NT-proBNP. Trambas et al,[2] on the other hand, found the interference of biotin caused proportional changes in sandwich immunoassays regardless of initial analyte concentrations, whereas competitive immunoassays showed a particularly exaggerated positive bias in samples with low analyte concentrations. Additionally, Trambas et al[32] reported almost 100% recovery of relative C-peptide concentration after adsorption. The discrepancy between these observations might be due to the difference in evaluated assays, because not all immunoassays are equally susceptible to biotin interference, even if the manufacturer-supplied thresholds for biotin interference are identical. Additionally, Trambas et al[2] started their evaluation of biotin interference in several competitive immunoassays (eg, for testosterone, estradiol, and progesterone) at very low analyte concentrations, and sandwich immunoassays were not evaluated at the extreme lower range of quantification. This might have biased the results.

Table 3. Percentage Means, Standard Deviations, and Coefficients of Variation (CVs) Postspiking and After Biotin Depletion and 95% Confidence Intervals (CIs) Original Versus Biotin-Spiked and Original Versus 15-Minute Adsorption in Evaluated Sandwich Immunoassays on Roche's cobas 8000 Modular Analyzer Series

Assay	Biotin-Spiked, %			95% CI Original Versus Biotin-Spiked, %	After Depletion, %			95% CI Original Versus 15-min Adsorption, %	Manufacturer-Supplied CV for Comparable Analyte Ranges, % (Value)[38]
	Mean	SD	CV		Mean	SD	CV		
β-HCG	87	7.3	8.4	81.9–92.3	97	4.3	4.4	94.1–100.3	2.4–2.8 (5.8–2592 U/mL)
C-peptide	48	9.8	20.4	40.5–54.6	92	2.7	2.9	90.4–94.2	0.9–2.1 (1.01–19.1 ng/mL)
HIV Duo	9	0.9	10.0	8.2–9.5	97	13.8	14.2	87.4–107.2	1.2–2.1 (2.12–53.0 COI)
Insulin	39	4.6	11.8	35.8–42.4	85	6.0	7.1	81.0–89.6	1.4–4.3 (3.25–22.1 mU/L)
NT-proBNP II	31	17.0	54.8	19.2–43.5	94	3.3	3.5	91.2–95.9	2.9–10.3 (16.9–4433 ng/L)
PCT	11	3.1	28.2	8.4–12.9	92	3.3	3.6	89.5–94.2	1.5–3.9 (0.082–39.3 µg/L)
Toxoplasma IgM	104	1.7	1.6	102.3–104.7	102	1.4	1.4	100.6–102.5	0.9–1.0 (1.18–3.87 COI)
Toxoplasma IgG	101	2.8	2.8	98.5–102.6	96	3.5	3.7	93.7–98.7	1.3–2.6 (46.6–324 IU/mL)
Troponin T hs	5	0.7	14.0	4.2–5.3	92	3.3	3.6	89.8–94.6	0.9–2.1 (19.8–9886 pg/mL)
TSH	2	2.5	125.0	0.7–4.2	95	2.2	2.3	93.5–96.6	1.4–1.6 (0.209–1.88 µU/mL)

Abbreviations: β-HCG, β human chorionic gonadotropin; COI, cutoff index; HIV, human immunodeficiency virus; hs, high sensitive; Ig, immunoglobulin; NT-proBNP, N-terminal pro-B-type natriuretic peptide; PCT, procalcitonin; TSH, thyroid-stimulating hormone.

[a] All calculated CVs are compared with manufacturer-supplied CVs for analyte ranges most closely matching the analyte ranges evaluated in this study as stated in Table 1. Because of the study design, the calculated CVs include the imprecision from the adsorption protocol and the analytical imprecision of the given assay.

In our study, the assays for FT3, FT4, and cortisol showed considerably less positive bias than would have been expected according to the studies by Piketty et al[4] and Trambas et al.[32] At 1000 pg/mL biotin, they found a widely spread relative positive bias of 237% to 328% for FT3, 431% or more for FT4, and 153% to 212% for cortisol.[4,32] However, it should be noted that these authors used different platforms: Roche cobas e 411 (Piketty et al[4]) and Roche cobas e 602 (Trambas et al[32]). Also, the FT4 III assay with increased resistance to biotin was introduced in 2018 and therefore could not have been used in these studies submitted for publication in 2016 and 2017. Differing reference and lot numbers as well as lower sample sizes further complicate direct comparability.

Some case studies also showed considerably less positive bias in FT3 and/or FT4 than would have been expected. In 2018, Ardabilygazir et al[34] evaluated a patient taking 200 mg biotin daily for more than 10 months whose FT3 and FT4 showed relative positive biases of 40% and 129% when compared with the hormone assessment before the initiation of biotin supplementation. Information about the Roche platform used was not provided.

Al-Salameh et al[35] observed relative positive biases of 73% and 258% for FT3 and FT4 in a patient taking 300 mg biotin daily when they compared the results on their Roche cobas e 170 with measurements obtained on the Siemens ADVIA Centaur in 2017. The biotin concentrations in the patients' blood were not assessed in these case reports, there was no attempt of biotin adsorption, and no information about the assay lot and reference numbers was given.[34,35]

The lack of information about assays used is common in many case studies, which mostly date back to the time before generation III of the FT4 assay was introduced. Often, the biotin concentration within the patients' blood was not assessed and the authors did not use any adsorption protocol. The use of varying Roche platforms or the complete lack of information about Roche platforms used complicates direct comparisons further.

The biotin resistance in the assays for toxoplasma IgM and toxoplasma IgG was remarkable. The experiment for these analytes was repeated with a freshly thawed aliquot of biotin stock solution as well as other patient samples and came to the same result (data not shown).

Our observations in FT3, FT4, cortisol, toxoplasma IgM, and toxoplasma IgG might suggest that we did not reach the desired target concentration for biotin when spiking these samples. However we find that unlikely, because achievement of the biotin target concentration was confirmed via ELISA, aliquots of the same stock solution were used throughout the study, the results in different analytes (eg, TSH, troponin T hs) matched observations made by other investigators, and pipetting was always performed by the same person. This could suggest a higher-than-manufacturer-reported threshold for biotin interference in these assays. Several independent studies have already confirmed much higher-than-manufacturer-reported interference thresholds in other assays.[36] To our knowledge, no other work group has evaluated biotin interference in Elecsys assays for toxoplasma IgM and toxoplasma IgG. Additional investigation will be required to further evaluate the results obtained for FT3, FT4, cortisol, toxoplasma IgM, and toxoplasma IgG.

Like all currently available countermeasures for biotin interference, the evaluated method has certain weaknesses. The adsorption caused an increase in the CVs and analytical imprecision in all tested assays, with the exception of cortisol, FT3, NT-proBNP, and procalcitonin, when compared with the manufacturer-supplied CVs in a comparable parameter range (Tables 2 and 3). Schrapp et al[33] made the same observation in their evaluation of biotin depletion in the Elecsys assay for troponin T hs. The increased CVs should not be problematic in most analytes, with the exception of troponin T hs and HIV. The H0/H1 protocol in suspected myocardial infarction as suggested by the European Society of Cardiology should not be used when biotin depletion is performed. The H0/H3 protocol is a suitable alternative.[33,37] In suspected HIV, low-level positive or high-level negative results for samples treated with the depletion protocol should be repeated on an alternate platform because of the decreased precision.

The observed increase in CV might partly be due to minimal dilution effects or matrix changes caused by

residual preservative in the Eppendorf tubes during the incubation with reagent M. Leftover preservative can also distort free to total hormone equilibrium, and thus measured values.[33] A further important source of possible error is inadequate immobilization of the streptavidin microparticles and contamination of the aspirated sample with streptavidin microparticles during pipetting.[33] The biotin depletion procedure requires manual handling, care, and attentiveness, and can therefore be prone to human error.[33]

The experimental setup in this study resulted in prolonged air exposure in tacrolimus and cyclosporine A assays, which is cautioned against in Roche's method sheets and might have resulted in a reduced recovery in these analytes.[38]

Because of the manual work steps, the depletion protocol cannot be applied indiscriminately to all samples. At the same time, a laboratory cannot determine the biotin concentration within all samples to screen out the ones needing pretreatment and still be expected to work in a time- and cost-efficient manner. Unfortunately, high-dose biotin supplements are also not traced via pharmacies in Germany and laboratories do not receive a list of patients who are taking biotin, unlike in France.[33] Therefore, the successful implementation of the suggested countermeasure in selected samples is dependent on close communication with clinicians who must be aware of the interference caused by biotin and inform the laboratory of patients using biotin supplements (including time of last intake, dose, and duration). Ideally, that information should be provided to the laboratory before the samples are taken, so that the appropriate course of action with regard to the specific situation can be determined. We found an informative letter very helpful in this regard.

Our study relied on sample spiking using a biotin stock solution and cannot account for the interference caused by biotin metabolites, primarily bisnorbiotin and biotin sulfoxide, in vivo. However, the majority of biotin within plasma remains native biotin, of which more than 50% is secreted unchanged renally, and biotin metabolites are thought to affect immunoassays much less than native biotin.[2,4] Also, our adsorption protocol would eliminate these metabolites at least partially from samples.[4,32] Therefore, we feel confident that the obtained adsorption results are comparable with the situation in vivo. In fact, we have already succeeded in correcting biotin interference in thyroid function tests and thyroid antibody assays of 2 MS patients taking 300 mg biotin daily (R.S., M.U., and I.M., unpublished data, April 1, 2019).

Recently, Roche started the distribution of a new test kit for troponin T hs and TSH with a supposed resistance to biotin interference up to biotin concentrations of 1.2×10^6 pg/mL.[39,40] Pending laboratory validation, this is an encouraging development for immunoassay reliability. It may take several years for Roche to complete the step-by-step replacement of further test kits. Until the new kits are delivered, the evaluated adsorption protocol will provide laboratories with a safe and easily implemented countermeasure for bias caused by biotin in their repertoire of solutions. When using adsorption protocols, successful biotin depletion should be confirmed by parallel analyzation of paired control samples, unspiked and spiked with a known concentration of biotin.[32]

Alternatives to biotin depletion for the evaluation of discrepant assay results in non–time-critical situations include discontinuation of biotin supplementation and repeated analysis after biotin clearance, serial dilution of samples, and repeat testing on an alternate platform.[36] As described above, each of these approaches suffers from specific drawbacks: serial dilutions can be time-consuming and overdilution can lead to inaccurate results, whereas repeat testing on an alternate platform usually requires sending samples to a reference laboratory for testing. Repeated analysis after biotin discontinuation and clearance might not be possible, because patients may not be available upon recognition of potential biotin interference.[36] Regardless of the chosen evaluation method, direct measurement of biotin concentrations should be performed to confirm biotin as the likely cause of interference.[36]

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