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Clinical risk assessment of biotin interference with a high-sensitivity cardiac troponin T assay

Short title: Biotin interference risk with high-sensitivity TnT

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Abstract

Background: Biotin >20.0 ng/mL (81.8 nmol/L) can reduce Elecsys® Troponin T Gen 5 (TnT Gen 5; Roche Diagnostics) assay recovery, potentially leading to false-negative results in patients with suspected acute myocardial infarction (AMI). We aimed to determine the prevalence of elevated biotin and AMI misclassification risk from biotin interference with the TnT Gen 5 assay.

Methods: Biotin was measured using an Elecsys assay in two cohorts: (i) 797 0-h and 646 3-h samples from 850 US emergency department patients with suspected acute coronary syndrome (ACS); (ii) 2023 random samples from a US laboratory network, in which biotin distributions were extrapolated for higher values using pharmacokinetic modeling. Biotin >20.0 ng/mL (81.8 nmol/L) prevalence and biotin 99th percentile values were calculated. AMI misclassification risk due to biotin interference with the TnT Gen 5 assay was modeled using different assay cutoffs and test timepoints.

Results: ACS cohort: 1/797 (0.13%) 0-h and 1/646 (0.15%) 3-h samples had biotin >20.0 ng/mL (81.8 nmol/L); 99th percentile biotin was 2.62 ng/mL (10.7 nmol/L; 0-h) and 2.38 ng/mL (9.74 nmol/L; 3-h). Using conservative assumptions, the likelihood of false-negative AMI prediction due to biotin interference was 0.026% (0-h result; 19 ng/L TnT Gen 5 assay cutoff). US laboratory cohort: 15/2023 (0.74%) samples had biotin

>20.0 ng/mL (81.8 nmol/L); 99th percentile biotin was 16.6 ng/mL (68.0 nmol/L). Misclassification risk due to biotin interference (19 ng/L TnT Gen 5 assay cutoff) was 0.025% (0-h), 0.0064% (1-h), 0.00048% (3-h), and <0.00001% (6-h).

Conclusions: Biotin interference has minimal impact on the TnT Gen 5 assay's clinical utility, and the likelihood of false-negative AMI prediction is extremely low.

Keywords: Acute myocardial infarction; biotin; high-sensitivity cardiac troponin T; false negative; immunoassay interference; risk of misclassification

List of abbreviations: ACS, acute coronary syndrome; AMI, acute myocardial infarction; CI, confidence interval; CLSI, Clinical and Laboratory Standards Institute; cTn, cardiac troponin; cTnI, cardiac troponin I; cTnT, cardiac troponin T; ISO, International Organization for Standardization; IVD, in-vitro diagnostic; MS, multiple sclerosis; NPV, negative predictive value; TnT Gen 5, Troponin T Gen 5

Introduction

Biotin is a water-soluble vitamin with an adult recommended adequate intake of 30 µg per day [1]. Biotin–streptavidin coupling has been used for decades by manufacturers of in-vitro diagnostic (IVD) devices to immobilize biotinylated proteins [2, 3]; however, these immunoassays are susceptible to interference from excessive blood biotin concentrations.

The biotin–streptavidin-based Elecsys® Troponin T Gen 5 (TnT Gen 5; marketed outside the United States [US] as Elecsys Troponin T-high sensitive; Roche Diagnostics International Ltd, Rotkreuz, Switzerland) assay provides a high negative predictive value (NPV; ≥99%) for ruling out acute myocardial infarction (AMI) [4–8]. Biotin concentrations >20.0 ng/mL (81.8 nmol/L) can reduce TnT Gen 5 assay recovery [9], which may lead to lower reported cardiac troponin T (cTnT) concentrations, and thus false-negative AMI prediction. However, the incidence of biotin interference and its clinical implications in the TnT Gen 5 assay intended-use population is unknown.

Until recently, immunoassay interference from biotin was considered extremely rare, as interference thresholds are considerably higher than blood concentrations associated with the recommended dietary biotin intake. However, very high biotin doses (up to 300 mg daily) have been used in clinical trials for treating multiple sclerosis (MS) and high-dose biotin supplements (up to 10 mg in single-ingredient preparations) have been marketed for cosmetic purposes, which may increase the risk of biotin interference [10–16]. In 2011–2012, 29% of US adults reported

using biotin-containing supplements [17]. In a 2017 US prevalence study, biotin use was reported by 7.7% of outpatients [18]. Biotin doses ranged from <1 to 50 mg; 47.0% of respondents reported taking ≤10 mg, and 44.9% did not know the dose they were taking or did not respond [18]. In the same study, 7.4% of emergency department patients had plasma biotin concentrations ≥10 ng/mL (40.9 nmol/L) [18]. Nielsen US retail sales data for July 2014 to June 2017 showed a slight increase in biotin sales. However, the data also suggest most consumers are taking biotin doses that pose a low interference risk, with the steadiest growth in ≤2.5 mg doses; sales of 5 mg biotin declined [19].

We aimed to determine the prevalence of elevated biotin concentrations and the associated patient misclassification risk due to biotin interference with the TnT Gen 5 assay in the US intended-use population. We also performed a second risk analysis using extrapolated biotin prevalence data based on random samples from a US laboratory network representative of the general US population. Patient misclassification risk was evaluated according to International Organization for Standardization (ISO) 14971 and Clinical and Laboratory Standards Institute (CLSI) guidelines [20, 21].

Material and methods

TnT Gen 5 test principle

The Elecsys Troponin T Gen 5 assay is an electrochemiluminescence sandwich immunoassay, comprising a biotinylated monoclonal anti-cTnT-

specific antibody and a monoclonal anti-cTnT-specific antibody labeled with ruthenium [9]. These antibodies react to form a sandwich complex with cTnT, which is then bound to the solid phase via biotin–streptavidin interaction [9]. The TnT Gen 5 assay has previously demonstrated good analytical performance and met precision requirements [9]: limit of detection 5 ng/L (cobas e 411 analyzer) and 3 ng/L (cobas e 601 analyzer); measuring range 6–10,000 ng/L (both analyzers); repeatability and intermediate precision coefficients of variation (CVs) 0.7–5.6% and 1.4–10.3%, respectively (cobas e 411 analyzer; mean cTnT concentrations 7.3–9341 ng/L in lithium-heparin plasma samples), and 0.7–3.0% and 1.5–6.4%, respectively (cobas e 601 analyzer; mean cTnT concentrations 7.4–9455 ng/L in lithium-heparin plasma samples); 10% CV (total imprecision) 11 ng/L; CV at 99th percentile upper reference limit 3.92 (cobas e 411 analyzer) and 3.18 (cobas e 601 analyzer) ~~with a coefficient of variation <10% at the 99th percentile upper reference limit, meeting precision requirements [9]~~. TnT Gen 5 assay recovery can fall to 90% at biotin concentrations of 20.0 ng/mL (81.8 nmol/L); higher biotin concentrations can reduce assay recovery further (Figure 1). No interference has been observed with biotin ≤20.0 ng/mL (81.8 nmol/L) [9].

Study design

The prevalence of elevated biotin and the clinical risk of biotin interference with the TnT Gen 5 assay was evaluated using two distinct study cohorts and risk assessment models (Figure 2). In each model, the impact of biotin interference on the NPV of the TnT Gen 5 assay and the

likelihood of false-negative AMI prediction was estimated, based on the prevalence of biotin >20.0 ng/mL (81.8 nmol/L) in each cohort, the distribution of cTnT concentrations (as specified in each model below), and the biotin interference curve for the TnT Gen 5 assay.

Model 1: Risk calculation based on biotin prevalence data from a cohort of patients with suspected acute coronary syndrome (ACS cohort)

The ACS cohort comprised 850 patients presenting to 16 US emergency departments with suspected ACS from July 2014 to October 2015 and aimed to represent the TnT Gen 5 assay intended-use population in the US; this cohort has been previously described [8]. The original study received ethics approval from all relevant institutional review boards, and was conducted in accordance with the principles of the Declaration of Helsinki and the International Conference on Harmonization guidelines for Good Clinical Practice. All patients provided informed consent.

In the original study, 1679 patients (48% female; median age 55 years [interquartile range: 47–64]) had a TnT Gen 5 assay result available at one or more time point; all patients had an available result on the cobas e 411 analyzer and 1675 patients had an available result on the cobas e 601 analyzer. Of these, 850 patients who had sufficient residual 0-h (admission) and/or 3-h sample volume to measure biotin, and consented to future use of their samples, were included in the present analyses. Samples were stored for 28 months at −80°C and protected from light prior to biotin analysis. Biotin has been shown to be stable following

frozen storage and freeze/thaw cycles [22] and has an effective half-life of 15 hours [23]. Biotin was quantified using a competitive Elecsys research assay on the cobas e 411 analyzer (Roche Diagnostics International Ltd, Rotkreuz, Switzerland): limit of detection 4.88 ng/L (cobas e 411 analyzer) and 2.05 ng/L (cobas e 601 analyzer); measuring range 3–10,000 ng/L (both analyzers); intermediate precision CV 2.81 ng/L (cobas e 411 analyzer) and 2.20 ng/L (cobas e 601 analyzer); 10% CV (total imprecision) 5.03 ng/L (cobas e 411 analyzer) and 4.49 ng/L (cobas e 601 analyzer); CV at 99th percentile upper reference limit <10% (both analyzers) [24]. This assay detects total serum biotin (free biotin, bound biotin/biocytin, and biotin metabolites) with a lower limit of detection of 0.1 ng/mL (0.41 nmol/L), and has been validated against a liquid chromatography-tandem mass spectrometry method at biotin concentrations of 40.0 to 300 ng/mL (164 to 1228 nmol/L) (Supplemental Figure 1). A comparison of these methods for biotin concentrations <40.0 ng/mL (163.7 nmol/L) was not assessed.

A risk calculation model was built based on the measured prevalence of biotin >20.0 ng/mL (81.8 nmol/L) and distribution of cTnT concentrations in the ACS cohort, and the biotin interference curve for the TnT Gen 5 assay. The prevalence of cTnT concentrations around the 99th percentile upper reference limit for the TnT Gen 5 assay was evaluated, respecting the diagnostic criteria of the Third Universal Definition of Myocardial Infarction (the adjudication process for diagnosing AMI was performed before the publication of the updated fourth definition). This requires detection of a rise and/or fall in cardiac troponin (cTn) values,

with at least one value above the 99th percentile upper reference limit, alongside clinical evidence of AMI [~~24~~25]. The misclassification risk was determined using the US-specific 19 ng/L TnT Gen 5 assay cutoff. A misclassification was defined as a cTnT above the cutoff, which could be reported as below the cutoff due to biotin interference, and thus lead to a false-negative result.

The following assumptions were used: (i) the highest anticipated biotin concentration was derived by multiplying the highest observed concentration in the ACS cohort by three, per CLSI EP07 guidelines [~~25~~26]; (ii) the prevalence of 0-h biotin >20 ng/mL (81.8 nmol/L) and ≤100 ng/mL (409 nmol/L) was derived from the upper confidence limit of the observed prevalence in the ACS cohort; (iii) the maximal reduction in TnT Gen 5 assay recovery at a biotin concentration of 100 ng/mL (409 nmol/L) was 42%; and (iv) the AMI prevalence was extrapolated to 15%, a more conservative, yet realistic, estimate based on the measured prevalence of 10.3% in the ACS cohort [8].

Model 2: Risk calculation based on extrapolated biotin prevalence data in random samples from a US laboratory network (US laboratory cohort)

To enable a more comprehensive assessment of the risk of biotin interference with the TnT Gen 5 assay, a second study cohort was utilized to provide biotin prevalence data from a broader population than the ACS cohort. The US laboratory cohort was intended to reflect the general US population and comprised 2023 routine blood samples randomly selected

from a US commercial laboratory network in 2016. Samples were stored for 2 weeks at −20°C and protected from light prior to biotin analysis. Biotin was quantified using a competitive Elecsys research assay on the cobas e 411 analyzer (Roche Diagnostics International Ltd, Rotkreuz, Switzerland). For a more conservative approach and to evaluate a worst-case scenario, the measured prevalence of elevated biotin >20 ng/mL in the US laboratory cohort was extrapolated to higher biotin values, such as patients taking very high biotin doses for MS. This extrapolation was based on previous pharmacokinetic studies and a biotin prevalence study of US emergency department patients [10, 18, 23, ~~26~~27].

A risk calculation model was built based on the extrapolated prevalence of biotin >20.0 ng/mL (81.8 nmol/L), a US-specific distribution of cTnT concentrations extracted from a global Roche Diagnostics data collection system of participating customers, and the biotin interference curve for the TnT Gen 5 assay. The misclassification risk was determined using TnT Gen 5 assay cutoffs of 14, 19, and 22 ng/L (to cover non-US combined, US-combined, and US sex-specific cutoffs), and following biotin washout times of 1, 3, and 6 h by applying the pharmacokinetic data described previously [23]. These washout times were chosen to reflect commonly used time points for serial TnT Gen 5 testing. We also evaluated the misclassification risk of biotin interference if using a TnT Gen 5 assay cutoff of 6 ng/L (i.e. the assay's limit of quantitation) for 0-h result. Specifically, we assessed the risk of biotin interference causing a true TnT Gen 5 result of ≥6 ng/L to be recorded as <6 ng/L. The rationale behind this analysis was that a cutoff of 6 ng/L for 0-h TnT Gen 5 result is

commonly used in US emergency departments to decide whether a patient should be ruled out for AMI (<6 ng/L) or undergo serial cTn testing (≥6 ng/L), although it should be emphasized that this is an off-label use of the TnT Gen 5 assay.

Further details on this second risk calculation model are provided in the online Supplemental material.

Risk assessment per ISO 14971 guidelines

A risk assessment was performed to evaluate the probability and clinical consequences of misclassification due to biotin interference with the TnT Gen 5 assay, according to ISO 14971 guidelines [20].

Results

Model 1: Risk calculation based on biotin prevalence data from the ACS cohort

Prevalence of elevated biotin

Biotin was undetectable (<0.1 ng/mL; <0.41 nmol/L) in 471/797 (59%) 0-h samples and 399/646 (62%) 3-h samples (Supplemental Figure 5). The 99th percentile biotin concentrations were 2.62 ng/mL (10.7 nmol/L) at 0-h and 2.38 ng/mL (9.74 nmol/L) at 3-h, seven times lower than the biotin interference threshold for the TnT Gen 5 assay of 20.0 ng/mL (81.8 nmol/L). Biotin >20.0 ng/mL (81.8 nmol/L), which might influence TnT Gen 5 results by >10%, was identified in one sample at each time point (0-h,

30.23 ng/mL; 124 nmol/L) and (3-h, 24.48 ng/mL; 100 nmol/L); the corresponding prevalence of biotin >20.0 ng/mL (81.8 nmol/L) was 0.13% (0-h; 95% confidence interval [CI] 0–0.70) and 0.15% (3-h; 95% CI 0–0.86). Both samples were from a 60-year-old female patient who was correctly not diagnosed with AMI; 0-h TnT Gen 5 results for this patient were 5.70 ng/L (cobas e 411) and 5.51 ng/L (cobas e 601); 3-h TnT Gen 5 results were 5.15 ng/L (cobas e 411) and 5.74 ng/L (cobas e 601).

Among the 850 patients included in this analysis, 257 (30%) had a single biotin measurement available, 325 (38%) had undetectable biotin (<0.1 ng/mL; 0.41 nmol/L) in both samples, and 73 (9%) had undetectable biotin in one of the two samples. Thus, 195 (23%) patients had detectable biotin in both samples available to calculate biotin kinetics in the ACS cohort. The median change in biotin between 0-h and 3-h serial samples was −0.015 ng/mL (0.061 nmol/L) (interquartile range: −0.050 to 0.002 ng/mL).

Risk calculation

The following assumptions were used: (i) the highest biotin concentration was 100.0 ng/mL (409 nmol/L) which is approximately three times the highest observed biotin concentration of 30.23 ng/mL (124 nmol/L) in the intended use population; (ii) the prevalence of 0-h biotin >20.0 ng/mL (81.8 nmol/L) and ≤100 ng/mL (409 nmol/L) was 0.7% (upper confidence limit of observed prevalence); (iii) the maximal reduction in TnT Gen 5 assay recovery at a biotin concentration of 100 ng/mL (409 nmol/L) was 42%; and (iv) the AMI prevalence was 15%. Based on these assumptions,

only a 0-h TnT Gen 5 result between 19 and 45.24 ng/L could lead to false-negative AMI classification, using the US overall 19 ng/L cutoff. As 25% of patients diagnosed with AMI in the ACS cohort had a 0-h TnT Gen 5 result within this range, the likelihood of false-negative results due to biotin interference was estimated as 0.026% (Figure 3).

Model 2: Risk calculation based on extrapolated biotin prevalence data from the US laboratory cohort

Prevalence of elevated biotin

Biotin >20.0 ng/mL (81.8 nmol/L) was identified in 15/2023 (0.74%) samples; the highest measured biotin concentration was 92.7 ng/mL (379 nmol/L). The measured biotin prevalence was extrapolated anticipating patients receiving high-dose biotin treatment for MS. This resulted in extrapolated biotin concentrations of 100–600 ng/mL (409–2456 nmol/L) (Figure 4), which is higher than has been previously observed in the intended-use population for cTn testing [18].

Risk calculation

Based on extrapolated biotin data, predicted elimination of biotin in blood, and US-specific cTnT distribution data from the Roche data collection system, the misclassification risk due to biotin interference with the TnT Gen 5 assay, using the US-specific 19 ng/L cutoff, was 0.025% (0-h), 0.0064% (1-h), 0.00048% (3-h), and <0.00001% (6-h). Using different TnT Gen 5 assay cutoffs in the modeling produced the following

misclassification risk estimates: 14 ng/L (non-US combined cutoff/US female-specific cutoff), 0.026% (0-h) and 0.0067% (1-h); 22 ng/L (US male-specific cutoff), 0.029% (0-h) and 0.0075% (1-h). Comparable misclassification estimates were obtained when applying a global distribution of cTnT data to the modeling, rather than a US-specific distribution alone: 0-h 19 ng/L cutoff, 0.025%; 0-h 14 ng/L cutoff, 0.027%. This risk calculation model was also applied to the ACS cohort biotin prevalence data, which showed a misclassification risk of <0.00001% (0-h). The risk of biotin interference causing a true TnT Gen 5 result of ≥6 ng/L to be recorded as <6 ng/L was 0.063% (0-h).

Risk assessment per ISO 14971 guidelines

The severity of biotin interference with the TnT Gen 5 assay can be described as high due to the risk associated with AMI misclassification. However, the probability of misclassification occurring was judged to be low (0.026%).

Discussion

Biotin interference with biotin–streptavidin-based assays is of increasing interest due to biotin supplementation marketed for cosmetic use and trials of high-dose biotin for treating MS [10–16]. CLSI EP07 guidelines recommend that potential assay interferents are tested at the highest concentration expected in the intended-use population [~~25~~26]. However, manufacturer-reported assay interference thresholds for biotin are based on historic reference ranges (<1.0 ng/mL; 4.09 nmol/L) [~~27~~28, ~~28~~29], and

attempts to address biotin interference are limited by the poorly documented pharmacokinetic profile for biotin [23]. We explored the impact of biotin interference on the biotin–streptavidin-based TnT Gen 5 assay. At least one cTn measurement above the 99th percentile upper reference limit is necessary, although not sufficient alone, for AMI diagnosis [~~24~~25]. As such, false-negative TnT Gen 5 results due to biotin interference have potential clinical implications.

The prevalence of elevated biotin in our study (ACS cohort, 0.13–0.15%; US laboratory cohort, 0.74%) is consistent with that reported in routine cTnT samples from an Australian population (0.2%) [~~29~~30], but is lower than observed in previous research of patients presenting to a US emergency department (1.7%) [~~30~~31]. Differences may be due to geographic differences in biotin consumption and a lack of standardization for all current, biotin assays. For instance, in an Australian study [~~29~~30], biotin was measured by liquid chromatography–mass spectrometry (LC-MS)/MS (Shimadzu), whereas, in our study, biotin concentration was analyzed by LC-MS and an in-house Elecsys research assay on a cobas e 411 analyzer [23]. The differences in methods and inter-laboratory instrument calibration can result in discrepancies and there is currently no standard approach. By defining biotin concentration and interference with the same assay, our methods are comparable and valid.

We demonstrated that the likelihood of false-negative AMI prediction due to biotin interference with the TnT Gen 5 assay is very low in the intended-use population (0.026%, based on 0-h TnT Gen 5 result). This is

lower than reported in a previous study, which estimated that up to 0.8% of US emergency department patients would be at risk of a clinically significant cTnT decrease (defined as any change in cTnT of 4 ng/L or 10%) caused by biotin interference [~~30~~31]. Our definition of a clinically significant cTnT decrease is more stringent: 10% decrease at the US overall 19 ng/L TnT Gen 5 assay cutoff (approximately 2 ng/L). Importantly, our results show that the misclassification risk with the TnT Gen 5 assay is not determined by the assay biotin interference threshold alone. The prevalence of elevated biotin in the intended-use population, the shape of the assay-specific biotin recovery curve, and the test analyte distribution with respect to the assay cutoff are also key factors.

We developed a second risk model, which included more recent samples from a US commercial laboratory network and aimed to address a worst-case scenario by anticipating very high biotin concentrations in patients with MS taking high-dose biotin treatments/supplements. The misclassification rate due to biotin interference in this model, based on a US-specific distribution of cTnT data, the US-specific TnT Gen 5 assay cutoff of 19 ng/L, and a strict and conservative risk assessment, remained extremely low: 0.025% (0-h) to <0.00001% (6-h). Similar misclassification risk estimates were obtained when using non-US and US sex-specific TnT Gen 5 assay cutoffs (14 and 22 ng/L).

The present misclassification rates due to biotin interference are considerably lower than those caused by other factors related to the clinical performance of cTn assays. For the TnT Gen 5 assay, the

misclassification rate would be 0.7%, based on an NPV of 99.3% at 3 h [8], or 0.6%, based on an NPV of 99.4% [~~31~~32]. For contemporary cTn assays, a meta-analysis reported an NPV of 98.2%, which equates to a misclassification rate of 1.8%, and reflects the current standard of care for cTn assays and thus user expectations [~~32~~33]. The misclassification rate of a high-sensitivity cardiac troponin I (cTnI) assay would be 1.5%, based on an NPV of 98.5% [~~31~~32]. A typical assay imprecision of 3–6% would translate into a misclassification risk of 0.30–0.64%; coefficients of variation for intermediate precision with the TnT Gen 5 assay range from 1.4% to 10.3% (cobas e 411 analyzer) and from 1.5% to 6.4% (cobas e 601 analyzer) [9].

The American Heart Association/American College of Cardiology guidelines recommend serial cTn testing, with an additional draw after 3–6 h [~~33~~34]. Biotin concentrations should decrease during this period, thus reducing the risk of interference and a false-negative result. However, the introduction of high-sensitivity cTn assays has prompted an increasing trend for accelerated diagnostic protocols and faster clinical decision-making than the traditional 3–6-h algorithms. The European Society of Cardiology guidelines include a 0/1-h algorithm for high-sensitivity cTn assays [~~34~~35], and some AMI diagnostic algorithms incorporate risk scores before cTn testing or with the initial cTn result. Risk scores and clinical judgment may provide an additional safety layer, as high-risk patients should be investigated further, despite a non-elevated cTn.

Although our findings show that the misclassification risk from biotin interference with the TnT Gen 5 assay is low, special attention should be paid to patients taking high-dose biotin and those with inherited biotin metabolism disorders (e.g., biotinidase and holocarboxylase synthetase deficiencies). Clinicians should ask patients about recent biotin consumption, perform serial cTn measurements, and be aware that TnT Gen 5 results may be falsely depressed. TnT Gen 5 assay measurements should be repeated in patients with clinical signs of AMI, but a negative initial TnT Gen 5 result, taking into account expected biotin clearance times. In contrast to high-dose biotin, biotin doses <5 mg, which are commonly found in multivitamin and biotin supplements, are unlikely to lead to blood biotin concentrations that pose an interference risk with the TnT Gen 5 assay [23]. However, patients with renal impairment may have altered biotin kinetics and receive supplements of water soluble vitamins especially under dialysis [~~35~~36]. The interpretation of cTn in patients with renal failure is complicated by concomitant chronic structural heart disease rather than acute injury [~~36~~37], and cTn is often persistently elevated and affected by hemodialysis timing and membrane used in this population. Further research is required to examine the effects of biotin in patients with chronic kidney disease.

All immunoassays can be affected by interferences [~~37~~38]; collectively, these likely contribute to the fact that NPVs of cTn assays using the 99th percentile upper reference limit during serial sampling are 95–99% [~~32~~33]. Our findings suggest that biotin interference is far less prevalent than other interferences that can affect cTn assays [~~38~~39,

~~39~~40]. In spite of the low risk, the TnT assay has recently been updated (Gen 5 reformulated) to a biotin interference threshold of 1200 ng/mL (4.91 umol/L).

Strengths of the present analyses are that the biotin interference risk with the TnT Gen 5 assay was assessed using a conservative approach for estimating the probability of false-negative AMI prediction, and that biotin prevalence data were determined from a range of populations. The risk of biotin interference is dependent on the prevalence of elevated biotin in the target population, the assay's biotin interference tolerance, and the test analyte concentration. Therefore, our findings are specific to the TnT Gen 5 assay and the US population, and are not generalizable to other assays or populations. Biotin interference thresholds for cTn assays range from 2.5 to 10,000 ng/mL (10.2 nmol/L to 40.9 umol/L) [~~40~~41], and the threshold for the TnT Gen 5 assay of 20.0 ng/mL (81.8 nmol/L) is on the lower end of this range. It should also be noted that cTnT measured using high-sensitivity assays may differ depending on the assay equipment used for analysis. Although samples were stored frozen for prolonged periods and were not freshly analyzed, biotin has been shown to be stable following frozen storage and freeze/thaw cycles [22]. A limitation of the study was that the samples used were not specifically collected for the purpose of measuring biotin levels. Prior studies have shown that biotin is stable under such conditions [~~41~~42]. Our data reflect biotin distribution in the intended use population for troponin testing, but might differ from broader or more selected populations. In the ACS cohort, biotin was undetectable in the majority of samples. This may suggest that the ACS

cohort had lower biotin concentrations than might be expected in the general population; however, biotin reference intervals vary and there is no standardization of biotin assays. Another limitation of the study is that the biotin assay was validated against a liquid chromatography-tandem mass spectrometry method for biotin concentrations of 40.0–300 ng/mL (164–1228 nmol/L), but not below. While values <40.0 ng/mL (164 nmol/L) may therefore be insufficiently checked between methods, the most important factor was to check for reliability of comparability of higher concentrations, which are critical in terms of interference. The critical concentration range of biotin has been recently confirmed in external studies [~~42~~43, ~~43~~44].

Patients with renal insufficiency, pregnancy, and recent hospitalization were excluded from the original ACS cohort; therefore, our findings may not be generalizable to these groups. This is in keeping with standard algorithms for diagnosing AMI, which are not applicable to patients with renal dysfunction [~~24~~25]. Our worst-case scenario modeling was based on assumptions rather than measured data, and we do not know the proportion of patients with MS in the study cohorts who may have been taking high-dose biotin. The ACS cohort data suggest that no patients with MS taking high-dose biotin treatment were included in this cohort. In the risk model derived from the US laboratory cohort, the measured biotin prevalence data were extrapolated to anticipate very high biotin concentrations resulting from high-dose biotin treatment for MS.

In conclusion, biotin interference has a minimal impact on the clinical utility of the Elecsys Troponin T Gen 5 assay, and the likelihood of false-negative AMI prediction in the intended-use population is low. However, further research is required to understand completely biotin interference as a concern. Medical and laboratory staff should be aware of the potential risk for biotin interference, and pay particular attention to results from high-risk groups, such as patients with MS. It is important that clinicians evaluate results in the context of the wider clinical picture, ask patients about recent biotin/multivitamin supplement use, and perform serial cTn testing.

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Author contributions: All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

Conflicts of interest disclosure

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Employment or leadership: AZ is an employee of Roche Diagnostics International Ltd and holds stocks in F. Hoffmann-La Roche; AS is an employee of Roche Diagnostics GmbH; DK is an employee of Roche Diagnostics International Ltd.

Honorarium: BM has received an honorarium from AACC for a high-sensitivity troponin talk; RT reports speaker honoraria/consulting honoraria from Abbott, Amgen, Astra Zeneca, Roche, Siemens, Singulex and Thermo Scientific BRAHMS; NT has received an honorarium from AACC for a high-sensitivity troponin talk and served on a Roche advisory board related to biotin immunoassay interference.

Competing interests: The sponsor was involved in study design, the collection and interpretation of the data, and writing of the manuscript.

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<FIGURES>

Figure 1. Impact of biotin interference on Elecsys Troponin T Gen 5 assay recovery.

Troponin recovery was measured on the cobas e 411 analyzer using samples with a cardiac troponin T concentration of 16.2 ng/L, which were spiked with measured concentrations of biotin. A non-linear dose-response model was fitted to the measured data and was used to predict recovery values in samples with up to 100 ng/mL (409 nmol/L) biotin.

Figure 2. Overview of the two study cohorts/risk assessment models used to determine the prevalence of elevated biotin and the clinical risk of biotin interference with the TnT Gen 5 assay. *A risk calculation model was built based on biotin prevalence data, distribution of cTnT concentrations, and the biotin interference curve for the TnT Gen 5 assay. A misclassification was defined as a cTnT above the cutoff, which could be reported as below the cutoff due to biotin interference, and thus lead to a false-negative result. ACS, acute coronary syndrome; cTnT, cardiac troponin T; TnT Gen 5, Troponin T Gen 5; US, United States.

Figure 3. Estimating the probability of false-negative AMI prediction due to biotin interference with the TnT Gen 5 assay (based on 0-h result).

ACS, acute coronary syndrome; AMI, acute myocardial infarction; CI, confidence interval; TnT Gen 5, Troponin T Gen 5; US, United States.

Figure 4. Measured and extrapolated biotin prevalence data based on random samples from a US commercial laboratory network and scientific literature.

The simulated biotin prevalence assumed a higher biotin prevalence, and thus higher degree of risk, than observed in the laboratory samples. US, United States.