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Biotin interferences: Have we neglected the impact on serological markers?

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ABSTRACT

Background: Biotin has been reported to be a leading cause of interference on several immunoassay platforms using the streptavidin-biotin immobilization system. While biotin interferences have now been well characterized for several assays, only few data are available on their impact on serological markers of infectious viral diseases.

Methods: Overall, 10 healthy volunteers (HVs) received a single 100 mg dose of biotin to evaluate its effect on hepatitis B serological markers. Blood samples were taken several times before and after biotin intake. In addition, spiking experiments were applied to investigate biotin's impact on anti-HIV/p24 Ag and anti-HCV antibody levels. Several procedures designed to overcome this interference were evaluated.

Results: Biotin intake resulted in a false-negative anti-HBs immunological status (< 10 mIU/mL) in 40.0% of cases. According to our anti-HBc and anti-HBe results, biotin intake was associated with 90.0% and 80.0% of false positive results, respectively. At the theoretical biotin peak concentration following a 100 mg intake, 50.0% and 66.6% of anti-HIV and anti-HCV results were false negatives, respectively. All the procedures evaluated to overcome the interference were proven effective.

Conclusion: HBV, HCV, and HIV serological markers are likely to be highly sensitive to biotin. Our data confirm that the scope of biotin interference is broader than commonly described.

1. Introduction

Biotin (vitamin H or B7) is a small, soluble carboxylase enzyme cofactor bioavailable from food intake or locally synthesized by gut microbiota [1]. Today, this molecule is widely available over the counter (OTC) and increasingly used for cosmetic or therapeutic purposes [2,3]. Recently, Katzman et al. showed that 7.4% of an American outpatient population were taking biotin supplementation at a dose that could potentially interfere with immunoassays (> 10 ng/mL) [4]. Due to biotin's high affinity to streptavidin, biotin has been extensively employed in immunoassays as an immobilizing system. A recent study revealed that approximately 60% of the parameters assessed using eight of the most popular immunoassays in the USA were biotin-based assays [5]. However, this small molecule is increasingly described as a leading cause of interference in the latter immunoassay systems [6–9]. In two-site assays, an excess of biotin displaces biotinylated antibody-antigen complexes from the streptavidin-coated solid phase, resulting in falsely low analyte levels (as the assay signal is directly related to the analyte concentration). Conversely, in competitive assays, an excess of biotin produces falsely positive interference given that the signal is inversely proportional to the analyte concentration [10]. While biotin interferences have now been well described for hormones, including thyroid-stimulating hormone (TSH), free thyroxine (FT4), free triiodothyronine (FT3), parathyroid hormone (PTH), and 25-hydroxy vitamin D (25OHD), serological markers have received less attention in the literature, although several manufacturers have highlighted the risk of biotin interference in their package inserts [11]. Among these, human immunodeficiency virus- (HIV), hepatitis B virus- (HBV) and hepatitis C virus-related (HCV) serological markers are commonly prescribed to determine whether a patient has been infected by these

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viruses or has acquired immunity as a consequence of prior vaccination (HBV) [12,13]. The aim of this study was to evaluate the impact of biotin supplementation on serological misinterpretations and propose a solution to overcome this issue.

2. Material and methods

2.1. Healthy volunteers

Overall, 10 healthy volunteers (HVs) (four females and six males aged 24 to 48 years [median age = 27 years]) were asked to take a single dose of biotin (100 mg). Creatinine measurements were conducted using an enzymatic isotope dilution mass spectrometry traceable assay (Roche Cobas 8000 e502® module; Roche Diagnostics, Mannheim, Germany). All HVs had a normal (≥90 mL/min/1.73 m^2) glomerular filtration rate according to the CKD-EPI creatinine equation [14]. HVs were fasting at the various collection times and none were taking dietary supplements at the time of the experimentation. Each patient was vaccinated against HBV. HVs underwent blood sampling, as follows: (i) at baseline (prior to the intake of biotin); (ii) 1.5 h after the intake of biotin; (iii) 24 h after the intake of biotin (washout period). Blood was collected in serum-gel tubes (S Monovette® 7.5 mL tubes, Sarstedt, Nümbrecht, Germany). Samples were then centrifuged at room temperature (RT) for 10 min at 3500 rpm (Eppendorf® 5810 benchtop centrifuge, Hamburg, Germany), with the serum immediately stored at −80 °C until analysis.

2.2. Measurement principles of the serological markers

The impact of biotin intake on measured serological markers was assessed using the Roche Cobas 8000 e602® module, with all assays based on the streptavidin-biotin immobilization system. On this platform, sandwich (immunometric) assays use one detection antibody labeled with ruthenium complex (which produces the chemiluminescent signal to be measured) and one capture antibody labeled with biotin. Once the analyte has bound to the antibodies, the complex binds to the streptavidin-coated microparticles due to the biotin-labeled antibody. The signal generated is directly proportional to the analyte concentration. As a result, an excess of exogenous biotin reduces the binding of the antibody complex to the latter microparticles, leading to a reduced signal and falsely decreased results. Conversely, for competitive assays, ruthenium-labeled antigen competes with the analyte to bind biotinylated antibodies, which are thereafter fixed by the streptavidin microparticles. The signal thus generated is inversely proportional to the analyte concentration. Consequently, exogenous biotin will directly bind the streptavidin-coated microparticles and thus reduce the generated signal by preventing the interaction of the ruthenium-labeled antigens (falsely elevated results) [8].

2.3. Quantification of hepatitis B-related serological markers

Once thawed, anti-hepatitis B surface antibody (anti-HBs), anti-hepatitis B core total antibody (anti-HBc), and anti-hepatitis B e-antibody (anti-HBe) levels were measured. Since anti-HBs antibodies are measured using a sandwich immunoassay, it was expected that biotin ingestion would lead to a decrease in the anti-HBs signal in vaccinated HVs [5]. Unlike anti-HBs, anti-HBc and anti-HBe antibodies, presumed to be absent in HVs, are measured with a competitive immunoassay. Biotin ingestion therefore has the potential to lead to false positive results [5]. For the latter, the signal is inversely proportional to the concentration of the antibodies, with a result below 1 relative unit (RU) considered to be positive. RU values are obtained by dividing the sample signal by the manufacturer's cutoff signal.

2.4. Biotin spiking experiments and anti-HIV/anti-HCV measurements

For serological markers measured using an immunometric assay, which are expected to be negative in HVs, a spiking experiment was performed. For that purpose, 12 residual sera of patients who were positive for anti-HIV antibodies, HIV-1 p24 antigen (n = 6), or anti-HCV antibodies (n = 6) were used. The biotin (Fagron NV, Nazareth, Belgium; Lot No. 18G03-B01-357651, purity: > 99.5%) solutions' preparation was adapted from the protocol previously described by Trambas et al. [15]. Briefly, a biotin stock solution (1 g/mL) in 0.01 M NaOH was prepared and stored at 4 °C for one day. The stock solution was then diluted in distilled water to achieve peak biotin concentrations of 90.6 ng/mL, 494.9 ng/mL and 823.8 ng/mL corresponding to the intake of 10 mg, 100 mg or 300 mg of biotin, respectively [16,17]. The spiked volume was minimized to 5% of the final volume, with control serum samples spiked with an equal volume of distilled water. After spiking and mixing procedures, the measurements were performed using the Roche Cobas 8000 e602® analyzer.

2.5. Biotin neutralization protocols: Proof of concept with anti-HBs measurement

In HVs' samples, streptavidin bead treatment, adapted from the procedure previously described by Piketty et al., was used to overcome the biotin interference [18]. Briefly, five volumes of streptavidin reagent (0.72 mg/mL in HEPES-bovine serum albumin buffer, pH 7.4; Roche Diagnostics®) were centrifuged at 3500 rpm, and the supernatant was discarded. Thereafter, one volume of serum was added to the streptavidin particles and incubated for one hour at RT under continuous agitation (rotamix, Ingenieurbüro CAT GmbH, Ballrechten-Dottingen, Germany). Finally, streptavidin beads were removed by centrifugation (10 min at 3500 rpm); treated sera were retested using the Cobas 8000® analyzer.

Collected samples were measured using three alternative analyzers that did not employ the streptavidin-biotin immobilization system for anti-HBs measurement, namely the Vitros 5600® (Ortho Diagnostics®, New Jersey, USA), Architect i2000® (Abbott®, Illinois, USA), and Liaison XL® (DiaSorin, Saluggia, Italy) analyzers. Bias (%) from baseline was calculated as follows: [bias = (value at specific condition – baseline value)/(baseline value) * 100]. Values superior to 1000 mIU/mL were diluted exclusively for measurements performed on the Cobas 8000® platform to accurately assess the effect of biotin intake. In other cases, values above 1000 or below 2 mIU/mL were rounded up to the latter values.

The Wilcoxon matched-pairs signed rank test, with a significance level set at 0.05, was conducted to assess the effect of biotin ingestion on each platform. GraphPad Prism 6.0e (San Diego, California, USA) was employed for all statistical analyses. Our study fulfilled the ethical principles set out by the Declaration of Helsinki, and all HVs signed an informed consent form.

3. Results

3.1. Impact of biotin ingestion on hepatitis B-related serological markers

As expected, all HVs were negative for anti-HBe and anti-HBc antibodies at baseline (Table 1), with anti-HBs levels > 10 mIU/mL on the Cobas 8000® and Liaison XL® platforms, corresponding to a vaccinal immunity status against HBV (Tables 1 and 2) [19]. However, one HV displayed lower anti-HBs levels when measured on the Architect® and Vitros® analyzers (6.0 and 8.1 mIU/mL, respectively). Values on the Cobas 8000® and Liaison XL® analyzers were near the vaccinal status cutoff (10.8 and 11.8 mIU/mL, respectively) (Table 2), while variability between manufacturers has already been reported for anti-HBs measurements [20].

Biotin ingestion significantly affected anti-HBs (p = 0.008), anti-

Table 1
Impact of biotin supplementation on anti-HBs, anti-HBc and anti-HBe measurements.

HVs	Anti-HBs (mIU/mL)			Anti-HBc (Cut-off: 1.00 RU)			Anti-HBe (Cut-off: 1.00 RU)		
	Baseline	Post-biotin (1.5 h)	Wash-out (24 h)	Baseline	Post-biotin (1.5 h)	Wash-out (24 h)	Baseline	Post-biotin (1.5 h)	Wash-out (24 h)
1	37,300	13,500	26,500	2.05	1.02	2.01	1.69	1.33	1.74
2	168	15	142	2.28	0.30	2.22	1.63	0.38	1.48
3	439	84	316	2.28	0.50	1.89	1.58	0.61	1.67
4	52	7	41	2.09	0.45	1.65	1.77	0.78	1.81
5	10	< 2	6	2.04	0.16	1.80	1.62	0.24	1.75
6	17	< 2	13	2.08	0.15	1.83	1.63	0.21	1.81
7	1357	168	1180	2.25	0.30	1.99	1.71	0.44	1.79
8	37	3	32	2.12	0.35	1.94	1.71	0.49	1.74
9	28,120	4210	22,000	1.93	0.63	1.67	1.75	1.24	1.81
10	1850	251	801	2.05	0.62	1.96	1.64	0.87	1.89

Post-biotin serum samples were collected 1.5 h and wash-out samples 24 h after biotin intake. A positive sample for anti-HBc or anti-HBe is defined by a measured level lower than 1.0 RU. These results applied to the Cobas 8000® analyzer.
RU: relative unit; HV: healthy volunteer.

HBc ($p = 0.002$), and anti-HBe ($p = 0.002$) levels on the Cobas 8000® analyzer. For anti-HBs, in four HVs, values < 10.0 mIU/mL were reached (Fig. 1 and Table 3). The maximal bias observed was −93.4% (from 37.9 to 2.5 mIU/mL). Following the 24-hour washout period, nine of the ten HVs showed values > 10.0 mIU/mL (Table 1**).** Therefore, one HV presented a value that was still under the immunological cutoff (10.8 to 6.3 mIU/mL; bias: −41.6%).

For anti-HBc and anti-HBe measurements, biotin ingestion caused a drop in the signals measured in nine (90%) and eight (80%) cases, respectively. All results returned to levels corresponding to baseline values (values higher than 1 RU) following the 24-hour washout period (Table 1). The calculated bias between baseline and 1.5-hour post-biotin values ranged from −50.2% to −92.6%, with a median of −81.2% for anti-HBc and from −21.3% to −87.1% with a median of −66.2% for anti-HBe. Following the washout procedure, a negative bias was observed for all anti-HBc dosages (−2.0% to −21%; median −11.7%), whereas this was not the case for anti-HBe (median bias: +4.0%).

Streptavidin bead treatment was able to overcome the biotin interference in anti-HBs measurement (Fig. 1). This procedure showed little impact on our baseline samples results (free from biotin) when measured with the Cobas 8000® analyzer (bias ranging from −12.4% to 11.5%). Finally, anti-HBs levels on the Architect®, Vitros® and Liaison XL® analyzers were not statistically affected by the intake of biotin supplementation ($p > 0.05$) (Table 2).

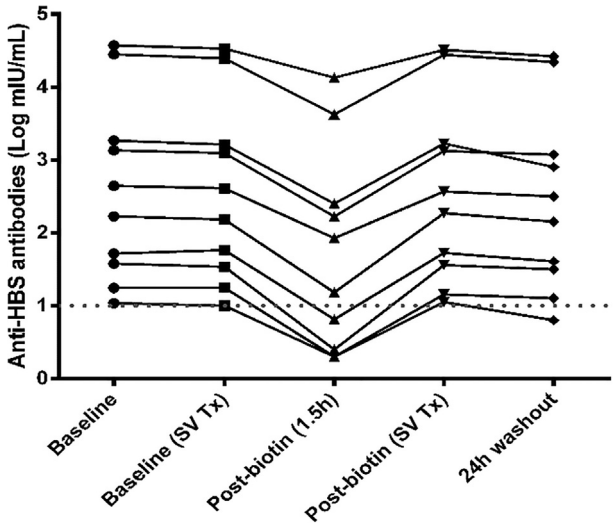

Fig. 1. Evolution of anti-HBs levels (Log mIU/mL) in 10 HVs under various conditions. SV Tx: Streptavidin beads treatment. Values below the limit of quantification (2.00 mIU/mL) were rounded to this latter value. Dotted lines represent the 10 mIU/mL cut-off.

3.2. Impact of biotin spiking on measurement of anti-HIV and anti-HCV antibodies

At 90.6 ng/mL, a limited but systematic negative bias was observed for anti-HIV/p24 measurement (median bias: −15.8%). This impact was likely higher for anti-HCV antibodies (median bias: −49.1%), with one sample passing beyond the positive threshold (1.84 to 0.96 RU). At 494.9 ng/mL, both anti-HIV/p24 and anti-HCV results were highly impacted with median bias of −94.2% and −98.2%, respectively. This concentration led to three HIV and four HCV false negative cases, respectively. Finally, at 823.8 ng/mL, measured indexes were still lower

Table 2
Measured anti-HBs values from 10 healthy volunteers on four different analyzers.

HVs	*Cobas®*		*Liaison XL®*		*Architect®*		*Ortho Vitros®*	
mIU/mL	Baseline	Biotin	Baseline	Biotin	Baseline	Biotin	Baseline	Biotin
1	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000
2	168	15	293	316	241	229	144	147
3	439	85	558	509	260	247	360	345
4	52	7	42	39	33	31	24	22
5	11	< 2	12	14	6	7	8	7
6	18	< 2	29	31	16	16	12	14
7	> 1000	168	> 1000	> 1000	> 1000	> 1000	870	840
8	38	3	32	37	27	26	14	15
9	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000
10	> 1000	251	> 1000	> 1000	768	750	712	717

The second column of each assay (namely “Biotin”) corresponds to the results obtained 1.5-hour after ingestion of 100 mg of biotin.

Table 3
Evolution of anti-HBs levels in 10 healthy volunteers under various conditions.

HVs	*Baseline*	*Baseline (SV Tx)*	*Post-biotin*	*Post-biotin (SV Tx)*	*24 h wash-out*
1	37,300	33,800	13,500	32,400	26,500
2	168	153	15	186	142
3	439	410	84	370	316
4	52	58	7	53	41
5	10	10	< 2	11	6
6	17	18	< 2	14	13
7	1357	1240	168	1328	1180
8	37	34	3	36	32
9	28,120	24,800	4210	27,800	22,000
10	1850	1620	251	1680	801

Results obtained at baseline, 1.5 h after biotin ingestion and 24 h after the ingestion. Impact of streptavidin beads treatment (SV Tx) on baseline and post-ingestion values are also presented. These results applied to the Cobas 8000® analyzer. Units: mIU/mL.

for both anti-HIV/p24 (median bias: −96.0%) and anti-HCV (median bias: −99.1%). At this concentration, all initially HCV-positive samples became negative (Fig. 2 and Supplementary Table 1).

4. Discussion

Interference caused by biotin ingestion has now been widely reported, with several clinical consequences [10]. Commonly affected parameters are cardiac markers (Troponin T and I) and hormone parameters, such as TSH, FT4, FT3, 25OHD, and PTH [6,7,21–23]. Herein, we have demonstrated that biotin supplementation may lead to clinically relevant interferences concerning HBV-, HCV-, or HIV-related serological markers.

Four of the ten HVs had anti-HBs levels below the 10 mIU/mL cutoff following biotin intake, which may lead to an unnecessary booster dose vaccination program in clinical practice. Furthermore, eight and nine of the ten HVs showed false positive results following biotin ingestion for anti-HBe and anti-HBc, respectively. These results are likewise a matter of concern, given that they may result in unnecessary clinical investigations, with their additional costs, and can cause anxiety for the patient.

Accurate anti-HBs quantification is mandatory since non-responder or low-responder patients (with anti-HBs levels < 10 mIU/mL) usually require a special regimen consisting of an additional vaccine dose [19,24]. Conversely, when a viral infection occurs, high anti-HBc titers are produced along with anti-HBe antibodies, which are never seen without anti-HBc. These high titers offer additional information about the disease activity in chronic hepatitis cases [12].

Supra-physiologic biotin supplementation is used in various conditions, such as hair and skin problems, inborn errors of metabolism, mitochondrial disorders, and multiple sclerosis (up to 300 mg per day for this indication) or in the aim to alleviate muscle cramps in hemodialysis patients [2,3,25–28]. These patients are likely to undergo virological evaluations. Of note, dialyzed patients are of particular concern since they are at a higher risk of HBV infection or reactivation and significantly less responsive to the vaccine, exhibiting faster decline of the anti-HBs titers following vaccination [24,29–31].

Moreover, our spiking experiment revealed that the average peak serum concentration expected from a 100 mg dose of biotin proved to be sufficient to lead to false negative HIV and HCV serology results [17]. This impact on HIV and HCV markers is of concern for several clinical situations, such as virological screening of blood and organ donors or testing of individuals with high-risk behavior. Furthermore, unrecognized HIV or HCV infection increases the risk of transmission [32].

However, only little data is available on the impact of biotin ingestion on virological parameters [10]. One case series involving multiple sclerosis patients taking 300 mg of biotin reported results in line with our data concerning anti-HBs, anti-HBc, and anti-HCV antibodies [33]. Other teams reported insensitivity of the anti-HAV Vitros® assay or a reduction of up to 50% in measured HBs antigen (HBsAg) levels on a Sysmex immunoassay platform (Kobe, Japan) when a patient's serum was spiked with biotin at 100 ng/mL [34,35]. To the best of our knowledge, no data pertaining to HIV serology have so far been published.

The spiking methodology has been applied in several interference studies [23,36]. In these *in vitro* studies, the interference observed is expected to be exclusively due to biotin. These studies do not take into account biotin metabolites, such as bisnorbiotin, while these metabolites are also able to interfere with the assay, yet to a lesser extent [18]. The use of spiking samples in our study can therefore be considered as a limitation. However, since anti-HIV antibodies (and/or HIV-1 p24 Ag) and anti-HCV antibodies are negative in HVs and are immunometric assays. An *in vivo* study would, however, require infected patients to take biotin, which might be difficult for practical and ethical reasons. Therefore, there is no doubt that biotin spiking experiments are pertinent in order to easily and rapidly investigate the scope of biotin interference. Consquently, both approaches (*in vivo* and *in vitro*) should be considered relevant.

The magnitude of biotin interference correlates with serum biotin concentrations. Moreover, an impact on some analyzers has already been reported at concentrations below 5.0 ng/mL when biotin was measured with a microbiological growth assay [22,37]. Such levels are easily encountered, even at trough levels, in people taking over-the-counter supplements and following 10 mg per day regimens [16,22]. Finally, the concordance between biotin quantification kits requires

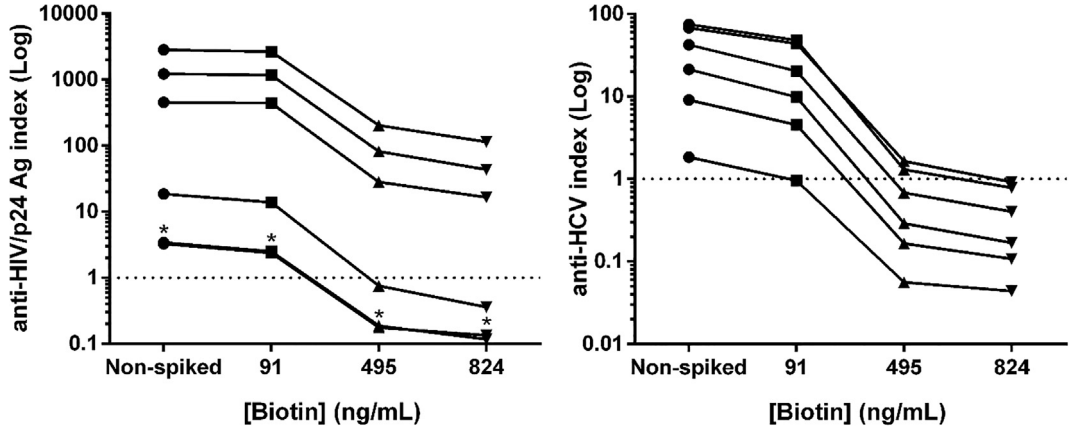

Fig. 2. Impact of biotin spiking on anti-HIV/p24 Ag and anti-HCV results. Dotted lines represent the cut-off index. *: values from two distinct samples, almost identical.

further investigation, whereas the comparison of study results should be avoided. Indeed, microbiological assays or ELISA, which are employed in some studies, are not comparable to liquid-chromatography coupled with mass spectrometry (LC-MS-MS) methods [38,39]. For these reasons and because it was clear that biotin was the etiology of our interference, we have estimated that biotin quantification was less relevant, as stated by the SIBioC working group on biotin interference (stage D recommendation) [40].

In this study, we chose to administer one unique high dose (100 mg) of biotin, likely to reach high serum levels (near to 500 ng/mL) [17]. Further studies are needed to investigate the interference extent at lower doses and following chronic supplementation under real-life conditions. The interference in cases of renal failure should likewise be investigated, given that biotin accumulation could be observed [16,17,41]. Of note, our spiking experiment suggests that an impact has occurred for anti-HIV and anti-HCV at peak concentrations following 10 mg administration.

To ensure that biotin concentrations are below the interference threshold, several manufacturers recommend a washout period following the last administration (*i.e.,* eight hours after the intake of more than 5 mg biotin according to Roche Diagnostics). While this washout procedure is a valuable option, no consensus has yet been reached on the washout period required. Indeed, taking into account biotin's long half-life (15 h), even a 24-hour washout period may not be sufficient in highly dosed supplementations, especially in the presence of renal failure, as explained above [41]. In our protocol, 24-hour post-ingestion levels were still affected, as reflected by constant lower anti-HBs and anti-HBc values. Utilization of non-biotinylated immunoassays or biotin purification processes, such as streptavidin bead treatment, is therefore preferred in practice. Indeed, the latter methodology can be easily used to rule out biotin interference [18]. It should, however, be kept in mind that variations in measured analyte levels may occur, with validation of such a process needed before implementation. These streptavidin beads can be recycled from the manufacturer's reagents and are effective in overcoming interference due to anti-streptavidin antibodies [42].

In this study, the only analyzer involving the streptavidin-biotin immobilization system for the anti-HBs quantification was the Cobas 8000®. Therefore, anti-HBs levels measured with the Liaison XL®, Architect®, or Ortho Vitros® analyzer were, as expected, not affected by biotin intake ($p > 0.05$). While the Architect® platform never uses the streptavidin-biotin immobilization process, it is important to keep in mind that some assays performed on the Ortho Vitros® (*i.e.,* anti-HBe antibodies, HBs or HBe antigens) and LiaisonXL® analyzers (*i.e.*, anti-HIV/p24 and anti-HCV antibodies) rely on this methodology [5]. Users should thus be careful when utilizing the latter platform in documented biotin intake cases, as the manufacturer only ensures the absence of interference in serum concentrations up to 10 ng/mL (anti-HCV antibodies) and 2.5 ng/mL (anti-HIV antibodies/p24 antigen).

Prevention of biotin interference cases begins outside the laboratories. Indeed, for any non-urgent requests, every time a daily (and superior to 5 mg per day) biotin intake has been documented, a 48-hour washout period is the simplest and safest way to avoid major interference in normal renal function patients. In case of urgent requests or undocumented intake with suspected interference, measurements on an analyzer that does not employ the biotin-streptavidin immobilization process should be preferred. If not available, the streptavidin bead treatment possibly represents a valuable alternative, but this technique needs to be validated locally before implementation.

5. Conclusion

Biotin proves to be a matter of concern for laboratory medicine. This study confirms that biotin affects virological parameters and could thus lead to misdiagnosis with potentially serious consequences for patients, their partners, or both. Clinical biologists and, particularly, manufacturers are now attempting to find feasible and rapid solutions to overcome this significant problem.

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CRediT authorship contribution statement

Jean-Louis Bayart: Conceptualization, Methodology, Investigation, Writing - original draft, Writing - review & editing. **Julien Favresse:** Conceptualization, Methodology, Writing - review & editing. **Anke Stoefs:** Investigation. **Mélanie Closset:** Investigation. **Tatiana Roy:** Investigation. **Catherine Fillée:** Supervision. **Hector Rodriguez-Villalobos:** Supervision. **Benoît Kabamba-Mukadi:** Supervision. **Damien Gruson:** Supervision, Writing - review & editing.

Declaration of Competing Interest

None.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cca.2020.01.012.

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