Clinical Biochemistry 74 (2019) 1–11

Contents lists available at ScienceDirect

Clinical Biochemistry

journal homepage: www.elsevier.com/locate/clinbiochem

Review

Best practices in mitigating the risk of biotin interference with laboratory testing

Raffick Bowen[a,*], Raul Benavides[b], Jessica M. Colón-Franco[c], Brooke M. Katzman[d], Alagarraju Muthukumar[e], Hossein Sadrzadeh[f], Joely Straseski[g], Ursula Klause[h], Nam Tran[i]

[a] Stanford Health Care, 300 Pasteur Dr, Stanford, CA 94305, USA
[b] Baylor University Medical Center, 3500 Gaston Ave, Dallas, TX 75246, USA
[c] Cleveland Clinic, 9500 Euclid Avenue, Cleveland, OH 44195, USA
[d] Mayo Clinic, 200 1st St SW, Rochester, MN 55905, USA
[e] University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd, Dallas, TX 75390, USA
[f] University of Calgary, 2500 University Dr NW, Calgary, AB T2N 1N4, Canada
[g] University of Utah, Department of Pathology, 15 N Medical Dr Suite 1100, Salt Lake City, UT 84112, USA
[h] Roche Diagnostics, 9115 Hague Rd, Indianapolis, IN 46256, USA
[i] UC Davis Health, 4610 X St, Sacramento, CA 95817, USA

ARTICLE INFO

Keywords:
Biotin
Biotin interference
Biotin supplements
Immunoassay

ABSTRACT

Dietary biotin intake does not typically result in blood biotin concentrations that exceed interference thresholds for in vitro diagnostic tests. However, recent trends of high-dose biotin supplements and clinical trials of very high biotin doses for patients with multiple sclerosis have increased concerns about biotin interference with immunoassays. Estimates of the prevalence of high biotin intake vary, and patients may be unaware that they are taking biotin. Since 2016, 92 cases of suspected biotin interference have been reported to the US Food and Drug Administration. Immunoassays at greatest risk from biotin interference include thyroid and reproductive hormones, cardiac, and immunosuppressive drug tests. Several case studies have highlighted the challenge of biotin interference with thyroid hormone assays and the potential misdiagnosis of Graves' disease. Biotin interference should be suspected when immunoassay test results are inconsistent with clinical information; a clinically relevant biotin interference happens when the blood biotin concentration is high and the assay is sensitive to biotin. We propose a best practice workflow for laboratory scientists to evaluate discrepant immunoassay results, comprising: (1) serial dilution; (2) retesting after biotin clearance and/or repeat testing on an alternate platform; and (3) confirmation of the presence of biotin using depletion protocols or direct measurement of biotin concentrations. Efforts to increase awareness and avoid patient misdiagnosis should focus on improving guidance from manufacturers and educating patients, healthcare professionals, and laboratory staff. Best practice guidance for laboratory staff and healthcare professionals would also provide much-needed information on the prevention, detection, and management of biotin interference.

1. Introduction: immunoassay interference from biotin

1.1. Case study

The following case illustrates the issue of biotin interference with immunoassays in patients taking high supplemental biotin doses, and the steps that can be performed to identify such interference.

A 44-year-old female with a history of type II diabetes mellitus, hypertension, acute myelogenous leukemia (in remission), and secondary hypoadrenalism was diagnosed with Graves' disease and treated with radioactive iodine ablation therapy and levothyroxine. Within a year, the patient's thyroid-stimulating hormone (TSH) level began to normalize with a TSH concentration of 4.7 mIU/L and free thyroxine (FT4) concentration of 1.4 ng/dL (assay reference intervals: TSH, 0.3–4.2 mIU/L; FT4, 0.9–1.7 ng/dL). During follow-up testing after 4 months, TSH dropped to 0.1 mIU/L and FT4 increased to 6.8 ng/dL.

Abbreviations: FDA, Food and Drug Administration; EMR, electronic medical record; FT4, free thyroxine; hCG, human chorionic gonadotropin; MAUDE, Manufacturer and User Facility Device Experience [database]; TSH, thyroid-stimulating hormone

* Corresponding author at: Department of Pathology, Stanford Health Care, Room H1401J, 300 Pasteur Drive, Stanford, CA 94305-5627, USA.
E-mail address: rbowen@stanfordhealthcare.org (R. Bowen).

https://doi.org/10.1016/j.clinbiochem.2019.08.012
Received 18 July 2019; Accepted 28 August 2019
Available online 29 August 2019

Her physician contacted the laboratory about these unexpected thyroid hormone test results. TSH results after serial dilutions were 1.1 mIU/L (×2); 3.4 mIU/L (×4); 5.1 mIU/L (×8); 5.8 mIU/L (×16); and 6.1 mIU/L (×32), indicating possible interference. Results on alternate platforms were TSH of 6.6 mIU/L and FT4 of 1.3 ng/dL. Treatment with a heterophile-blocking reagent did not significantly alter the results. The biotin concentration in the sample was found to be 240 ng/mL (Roche in-house method; not available for commercial use). The medication list in the patient's electronic medical record (EMR) did not list biotin; however, the patient confirmed she was taking biotin orally at a dose of 10 mg/day.

The objective of this review is to provide a summary of the current evidence on biotin interference, with the aim of increasing awareness of this issue. Best practice recommendations are also provided for suspected cases of biotin interference.

2. Biotin

Biotin is a naturally occurring vitamin found in foods such as eggs, pork, cereals, and leafy green vegetables and is also often found in multivitamin supplement preparations at low doses (e.g., 30–500 μg) [1,2]. It serves as an essential cofactor for a number of carboxylation reactions involved in the cellular metabolism of fatty acids, amino acids, and gluconeogenesis [3,4]. Biotin can exist in both free and protein-bound forms and is composed of a ureido ring, a tetrahydrothiophene ring, and a valeric acid side chain [5]. The recommended daily intake of biotin is 30 μg, which most people can easily obtain from their diet [6]. Therefore, biotin deficiency is extremely rare and is usually only observed in severely malnourished children or individuals with a profound biotinidase deficiency (incidence 1:112,271) [7–10]. Pregnant women are also potentially at risk, although the biotin deficiency tends to be marginal [11].

Biotin–streptavidin coupling is a common component of in vitro diagnostic tests, as the highly specific interaction of streptavidin with biotinylated antibodies aids in the detection and targeting of biologic analytes (Fig. 1) [12–21]. Biotinylation offers a number of advantages for use in the development of immunoassays: (1) the interaction between biotin and streptavidin is the strongest known non-covalent interaction between a protein and ligand; (2) the bond is formed rapidly and is resistant to extremes of temperature, pH, and denaturing agents; and (3) the relatively small size of the biotin molecule and the presence of a valeric side chain make it well suited for protein labeling. Unfortunately, biotin–streptavidin-based immunoassays are susceptible to interference from high blood biotin concentrations, which can result in patient misdiagnosis and/or inappropriate interventions if the interference is not detected [13,22–25]. Manufacturers that use biotin–streptavidin technology in some or all of their immunoassays include Beckman Coulter (Brea, CA, USA), Ortho-Clinical Diagnostics (Raritan, NJ, USA), Roche Diagnostics (Indianapolis, IN, USA), and Siemens Healthcare Diagnostics (Tarrytown, NJ, USA) [26].

Initially, biotin interference with immunoassays was thought to be rare, as immunoassay interference thresholds are considerably higher than blood biotin concentrations resulting from a dietary biotin intake. Estimates for normal plasma biotin concentrations vary (0.12–0.32 ng/mL [27]; 0.60 ± 0.15 ng/mL [12]), but are generally far lower than assay interference thresholds [28]. Pharmacologic uses of biotin were limited to treatments for biotin-responsive basal ganglia disease and some inherited metabolic diseases, such as biotinidase, multiple carboxylase, and holocarboxylase synthetase deficiencies [29,30]. However, the increased marketing of high-dose biotin up to 20 mg as a cosmetic hair, skin, and/or nail supplement [1,2], and recent European clinical trials of very high doses of biotin up to 300 mg/day for the treatment of multiple sclerosis [31–33], have raised concerns that the risk of biotin interference may be greater than previously thought.

Although biotin may offer some clinical benefits to patients with hair or nail disorders (e.g., brittle nail syndrome), there is limited evidence to support the use of biotin supplementation in healthy individuals [34–39]. The US Food and Drug Administration (FDA) regulates vitamin products and other dietary supplements once they have entered the marketplace [40–42]. However, unlike drug products, manufacturers of dietary supplements are only responsible for ensuring that the safety and labeling of their products meet FDA requirements; but the product efficacy does not need to be demonstrated prior to marketing. Concerns continue to be raised regarding the variability in quality and safety of dietary supplements, as well as the FDA's ability to effectively regulate these products [41,43,44].

2.1. Prevalence of elevated biotin

Estimates of biotin supplement use in the USA vary. In a study from the National Health and Nutrition Examination Survey, 29% of adults in the USA reported using biotin-containing supplements in 2011–12, with multivitamins containing biotin at or near to the recommended daily allowance accounting for the majority of cases [45]. A 2017 study of 1944 outpatients in the USA reported a lower prevalence of biotin use of 8% [1]. Retail sales of biotin supplements increased between 2014 and 2018 by 3.3–6.4% annually (9.7% overall) [46]. Comprehensive data are lacking on the biotin doses that the general population is taking, but retail sales data suggest that most consumers are taking biotin doses that pose a lower interference risk. The steadiest sales growth for the period 2014–17 was in biotin doses of ≤2.5 mg, while sales of 5 mg supplements declined [46].

One of the difficulties in obtaining accurate and reliable data on biotin supplement use is that healthcare professionals are less likely to be aware of patients taking biotin as a hair and/or nail supplement, compared with those who are under physician care and being prescribed biotin. Furthermore, biotin is sold under various commercial names and patients may not realize they are taking biotin or that it is relevant to report intake to their physician. In the USA outpatient survey described above, 5% of responders were unsure whether they were taking biotin [1].

Several studies have sought to quantify the prevalence of elevated biotin in the general public and patient populations. The prevalence of biotin concentrations > 20 ng/mL in 2023 routine samples from a USA commercial laboratory network was 0.74% [47]. In a study of 1442 patients who presented to a USA emergency department, 7.4% had biotin concentrations ≥10 ng/mL [1]. A similar study of biotin prevalence in an Australian emergency department population found that the concentration of biotin was < 1 ng/mL in 98% of samples and < 5 ng/mL in 99%; only four samples contained biotin concentrations exceeding 10 ng/mL [48].

The relationship between biotin intake and blood biotin concentration is an important consideration. Peak blood biotin concentrations occur 1–2 h after biotin ingestion and then rapidly decrease [49]. In healthy volunteers, median (minimum–maximum) peak serum biotin concentrations 1 h after the ingestion of 5, 10, and 20 mg biotin were 41 (10–73), 91 (53–141), and 184 (80–355) ng/mL, respectively (Fig. 2) [50]. Simulations based on characterization of biotin pharmacokinetics in these healthy individuals showed that for individuals who received biotin doses of 1–20 mg once daily for 5 days, the time taken to fall below a serum biotin concentration of 10 and 30 ng/mL ranged from 1.5–73 and 0–31 h, respectively [50]. Maximum plasma biotin concentrations following a single 300 mg biotin dose have been shown to be < 1200 ng/mL [13].

2.2. Impact of biotin interference with diagnostic assays

The risk of patient misclassification due to biotin interference can vary considerably between assays, and is dependent on the biotin and analyte concentrations present in the sample, as well as the specific assay in question. All biotin–streptavidin-based immunoassays are susceptible to biotin interference. Depending on the biotin dose, assay

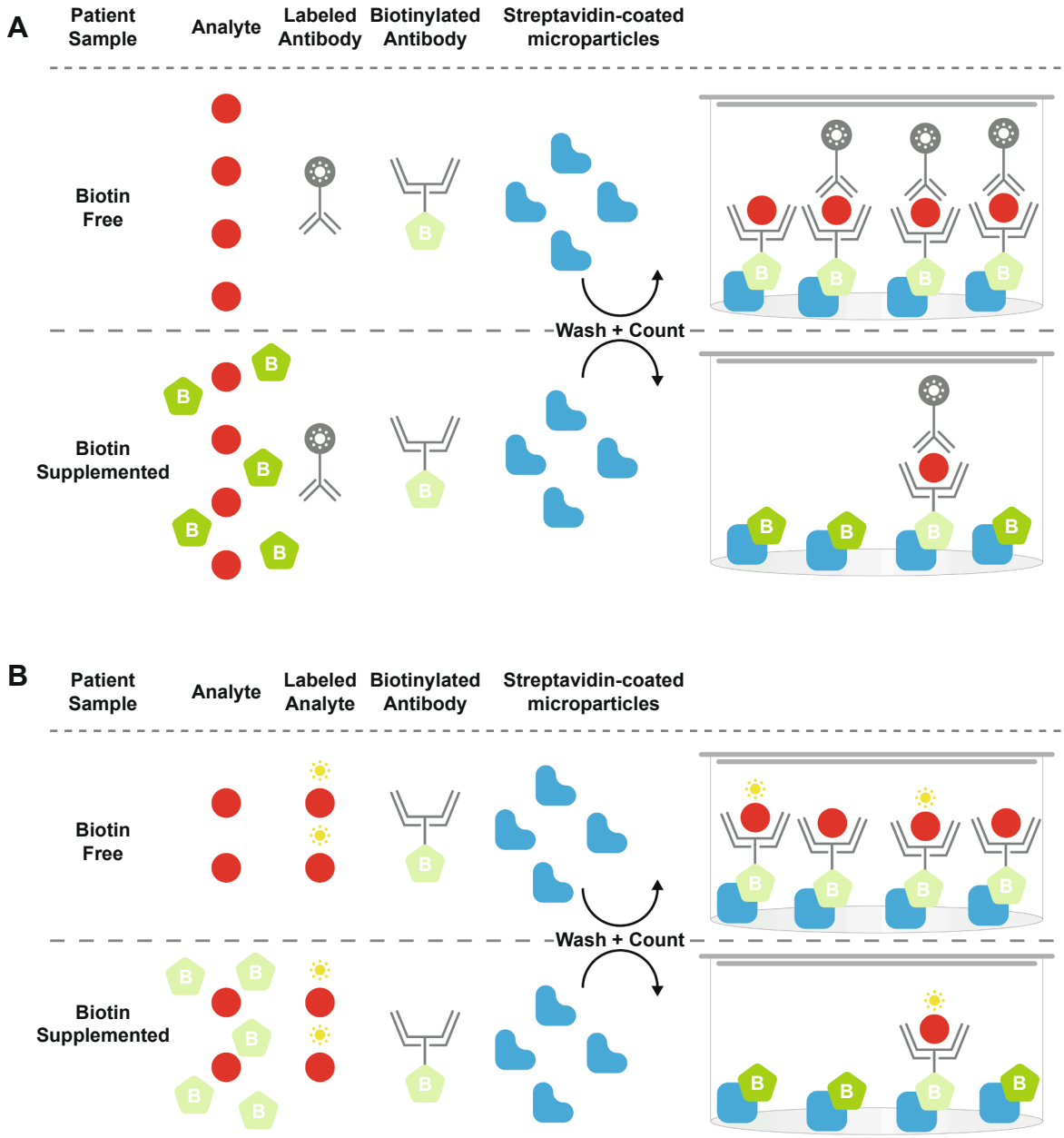

Fig. 1. Mechanism of biotin interference in (A) sandwich and (B) competitive immunoassays [2]. Reprinted from Endocrine Practice, Vol 23. Samarasinghe, et al, Biotin interference with routine clinical immunoassays: understand the causes and mitigate the risks. 989–998, ©2019, with permission from the American Association of Clinical Endocrinologists.

design, and assay interference threshold, biotin interference can cause falsely low (sandwich immunoassays) or falsely high (competitive immunoassays) results (Fig. 1) [2,26]. In sandwich (or non-competitive) immunoassays, the analyte is bound by the signal and biotinylated antibodies; a biotinylated antibody then links the analyte–antibody sandwich complex to a streptavidin-coated solid phase [26]. The signal increases as the analyte concentration increases. In the presence of high biotin concentrations, excess biotin saturates the streptavidin binding sites, preventing linking with the analyte–antibody sandwich complex and leading to falsely low assay results. In competitive immunoassays, endogenous and labeled (signal) analytes compete for a single biotinylated antibody binding site; the biotinylated antibody is then bound to the streptavidin-coated solid phase. The signal decreases as the analyte concentration increases. In the presence of high biotin concentrations, excess biotin binds to the solid phase and prevents the binding of antibody-endogenous and labeled analytes and unbound antibodies are removed in the wash step, leading to a falsely decreased signal and thus falsely high assay results.

Investigations performed with spiked samples and studies in healthy volunteers have demonstrated biotin interference affecting thyroid, reproductive hormones, cardiac, and immunosuppressive drug assays [51–58]. Notably, some manufacturers report conservative claims for biotin interference thresholds (i.e., in assay package inserts), and it is important for laboratories to verify a manufacturer's tolerance limits through replicate studies. There are several examples where independent studies have confirmed much higher interference thresholds [28,59], which may be due to differences in the experimental designs used to determine the interference thresholds. In a study of multiple manufacturer assays with varying thresholds for biotin interference, 5/8 biotinylated competitive immunoassays tested falsely high and 4/15 biotinylated sandwich immunoassays tested falsely low after a daily administration of 10 mg biotin for 7 days and blood sampling at peak

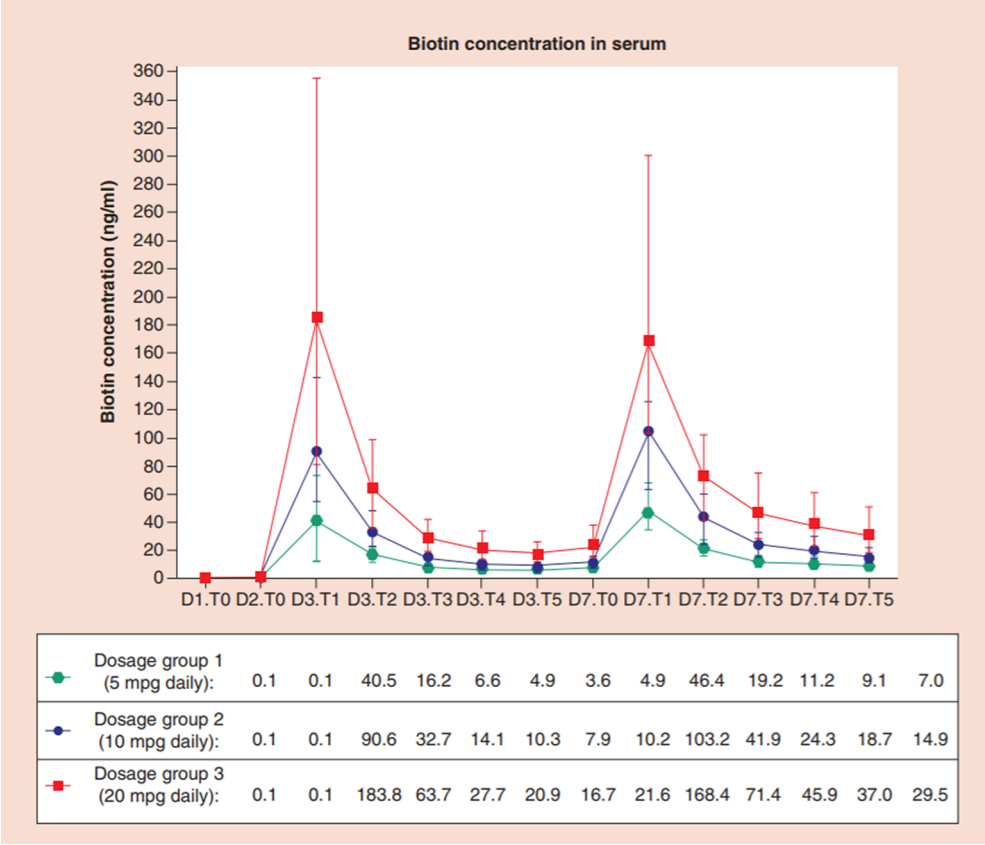

Fig. 2. Median serum biotin concentrations following daily 5, 10, and 20 mg biotin dosing in healthy individuals [50]. Reprinted from P. Grimsey, et al, Population pharmacokinetics of exogenous biotin and the relationship between biotin serum levels and in vitro immunoassay interference, Int. J. Pharm. 2 (2017) 247–256.

biotin concentration [56]. A study of seven qualitative point-of-care human chorionic gonadotropin (hCG) test devices for urine found that only one device was affected by biotin interference [57]. Cardiac troponin assays have a relatively low biotin interference threshold compared with other analytes. This is clinically relevant, as cardiac troponin concentrations are key to the differential diagnosis of acute coronary syndrome, and falsely low results could result in false-negative prediction of acute myocardial infarction [60,61]. In a study of 850 patients who presented to USA emergency departments with suspected acute coronary syndrome, biotin concentrations > 20 ng/mL were observed in 1/797 (0.13%) 0-hour samples and 1/646 (0.15%) 3-hour samples from the same patient [47]. Biotin interference has also been shown to affect the luminescent oxygen challenging technology-based digoxin assay (Siemens Healthcare Diagnostics, Tarrytown, NY, USA) [58].

Biotin interference is a particular issue with thyroid hormone assays (e.g., free triiodothyronine [T3], FT4, and TSH), with several case studies reporting the misdiagnosis of Graves' disease, an autoimmune disease of the thyroid gland (Table 1) [23–25,49,62–68]; five of these cases were patients with multiple sclerosis [23,24,62,64,68]. Moreover, many of the reports were published within the previous 3 years, suggesting an increased awareness of this issue. In these cases, biotin interference was identified because the thyroid function test results were not consistent with the clinical picture (e.g., elevated thyroid hormones and suppressed TSH, but no typical symptoms of hyperthyroidism and an unremarkable physical examination). Despite altered biotin clearance in patients with kidney disease, there is limited evidence of biotin interference in this patient group [69,70].

Cases of suspected biotin interference have also been submitted to the FDA's Manufacturer and User Facility Device Experience (MAUDE) database, which collates medical device reports submitted on a mandatory (e.g., manufacturers, importers, and device user facilities) and voluntary (e.g., healthcare professionals, patients, and consumers) basis. From 2016 until the time of writing (October 2018), 92 incidents of suspected biotin interference with various assays had been reported to the FDA. Biotin interference was deemed “most likely” or “not ruled out” in 78 of the 92 incidents [71]. Many of these cases also reported high biotin doses (100–300 mg daily) and biotin interference was most frequently reported among thyroid assays. However, a proportion of cases are likely to go unreported. There are several reasons why it can be challenging for laboratories to confirm interference. It can be difficult to identify blood samples containing biotin and limited methods are available for the quantitation of high biotin concentrations, which can make it difficult to determine if an interference threshold has been exceeded. Adequate troubleshooting of suspected interference can be cost or time prohibitive and may not be feasible at most hospitals; specimen stability and volume issues may also prevent follow-up testing. Furthermore, biotin interference must first be recognized by clinicians, and samples may be discarded before an investigation can proceed due to the length of time taken for an unexpected finding to be flagged to the laboratory. Therefore, the MAUDE database is likely to underrepresent the true number of biotin interference cases. To increase awareness and aid the evaluation of biotin interference risk, an FDA safety communication directed all assay manufacturers and developers who use biotin technology to communicate their interference thresholds to their customers [72].

Biotin interference thresholds can vary substantially between different manufacturers; for example, biotin interference thresholds for cardiac troponin assays range from 2.5–10,000 ng/mL [51,73]. Table 2 presents commonly used assays and biotin interference thresholds as reported in package inserts. Some immunoassays exhibit greater sensitivity to biotin interference than others. A recent study of Roche

Table 1
Summary of case reports describing biotin interference with thyroid assays.

Reference	Gender, age[a], reason for biotin use if known	Biotin dose	Impact			Assay(s)	Clinical implication	Solution implemented
			TSH	FT3	FT4			
Kwok, [49]	Female, 3	10 mg QID	↓	↑	↑	NR	NR	Tests repeated on different assays and showed normal results
Wijeratne, [67]	Male, 42	30 mg single dose	–	↑	↑	Beckman DxI	None	Biotin medication discontinued, tests repeated and showed normal results
Barbesino, [23]	Male, 55; MS treatment	100 mg TDS	↓	↑	↑	Roche Elecsys® FT4 II, T3, TSH	123I thyroid scan	Biotin medication discontinued, tests repeated and showed normal results
Kummer, [66]	Female, 9; female, 2; male 2; male, 5 months; male, 1 month; male, 1 month. All receiving treatment for inherited metabolic diseases	10; 14; 15; 2; 7; 8 mg OD	↓	↑	↑	Roche	Antithyroid medication was initiated in at least three children	Biotin medication discontinued, tests repeated and showed normal results
Minkovsky, [64]	Female, 74; MS treatment	100 mg TDS	↓	↑	↑	Roche	123I thyroid scan	Biotin medication discontinued, tests repeated and showed normal results
Al-Salameh, [25]	Male, 32; X-linked adrenomyeloneuropathy treatment	100 mg TDS	↓	↑	↑	Roche Elecsys®, Siemens ADVIA Centaur XP, BRAHMS (TRAK)	Misdiagnosis of Graves' disease; prescribed antithyroid drug	Tests repeated on different assays and showed normal results
Ardabilygazir, [62]	Female, 49; MS treatment	200 mg OD	↓	–	↑	NR	None	Biotin medication discontinued, tests repeated and showed normal results
De Roeck, [24]	Female, 60; MS treatment	100 mg TDS	↓	↑	↑	NR	Misdiagnosis of Graves' disease	Normal results obtained by chemiluminescent microparticle immunoassays
Giovanella, [68]	Male, 55; MS treatment	300 mg OD	↓	↑	↑	UniCell DxI®800 Beckmann; Roche Elecsys®; Architect®i2000SR Abbott	Misdiagnosis suggestive of Graves's disease	Tests repeated following withdrawal of biotin supplementation showed normal results
Koehler, [63]	Male, 47	300 mg OD	↓	↑	↑	NR	Misdiagnosis suggestive of Graves' disease	Tests repeated following withdrawal of biotin supplementation showed normal results
Charles, [65]	Female, 78; Female, 69; Female, 84; Female, 66	300–1000 μg OD	↓	–	–	Vitros 5600	Misdiagnosis of hyperthyroidism	Tests repeated following withdrawal of biotin supplementation showed normal/ improved results

FT3, free triiodothyronine; FT4, free thyroxine; OD, once daily; MS, multiple sclerosis; NR, not reported; QID, four times daily; TDS, three times daily; TSH, thyroid-stimulating hormone.
[a] Age in years unless otherwise stated.

Table 2
Biotin interference thresholds of commonly used assays with known biotin interferences.

Assay[a]	Demonstrate ≤10% change in results up to concentration specified (i.e., interference threshold)
Reproductive endocrinology	
Abbott Architect i2000® Testosterone	29 ng/mL
Ortho VITROS® 5600 FSH	10 ng/mL
Ortho VITROS® 5600 LH	5.1 ng/mL
Ortho VITROS® 5600 Testosterone	10 ng/mL
Ortho VITROS® 5600 Progesterone	20 ng/mL
Elecsys® Progesterone III	30 ng/mL
Elecsys® Testosterone II	30 ng/mL
Elecsys® LH	60 ng/mL
Thyroid assays	
Beckman Coulter Access®/DXI® FT3	10 ng/mL
Ortho VITROS® 5600 TSH	5.1 ng/mL
Ortho VITROS® 5600 iPTH	4.9 ng/mL
Roche Elecsys® FT4 II	20 ng/mL
Roche Elecsys® TSH	25 ng/mL
Roche Elecsys® FT3 III	70 ng/mL
Siemens Dimension® TSH	492 ng/mL
Siemens Dimension® FT4	50 ng/mL
Siemens Dimension® FT3	50 ng/mL
Cardiac assays	
bioMérieux hs-cTnI (VIDAS)	2000 ng/mL
ET healthcare hs-cTnI (Pylon 3d)	200 ng/mL
Ortho-Clinical Diagnostics Troponin I ES (ECi/ECiQ 3600, 5600)	2.5 ng/mL
Radiometer POC TnI (AQT90 FLEX)	3 ng/mL[b]
Roche Diagnostics cTnT-hs and TnT Gen 5 STAT (MODULAR E170, cobas e 411, e 601, e 602, e 801)	21 ng/mL[c]
Siemens Healthineers High Sensitivity Troponin I [TNIH] (ADVIA Centaur® XP/XPT Systems; Atellica™ IM analyzer)	3500 ng/mL
Siemens Healthineers High Sensitivity Troponin I [TNIH] (Dimension® EXL™ System; Dimension Vista® System)	300 ng/mL
Siemens Healthineers TnI-Ultra (ADVIA Centaur® CP/XP/XPT Systems; Atellica™ IM analyzer)	10 ng/mL
Siemens Healthineers TNI (Dimension® EXL™ System)	100 ng/mL
Siemens Healthineers Troponin-I (IMMULITE® 2000/2000 XPi Systems; IMMULITE®/IMMULITE® 1000 Systems; IMMULITE® Turbo System)	1500 ng/mL
Singulex hs-cTnI (Clarity)	10000 ng/mL

Thresholds are reported according to assay package inserts. Note that this list is not exhaustive, and other assays (including laboratory developed tests) may have biotin interferences.

[a] Please note that it is important to check the country-specific regulatory status of all assays prior to use.

[b] Not validated; under further investigation.

[c] Assay has been reformulated (cTnT-hs*) with a higher biotin threshold [91].

Diagnostics assays demonstrated that, although most were minimally affected by biotin concentrations of 15.6 and 31.3 ng/mL (simulating biotin intakes of 5 and 10 mg, respectively; 5% bias observed), the cardiac Elecsys® Troponin T Gen 5 assay (Roche Diagnostics, Indianapolis, IN) and thyroid assays showed greater analytical bias (10% negative bias) at these concentrations [28]. A patient with both biotin and analyte concentrations in plasma that would lead to clinical misclassification, and an assay sensitive to biotin, are required for a clinically relevant interference to occur. For example, despite the relatively higher sensitivity to biotin interference reported for the Elecsys® Troponin T Gen 5 assay [28], the risk of false-negative acute myocardial infarction prediction due to biotin interference with this assay has been estimated to be extremely low (0.026%) [47]. This is considerably lower than the misclassification risk due to the assay's clinical performance (0.7%, based on a negative predictive value of 99.3% at 3 h) [74].

Immunoassays can also be affected by a wide range of other interferences, including hemolysis [75,76], lipemia [75], icterus [75], heterophile antibodies [77–79], drugs [80], and endogenous substances [81–83], with the prevalence varying according to the particular assay. Hemolysis is a leading cause of unsuitable specimens for analysis in clinical laboratories [76], and is likely to be more prevalent than biotin interference. Hemolysis rates among blood samples used for cardiac troponin I testing were reported as 5–8% in a Canadian hospital network [84]. In contrast, the prevalence of biotin concentrations > 20 ng/mL (biotin interference threshold for the Elecsys® Troponin T Gen 5 assay) in the assay's intended-use population was 0.13–0.15% [47]. In the context of ~1.2 million hospital admissions annually for acute coronary syndrome in the USA, this would translate into a substantial number of cases [85]. Furthermore, hemolysis is relatively easy for laboratory staff to identify, with visual charts and analyzer hemolysis indices being widely used to determine degrees of hemolysis and prevent the reporting of erroneous results. The frequency of interference from heterophile antibodies, which is more difficult to identify due to its unpredictability, is estimated to be 0.1–3.1% in cardiac troponin testing [86]. Therefore, a systematic approach should be adopted when evaluating discrepant results rather than focusing on a particular interferent; a detection algorithm has been proposed previously to facilitate the identification of assay interference [22]. The potential impact of biotin interference also needs to be evaluated in the context of an assay's analytical performance as imprecision may cause variations in assay results and can lead to patient misclassification.

3. Methods for detecting biotin interference

For any diagnosis, to obtain a complete picture it is important to consider test results in the context of the patient's clinical evaluation, as well as any diagnostic imaging. When test results suggest a diagnosis inconsistent with patient symptoms, interference should be suspected and appropriate investigations initiated. If biotin interference is

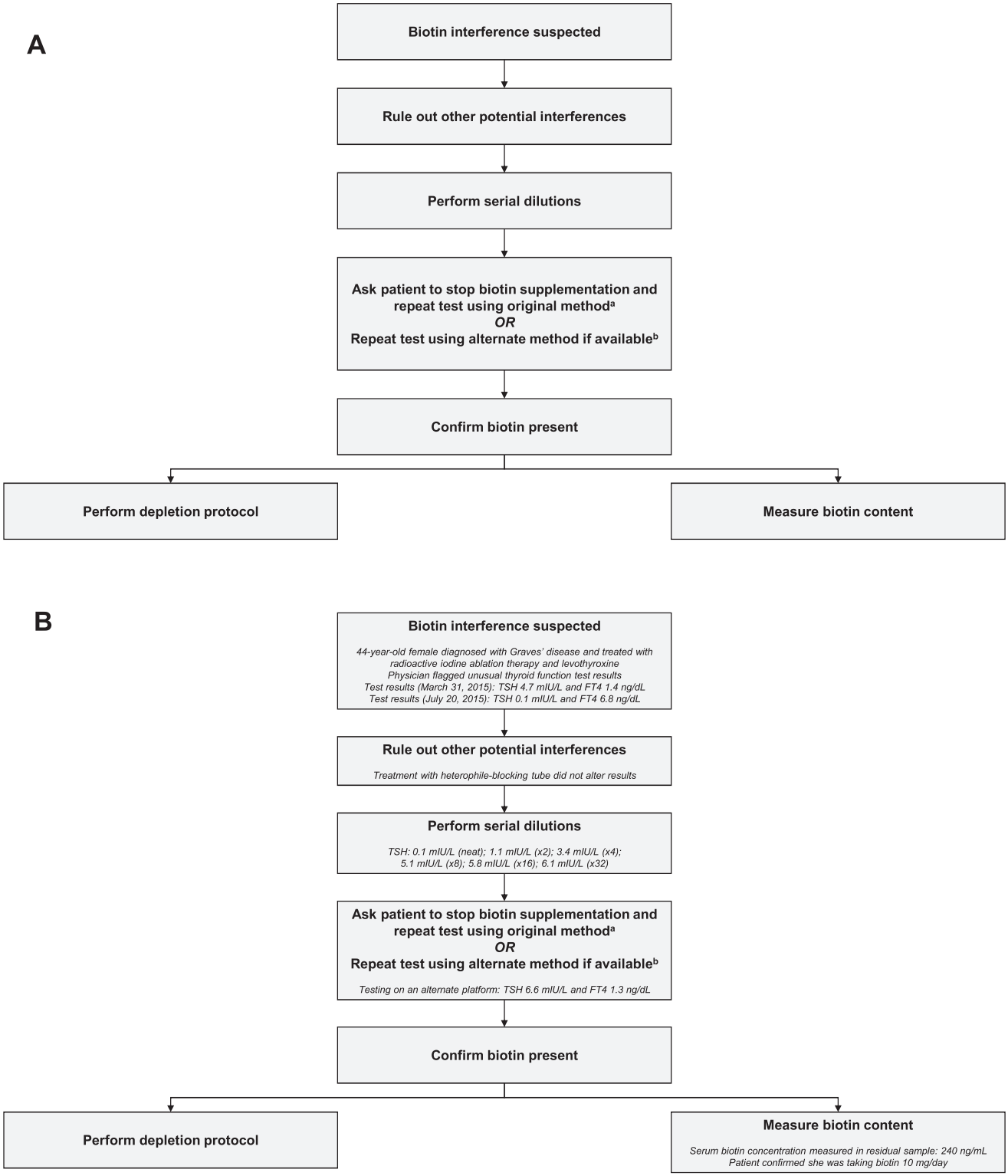

Fig. 3. (A) Suggested "best practices" workflow following suspected biotin interference and (B) using the workflow with an example case study.
[a]The interval between biotin supplement cessation and repeat testing is dependent on the biotin dose and assay interference threshold.
[b]The alternative method should not be susceptible to biotin interference and may require sending samples to regional or central reference laboratories, depending on available resources.
FT4, free thyroxine; TSH, thyroid-stimulating hormone.

suspected, biotin discontinuation and repeated analysis of test samples is recommended [23,64,66,67,87]. It is often appropriate to comprehensively review the patient's medical record and current medications for the presence of biotin or other interferents, and assess the direction of the suspected interference in the context of the assay format. Patient querying by their treating physician may also yield meaningful results. It is also important to recognize that apparent anomalies in assay results may be unrelated to biotin or other interferences, and could be a product of other analytical issues inherent to the test and/or analyte itself (i.e., analytical imprecision and biological variability).

Best practice recommendations following suspected biotin interference (summarized in Fig. 3) depend on sample availability and local resources. Typically, the first step is to perform a serial dilution; second, retesting is performed using a method that is not susceptible to biotin interference (either locally or at a central reference laboratory); and third, depletion protocols or direct assays for interferents are implemented. Details of these three approaches are outlined below.

3.1. Serial dilution study

A simple serial dilution study reduces the concentration of biotin and the analyte concentration adjusted for dilution will provide the correct result. A sample diluted serially is expected to recover according to the dilution factor. When an interferent is present, the recovery may not be linear. As the sample is diluted, so is the interferent, and thus interference decreases, providing a more reliable result. However, caution should be exercised when diluting free hormones or when overdiluting, which can lead to inaccurate results [88]. Additionally, when biotin levels in the sample are high, several serial dilutions may be required, which can be time consuming.

3.2. Repeating tests

Repeating tests after allowing sufficient time for biotin clearance in the patient and/or using alternative assays/methods (e.g., non-biotin–streptavidin-based immunoassays or liquid chromatography–tandem mass spectrometry). The former may not be feasible since patients may not be available upon recognition of potential biotin interference, while the latter usually involves sending samples to a reference laboratory for testing [25,49,89].

3.3. Depletion protocols

Depletion protocols, if available, involve the addition of materials to bind and remove any biotin in the sample prior to testing; for example, adding streptavidin agarose beads to the sample (10% sample volume) followed by incubation for 1 h with intermittent mixing. The sample is then centrifuged and the supernatant removed for testing. Biotin interference is indicated if the difference in the test results before and after the biotin block is significant. However, depletion protocols generally need thorough evaluation prior to implementation and, while the protocol may confirm the presence of biotin, results from streptavidin-treated samples should not be reported, but rather guide next steps on asking a patient to refrain from biotin and return for another blood draw. Piketty et al. found the use of streptavidin-coated microparticles to be effective in a study comprising 23 high-biotin samples from patients with multiple sclerosis and healthy volunteers receiving high-dose biotin and 39 biotin-unsupplemented patients [13]. Trambas et al. also implemented a depletion protocol using streptavidin-coated microparticles, which allowed the removal of biotin in samples from patients with multiple sclerosis receiving 300 mg/day biotin. Following depletion, previously affected Roche TSH, FT4, and free T3 levels returned to the biochemically euthyroid reference range [90].

It should be emphasized that the approaches described in Sections 3.1 and 3.2 are not exclusive tests for biotin interference and may demonstrate erroneous results due to other interferents (e.g., heterophile antibodies). Therefore, depletion protocols and biotin testing are recommended to confirm the presence of biotin. In practice, the avoidance of assays that use biotin–streptavidin technology may not be practical or necessary. A case in point is cardiac assays, for which timeliness requirements are important and there is often a limited availability of alternative methods within a laboratory. The approach described in 3.3 should be used only for troubleshooting, since depletion protocols are considered laboratory developed tests in the USA.

3.4. Potential technical solutions

Assay manufacturers continually seek ways to optimize assays and improve biotin interference thresholds. For example, Roche has developed a new cTnT assay (cTnT-hs*) with the aim of eliminating biotin interference (at the time of writing, not available in the US). The new assay showed substantially greater tolerance to biotin interference, with recoveries of ≥99% in the presence of biotin ≤500 ng/L and ≥96% for biotin ≤1250 ng/L. Furthermore, a method comparison using multiple platforms demonstrated high correlation of the cTnT-hs* assay with the predicate device, indicating that performance was not affected [91]. Clinical concordance with the predicate device was also high using the global (excluding USA) 14 ng/L cutoff, with 95.3% and 100% negative and positive percent agreements, respectively. Therefore, the cTnT-hs* could be used for patients with multiple sclerosis taking biotin up to 300 mg daily without any special precautions [91].

4. Raising awareness of biotin interference

In addition to the analytical strategies outlined above, several educational strategies aimed at increasing awareness of the risk posed by biotin interference on clinical immunoassays have been described for both healthcare professionals and patients [92]. Specifically, education is the “first line of defense” against biotin and other exogenous sources of testing interferences. Communications include newsletters/memos for healthcare professionals, presentations to specialist physician groups, posters/displays for patients, and website information for both healthcare professionals and patients. A number of online educational materials advise patients to check the labels of any supplements they are taking to see whether biotin is listed as an ingredient, and disclose this to their healthcare professional accordingly [93–96]. Healthcare professionals should actively ask patients about dietary supplement use and biotin intake, including dosages [97]. Recommendations for the period of time that patients should stop taking biotin supplements prior to the use of biotin–streptavidin-based assays vary widely in the literature, from 1 to 5 days [2,64,95,97]. Despite a lack of clear and consistent general guidance, assay package inserts may offer assay-specific advice. For example, the Roche Elecsys® TSH, FT3 III, and FT4 II assay package inserts state that samples should not be taken from patients receiving therapy with high biotin doses (i.e., > 5 mg/day) until at least 8 h after the last biotin administration.

To increase awareness and aid in the evaluation of biotin interference risk, an FDA safety communication includes advice for people taking or considering taking biotin, physicians, other healthcare providers who order laboratory tests, and laboratory staff. The FDA requires that manufacturers titrate biotin interference up to 1200 ng/mL, although this information is often missing from package inserts [98]. The International Federation of Clinical Chemistry has also published information detailing biotin interference thresholds for a range of cardiac troponin and natriuretic peptide assays, which can be used as a guide by laboratory staff (Table 2) [51,98]. Additionally, many manufacturers, laboratories, and healthcare providers have distributed bulletins to increase awareness of, and provide guidance regarding, biotin interference [65–67,99–101]. Several manufacturers have dedicated web resources with the aim of increasing awareness of this issue [94,102,103]. Some manufacturers have also prepared educational materials targeting healthcare professionals and patients, and referral laboratories have provided biotin interference information for laboratory staff and customers, including details regarding assays that are susceptible to interference [104].

Open communication between laboratory and clinical providers is essential to ensure that clinicians are aware of the potential for biotin interference, and that healthcare professionals can contact clinical laboratories for information regarding assays that may be affected by biotin interference. Accordingly, key laboratory contact information should be provided alongside assay results/request forms. Laboratory

Table 3
Recommendations for manufacturers, laboratory staff, healthcare providers, and patients to help reduce the risk of biotin interference with immunoassays.

Stakeholder	Recommendations
Manufacturers	• Determine biotin interference thresholds and include in product inserts. • Increase awareness of biotin interference and provide guidance via bulletins or online resources.
Laboratory staff	• Provide key contact information with assay results/request forms. • Advise healthcare providers on how to minimize the risk of inaccurate test results (see information for healthcare providers [below]). • Develop internal algorithms to investigate inaccurate test results.
Healthcare providers	• Ask patients about supplement/biotin use, including dosages. • Provide patient instructions to prepare for blood tests several days prior to appointment. • Contact laboratory if biotin interference suspected (e.g., test results that are not consistent with the clinical picture).
Patients	• Check supplement labels for biotin. • Report all supplement intake to healthcare provider. • Do not take biotin prior to undergoing blood tests.

staff can also advise clinicians on how to prepare patients and time blood draws to minimize the risk of inaccurate test results [2]. Patient instructions, including information on biotin interference, should be provided several days prior to an appointment.

It may be beneficial to implement a flagging procedure within health information systems (e.g., EMR) so that clinicians can alert laboratories to samples from patients taking high-dose biotin. Equally, a pop-up alert could be included in EMRs, alerting caregivers if biotin interference is relevant for a specific assay when ordering tests [2]. However, this approach is controversial, as warning comments may go unnoticed. A standard interpretive comment about biotin interference could also be added below patient results in EMR charts for affected biotinylated assays [64]. A summary of suggested recommendations for manufacturers, laboratory staff, healthcare providers, and patients to help reduce the risk of biotin interference with immunoassays is provided in Table 3.

5. Conclusions and future perspectives

An increasing number of people are now taking biotin supplements. When the doses are high, and if certain processes are not followed (e.g., a “washout” period prior to testing), biotin interference may affect immunoassays. This can cause both erroneous test results and false diagnoses. Biotin interference can potentially affect any immunoassay based on biotin–streptavidin technology, although most cases identified to date have been related to assays for thyroid function. Patients, healthcare professionals, and laboratory staff need to be aware of the potential risks of high-dose biotin and implement processes to prevent assay interference. The risk of false diagnosis can be mitigated by compliance with standard laboratory practice and guidelines, meticulous patient examinations, and efficient communication between laboratories, clinicians, and patients. Continuous improvement opportunities also exist, and ongoing efforts fostering collaboration between stakeholders will improve knowledge about laboratory testing as well as the understanding of concepts such as random error, imprecision, and standardization/harmonization [105]. However, universal best practice guidance (including alignment on when patients should stop taking biotin prior to a blood draw) is needed to enable laboratories and healthcare professionals to prevent, detect, and manage biotin interference. Herein, we presented a best practice workflow for laboratory staff to follow when biotin interference is suspected.

Additional information regarding the extent of biotin interference and further risk assessments would be beneficial. Therefore, research should continue to focus on the effects of high blood biotin concentrations on clinical immunoassays. Further research is also required to examine the accumulative effects of biotin in individuals taking biotin supplements over longer periods of time and in patients with chronic kidney disease (~14% of the USA population [106]), as limited data are currently available.

Funding

This work was supported by Roche Diagnostics. The sponsor was involved in the initiation of the review, the collection and interpretation of the data, and writing of the manuscript. All authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

Declaration of Competing Interest

Raffick Bowen – reports serving as an Advisory Committee member for Roche Diagnostics.

Raul Benavides – reports serving as an Advisory Committee member for Roche Diagnostics.

Jessica Colon-Franco – reports serving as an Advisory Committee member for Roche Diagnostics.

Brooke M. Katzman – reports serving as an Advisory Committee member for Roche Diagnostics.

Ursula Klause – employee of Roche Diagnostics.

Alagarraju Muthukumar – reports serving as an Advisory Committee member for Roche Diagnostics and non-compensated PI for studies funded by Abbott Diagnostics.

Hossein Sadrzadeh – reports serving as an Advisory Committee member for Roche Diagnostics.

Joely Straseski – reports serving as an Advisory Committee member for Roche Diagnostics.

Nam Tran – reports serving as an Advisory Committee member for Roche Diagnostics.

Acknowledgments

Medical writing support, under the guidance of the authors, was provided by Louise Kelly, BSc (Gardiner-Caldwell Communications, Macclesfield, UK) and funded by Roche Diagnostics International Ltd.

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