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FOCUSED REPORT

A Dilution Method to Mitigate Biotin Interference in Cardiac Troponin T Testing

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Background: Oral biotin supplementation is known to interfere with biotin-streptavidin–based immunoassays, including Roche's fifth-generation cardiac troponin T (cTnT) assay, which plays a critical role in the diagnosis of myocardial infarction (MI). The utility of dilution, a quick and easy method to detect and remove interferences, has not been published for biotin interference.

Methods: Concentrations of cTnT were measured in pooled serum from clinical samples. Serum samples were supplemented with biotin to known concentrations, then cTnT concentrations were remeasured to assess for biotin interference. Samples were then diluted to assess for effective removal of biotin interference.

Results: At cTnT values near the critical reporting range for our institution (100 ng/L) we observed significant interference in measured values with added biotin concentrations above 50 ng/mL. In specimens without added biotin, autodilution at a 1:10 ratio yielded a mean 157% capture of measured cTnT, precluding the use of autodilution for detecting and mitigating biotin interference. A 1:10 dilution with serum containing 20–30 ng/L cTnT yielded a mean capture of 107%, which was suitable for detecting underlying biotin interference in supplemented samples.

Conclusions: Biotin interference, at supraphysiologic concentrations, may create an artifactual reduction in measured cTnT to levels that could lead to delayed detection of an MI. Dilution with serum of known cTnT concentration of 20–30 ng/L is a fast and effective method to mitigate the analytical consequences of biotin interference.

IMPACT STATEMENT

Quantitative measurements of serum cardiac biomarkers are critical components in the timely diagnosis and management of suspected myocardial infarction (MI). The fifth-generation cTnT test provides an opportunity for earlier and more sensitive detection of MI events. Oral biotin supplementation can interfere with the test in a manner that may lead to missed or delayed MI diagnosis and subsequent patient morbidity. Here we demonstrate the efficacy of a simple method (dilution with serum with 20–30 ng/L cTnT) to detect and mitigate the effect of biotin interference.

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[2] **Nonstandard abbreviations:** FDA, Food and Drug Administration; cTnT, cardiac troponin T; MI, myocardial infarction.

Biotin is a water-soluble B vitamin that acts as a cofactor for multiple carboxylation reactions. Biotin supplementation at 2.5–10 mg/day is becoming increasingly popular in the US for cosmetic and medicinal uses despite limited efficacy data and the rarity of dietary deficiency (*1*). Additionally, high-dose biotin therapy (300 mg/day) is currently under investigation for treatment of secondary progressive multiple sclerosis, and some neurologists are prescribing high-dose biotin in anticipation of US Food and Drug Administration (FDA)[2] approval (*2*).

Biotin binding to streptavidin is a key component in many clinically used immunoassays (*3*). Oral biotin supplementation at both low and high dosages has been reported to interfere with many tests, resulting in significant morbidity (*4*–*7*). In November 2017, the FDA released a safety warning for biotin interference in laboratory testing (*8*). A method of using streptavidin-coated beads to remove biotin interference has been reported (*9*, *10*). However, no studies of dilution, a potentially quicker and easier approach to mitigate the effects of biotin interference, have been published to date (*11*).

Currently, UC San Diego Health System uses the fifth-generation cardiac troponin (cTnT) test to aid in the diagnosis of acute myocardial infarction (MI). Neither allowing for natural clearance of biotin nor manually removing biotin interference with streptavidin-coated beads is a feasible method of mitigating the effect of biotin interference owing to the time-sensitive setting of MI diagnosis. Thus, we sought to characterize the extent of biotin interference in the cTnT test and the utility of dilution for detecting and effectively mitigating the clinical effect of biotin interference.

MATERIALS AND METHODS

Pooled serum from routine patient care was collected with an approved institutional review board protocol for existing tissue. Quantitative measurements of cTnT were performed as singleton measurements using the Troponin T Gen 5 STAT kit on the Roche Cobas 8000 e602 (Cat. 07398000 160). The data shown in Figs. 1 and 2 represent the average recovery (±SD) from singleton measurements on 9 different patient specimens. Autodilution tests were performed with the system's "autodilution" function with the specified dilution ratio indicated. Dilution with serum was manually performed. Calculation of cTnT concentration in the test solution was derived with the formula $C_{test} = (C_{resulted} \times V_{resulted} - C_{diluent} \times V_{diluent}) / V_{test}$, in which C_{test} and V_{test} are the cTnT concentration and volume of the serum of interest, respectively, $C_{resulted}$ and $V_{resulted}$ are the cTnT concentration resulted by analysis and total volume, respectively, and $C_{diluent}$ and $V_{diluent}$ are the cTnT concentration (previously measured) and volume of serum used to dilute the interferent, respectively.

Biotin (Thermo Scientific, Cat. No. 29120, Lot No. SG251613; purity, 99.5%) was reconstituted into a 0.1 g/L stock solution in distilled water and stored at 4 °C. Biotin stock was diluted with 18.2 MΩ-cm water (Siemens PureLab Ultra) into working solutions that were then added into serum samples to achieve the indicated final concentration of biotin. All samples were supplemented with biotin solution or distilled water at 10% of the total volume of the sample.

Statistical analysis was performed using Microsoft Excel and Prism 8. Tukey's multiple comparison test was performed for all group comparisons.

RESULTS

To characterize the effect of biotin supplementation and autodilution on measured cTnT concentrations, we tested patient samples with known cTnT concentrations. We observed a mean capture of measured cTnT of 96%, 90%, 56%, and 11% at biotin concentrations of 25, 50, 125, and 250 ng/mL, respectively (Fig. 1).

We next sought to characterize the dilution characteristics of the assay at cTnT concentrations of approximately 100 ng/L cTnT, using the

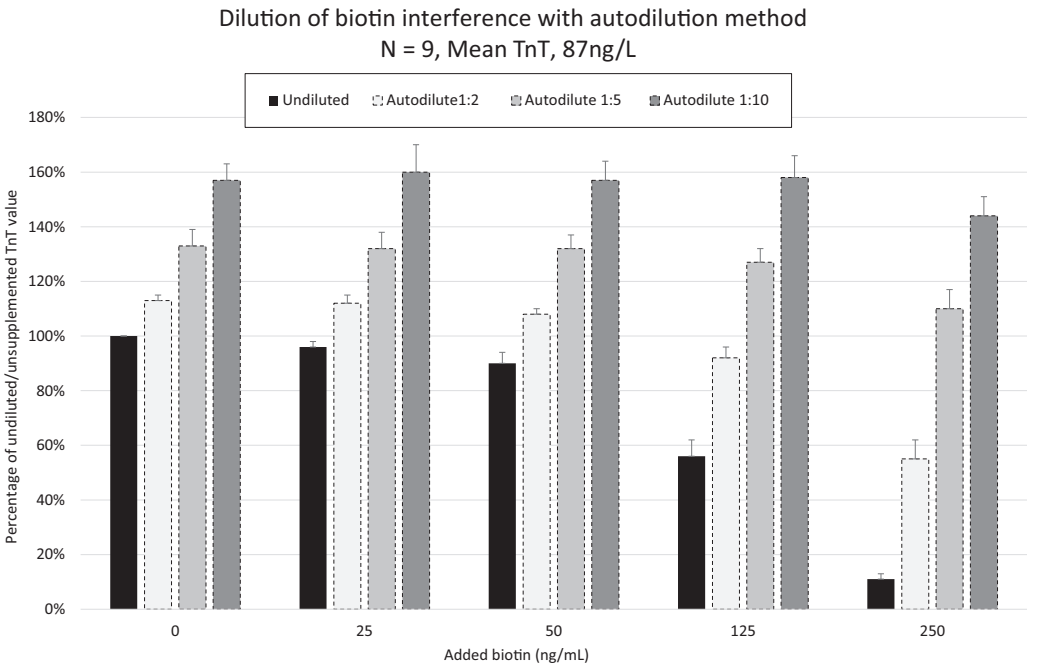

Fig. 1. Vertical axis displays percentage value of unsupplemented cTnT (serum supplemented with water only is 100%).
Horizontal axis displays added biotin concentration (ng/mL). Values displayed represent mean ± 1 SD of 9 independent results. Means with and without dilution at any ratio are all significantly different (adjusted $P < 0.01$, values not shown).

autodilution feature available on the COBAS system. We observed a mean capture of measured cTnT of 113%, 133%, and 157% after autodilution at 1:2, 1:5, and 1:10 ratios, respectively (Fig. 1). The values for all samples are available in the "Autodilution" column in Table 1 in the Data Supplement that accompanies the online version of this article at http://www.jalm.org/content/vol4/issue3. We also showed significant increases in captured value of similar magnitude when manually diluting samples with Roche diluent solution M or patient serum with measured cTnT values below the lower limit of quantification of 6 ng/L (see Fig. 1 in the online Data Supplement). The increase in measured troponin concentration with autodilution, manual dilution with the Roche diluent, or dilution with a serum specimen containing cTnT <6 ng/L would make it challenging to correct for biotin interference.

In an effort to find a diluent with approximately linear characteristics, we used patient serum with measured cTnT between 20 and 30 ng/L. Using a specimen with measured cTnT in this range, we observed a mean recovery of measured cTnT of 104%, 105%, and 107% after dilution at 1:2, 1:5, and 1:10 ratios, respectively (Fig. 2). Using this diluent at a 1:10 dilution ratio, we observed a mean recovery of cTnT of 107%, 100%, 104%, 95%, and 79% in the presence of 0, 25, 50, 125, and 250 ng/L of biotin, respectively (Fig. 2). The values for all samples are available in the "Dilution with serum containing approximately 20 ng/L cTnT" column in Table 1 in the online Data Supplement.

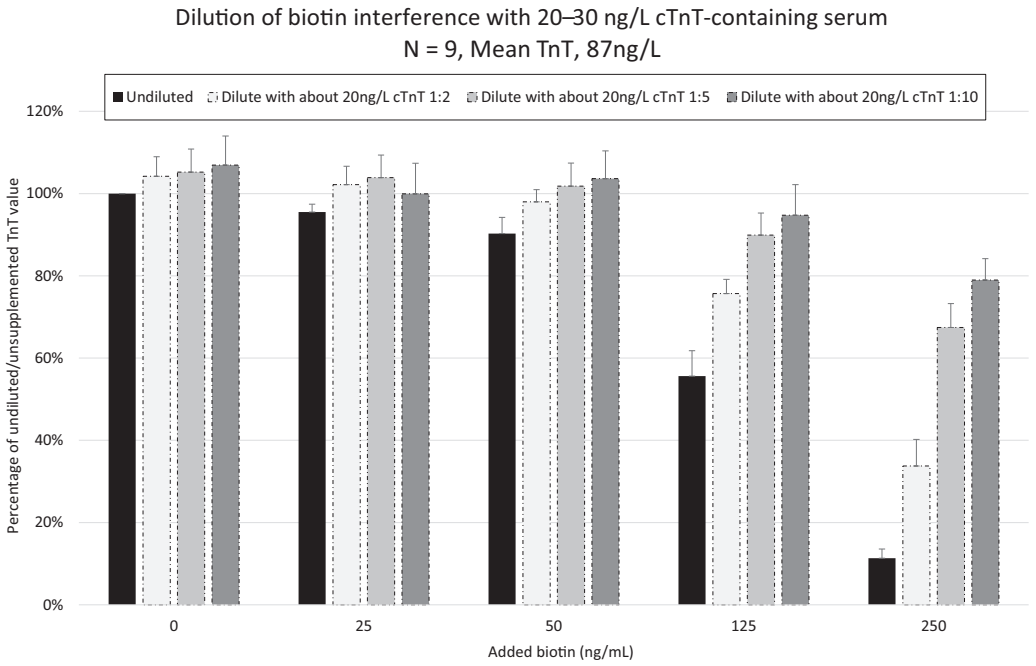

Fig. 2. Vertical axis displays percentage value of unsupplemented cTnT (serum supplemented with water only is 100%).
Horizontal axis displays added biotin concentration (ng/mL). Values displayed represent mean ± 1 SD of 9 independent results. Means with and without dilution are significantly different in the presence of added biotin at 50 ng/mL or more (adjusted $P < 0.01$, values not shown).

DISCUSSION

Biotin interference has been shown to cause false-negative results for fifth-generation cTnT assays because the assay uses biotin-streptavidin binding (*12*). In our hands, we see interference of cTnT at biotin concentrations >50 ng/mL that may be clinically significant. Pharmacokinetic studies suggest that serum biotin concentrations >50 ng/mL could be sustained for 2–3 h after low-dose (20 mg) biotin ingestion or up to 24 h after a single high-dose (300 mg) biotin ingestion (*13*, *14*). Thus, despite relatively rapid clearance, biotin may lead to clinically significant interference, especially in institutions practicing a variant of the 0-h/1-h troponin testing algorithm (*15*). Prevalence estimates of analytically significant concentrations of biotin in blood among patients differ between sources (*16*, *17*). However, because of the high risk of patient morbidity and mortality in such a scenario, clinical laboratories using biotin-streptavidin–based platforms should have a high level of suspicion for biotin interference and implement an effective evidence-based strategy to mitigate the potential risk.

Manufacturer guidelines suggest that patients ingesting oral biotin supplementation should wait for natural clearance of biotin before undergoing testing. However, the need for an expedient MI diagnosis necessitates the use of a faster method than natural clearance to obtain cardiac marker testing results. The 510k submission for the Roche 5th generation cTnT assay indicates that the test results did not deviate from linearity by more than

12.4% over the range of 3–10 000 ng/L (*18*). It should be noted that the linearity studies described in the package insert were performed "by diluting one high analyte plasma sample (spiked with recombinant cTnT) with native low analyte plasma." This process is consistent with the protocol we describe here. Unfortunately, our data and that published by other groups (*19*) indicate that the autodilution function of the COBAS platform would not be ideal for detecting an underlying interferent (e.g., biotin) because of the nonlinear dilution characteristics of the test for cTnT values <100 ng/L with the Roche diluent. In a patient with suspected biotin interference, it would be difficult to determine if an increase in measured troponin concentration after dilution (e.g., 18 ng/L cTnT result predilution becoming 28 ng/L cTnT after a 1:10 dilution is performed) is due to nonlinear dilution effects or diluting out an underlying interferent.

Using autodilution to uncover biotin interference would potentiate false-positive assessments of interference. As an example scenario: if a patient presents with a 0-h cTnT value of 60 ng/L and the clinician suspects that biotin is interfering with the assay, a 1:10 autodilution of the sample would be predicted to yield approximately 95 ng/L even in the absence of biotin interference. If this same sample is diluted at a 1:10 ratio with serum at a concentration of 20–30 ng/L cTnT, then a result of 60 ng/L (±10 ng/L) would result, indicating that significant biotin interference is unlikely. If the sample does indeed contain biotin at significant concentrations, then the results after dilution with serum of 20–30 ng/L would increase in proportion to the extent of biotin interference, indicating the presence of an underlying interferent.

The etiology of the observed nonlinearity of dilution and the mechanism by which our proposed method works are not clear. Data generated in our laboratory suggest that the increased measured concentration of troponin observed with autodilution, manual dilution using the Roche diluent, or dilution with a specimen containing <6 ng/L troponin is consistently about 4–5 ng/L cTnT above the expected value before correcting for dilution factors throughout the range of 7–90 ng/L of cTnT (see Fig. 2 in the online Data Supplement). Our conjecture is that dissociation of troponin complexes may accelerate with dilution, leading to increased cTnT; however, additional research will be needed to understand the molecular dynamics underlying the observed phenomena.

Integration of the described dilution method into institutional protocols will be a unique process at each institution owing to the heterogeneity of clinical and laboratory usages for the fifth-generation cTnT assay. Serum with known cTnT values is not a commonly stocked reagent in most clinical laboratories. Given the importance of the cTnT assay it may be necessary for laboratories to generate and maintain stocks of patient serum expressly for the purpose of dilution. Establishing a protocol will require (*a*) clinicians to communicate suspected biotin interference to the laboratory, (*b*) for the laboratory to have a supply of serum with known cTnT value to use as a diluent, (*c*) validate the process for all relevant predilution cTnT values as needed for the institution's clinical cTnT testing protocols. These steps will require institution-specific protocol development, validation, and implementation.

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N. Bevins, statistical analysis, provision of study material or patients; J.A. Hubbard, statistical analysis.

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