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Evaluating the performance of an updated high-sensitivity troponin T assay with increased tolerance to biotin

Abstract

Objectives: Biotin >20 ng/mL may interfere with the Elecsys® Troponin T-high sensitive assay (cTnT-hs; Roche Diagnostics International Ltd). We evaluated the performance of an updated assay, cTnT-hs*, which was designed to reduce biotin interference.

Methods: cTnT-hs* assay performance was assessed using up to two applications (18 min/9 min) on three analyzers (cobas e 411/cobas e 601/cobas e 801). Biotin interference was determined by measuring recovery in an 11-sample series dilution with biotin ranging from 0–3600 ng/mL. Repeatability/reproducibility were evaluated in five serum sample pools (n=75 each). Method comparisons tested: cTnT-hs* vs. cTnT-hs (18 min/cobas e 601); cTnT-hs* assay 18 vs. 9 min (cobas e 601); cTnT-hs* (18 min) on cobas e 601 vs. cobas e 411 and cobas e 601 vs. cobas e 801. Concordance at the 99th percentile decision limit between cTnT-hs* and cTnT-hs (9 min/cobas e 601) was calculated using 300 lithium-heparin plasma samples and a 14 ng/L assay cutoff.

Results: cTnT-hs* assay (18 min/cobas e 601) recovery was ≥96% for biotin ≤1250 ng/mL. Across all applications/analyzers, coefficients of variation for repeatability/reproducibility with the cTnT-hs* assay were <5% in most serum sample pools (mean cardiac troponin T: 8.528–9484 ng/L). High correlation (Pearson's r=1.000) was demonstrated for all method comparisons. Concordance at the 99th percentile decision limit was high between the cTnT-hs* and cTnT-hs assays.

Conclusions: The updated cTnT-hs* assay may provide greater tolerance to biotin interference, and shows good analytical and clinical agreement/concordance with the previous cTnT-hs assay.

Keywords: analytical performance; biotin interference; cardiac troponin; method comparison; preanalytics; 99th percentile.

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Introduction

Biotin-streptavidin coupling is one of the strongest non-covalent interactions in nature and has been used for decades by manufacturers of *in vitro* diagnostic devices to immobilize biotinylated proteins [1–3]. However, all immunoassays based on biotin-streptavidin technology are potentially susceptible to interference with biotin in the blood sample. The Elecsys® Troponin T-high sensitive assay (cTnT-hs; Roche Diagnostics International Ltd, Rotkreuz, Switzerland) is a biotin-streptavidin-based immunoassay, which provides a high negative predictive value (NPV) of 99.8% for ruling out a diagnosis of acute myocardial infarction (AMI) at 1 h after presentation using the 0/1 h algorithm [4].

All immunoassays that utilize biotin-streptavidin coupling are potentially susceptible to biotin interference, although the impact on test results differs depending on the assay's mechanism of action [5]. In non-competitive (or sandwich) immunoassays, such as the cTnT-hs assay, the target analyte is 'sandwiched' between the signal and biotinylated antibodies, of which the latter links the antibody-analyte complex to a streptavidin-coated solid phase; thus, the signal increases as the target analyte concentration increases. In the presence of excess biotin, the streptavidin binding sites are saturated by free biotin, which inhibits the binding of the antibody-analyte complex, resulting in a decreased signal and falsely low test result. In competitive

immunoassays, the target analyte competes with a labelled analyte for the biotinylated antibody binding site, which is captured to the streptavidin-coated solid phase; thus, the signal decreases as the target analyte concentration increases. In the presence of excess biotin, labelled and target analytes are prevented from binding to the streptavidin-coated solid phase by free biotin, and are removed during the wash step. This results in a decreased signal and falsely high test result. There is also a high degree of variability in biotin tolerance and interference thresholds between different assays; biotin interference thresholds for cardiac troponin (cTn) assays, for example, can range from 2.5 to 10,000 ng/mL [6, 7].

High blood biotin concentrations >20 ng/mL may interfere with the cTnT-hs assay and reduce recovery by >10%, which could potentially lead to falsely decreased cTnT-hs results and thus false-negative AMI prediction [8–11]. This interference threshold is sufficient for most routine clinical laboratory testing, as it is considerably higher than blood biotin concentrations associated with the adequate intake of dietary biotin (30 µg daily in adults) [12], and until recently, the risk of biotin interference with biotin-streptavidin-based immunoassays was deemed to be very low. However, societal trends for very high biotin supplementation and clinical trials of high-dose biotin up to 300 mg daily for treating multiple sclerosis may increase the risk of immunoassay interference from biotin [13–19].

Previous pharmacokinetic studies have shown that daily biotin dosing regimens of either 3×100 mg or 1×300 mg result in maximum plasma biotin concentrations of <700 ng/mL (1.45 h after intake) and 1127–1160 ng/mL (1–1.5 h after intake), respectively, thus exceeding the 20 ng/mL biotin interference threshold for the cTnT-hs assay [13, 20]. The use of very high biotin dosing regimens is still likely to be rare in the general population; a recent study found biotin concentrations ≥30 ng/mL in <0.5% of residual waste plasma samples collected from emergency department (ED) patients and no concentration >280 ng/mL [21]. However, given the trends for increased use of high-dose biotin supplementation we evaluated the performance of an update of the cTnT-hs assay, referred to here as cTnT-hs*, which was designed to reduce biotin interference by including a highly specific antibody to bind and neutralize free biotin.

Materials and methods

Analytical methods

The performance of the Elecsys cTnT-hs* assay was assessed at three sites (Kliniken Nordoberpfalz AG, Weiden, Germany; Labor Augsburg MVZ, Augsburg, Germany; Roche Diagnostics Research and Development, Penzberg, Germany) using two assay applications (18 and 9 min [STAT]) and three analyzers (cobas e 411, cobas e 601 and cobas e 801 analyzers; Roche Diagnostics International Ltd, Rotkreuz, Switzerland).

The Elecsys cTnT-hs assay is an electrochemiluminescence sandwich immunoassay, in which a biotinylated monoclonal anti-cardiac troponin T (cTnT)-specific antibody and an additional monoclonal anti-cTnT-specific antibody labelled with ruthenium react to form a sandwich complex with cTnT. The sandwich complex is bound to the solid phase by the addition of streptavidin-coated microparticles. The reaction mixture is aspirated and the microparticles are magnetically captured; a voltage is then applied to induce electrochemiluminescence, which is measured by a photomultiplier. The updated cTnT-hs* assay was designed to reduce biotin interference by including a highly specific antibody to bind and neutralize free biotin (a patent has now been filed for this updated design) [22]. Since the cTnT-hs assay is used as an emergency testing tool, an assay application that has only 9 min duration, the STAT application, was developed. The shorter turn-around time provides benefits to the patient since it allows for earlier decision making with the aid of the assay result compared with the 18 min application. With regards to biotin interference, no differences were expected between these applications; however, as the 18 min application represents the "standard" duration of the Elecsys portfolio, analysis was carried out on both applications for completeness.

The cobas e 411, cobas e 601, and cobas e 801 are fully automated analyzers for processing of electrochemiluminescence-based immunoassays.

Sample materials

Anonymized samples without further clinical or demographic information were used for all experiments in this study, which was conducted in 2018–2019. A native sample pool comprising single samples purchased from various vendors was used for biotin interference testing. Five native serum and five plasma sample pools, partially spiked with recombinant cTnT from *Escherichia coli*, and two control pools with low and high cTnT concentrations (PreciControl Troponin; Roche Diagnostics International Ltd, Rotkreuz, Switzerland) were used for precision testing; all sample material for the reproducibility experiment at the study sites was provided by the manufacturer. Native serum samples purchased from vendors in 2018 were used for method comparison testing; less than 10% were spiked with recombinant cTnT (Roche, in-house) in order to cover the whole measuring range. Samples were handled according to good laboratory practice and assays package inserts.

The study was conducted in accordance with all relevant national regulations, institutional policies, and the principles of the Declaration of Helsinki. The samples in this study (anonymized residual samples) were used in accordance with recommendations of members of the German Ethics Council (Deutscher Ethikrat).

Experimental design

To determine the biotin interference, a serum sample pool was spiked with biotin (Sigma-Aldrich) and serially diluted to obtain 11 biotin concentrations in the range 0–3600 ng/mL. The recovery of troponin T

in the sample was measured *in vitro* with the cTnT-hs* assay, using the 18 min application on the cobas e 601 analyzer. The troponin T concentration of the sample pool was 16.1 ng/L.

Assay reproducibility was evaluated *in vitro* according to Clinical Laboratory and Standards Institute EP05-A3 guidelines [23]: testing was conducted over 5 days at three different study sites; one run with five replicates was performed per day (n=75 results per sample pool). Repeatability data were generated in the context of the reproducibility testing.

The following method comparisons were conducted *in vitro* using serum samples: cTnT-hs vs. cTnT-hs* assays, using the 18 min application on the cobas e 601 analyzer; 18 vs. 9 min applications of the cTnT-hs* assay on the cobas e 601 analyzer; and the cTnT-hs* assay on the three different analyzers (cobas e 601 vs. cobas e 411 and cobas e 601 vs. cobas e 801) using the 18 min application.

Concordance at the 99th percentile decision limit between the cTnT-hs* and cTnT-hs assays was evaluated *in vitro* in 300 lithium-heparin plasma samples using the 9 min application on the cobas e 601 analyzer. Repeatability/reproducibility, method comparisons and concordance at the 99th percentile decision limit were tested using samples that were understood not contain biotin, as per information provided by the vendors.

Statistical analyses

Assay recovery was estimated by calculating the quotient of measured and expected cTnT concentrations. An analysis of variance approach was used to estimate coefficients of variation (CVs) for repeatability and reproducibility. Method comparison data were analyzed using Passing-Bablok regression, and Pearson correlation coefficients were calculated. Concordance at the 99th percentile decision limit was calculated using a 2×2 contingency table based on a cutoff of 14 ng/L for the cTnT-hs assay; positive and negative agreement for cTnT-hs* vs. cTnT-hs assays were estimated. R software (version 3.2.2) was used to conduct the statistical analyses [24].

Results

Biotin interference

In the presence of increasing biotin concentrations, recovery with the cTnT-hs* assay was high (≥99%) for biotin concentrations ≤500 ng/mL, decreased slightly at biotin concentrations 1000–1500 ng/mL, and only dropped below 90% for biotin concentrations ≥1750 ng/mL (Figure 1, Supplemental Figure 1).

Repeatability/reproducibility

Across all assay applications (18 and 9 min) and analyzers (cobas e 411, cobas e 601 and cobas e 801) tested, CVs for repeatability and reproducibility were low (<5%) for the majority of plasma sample pools
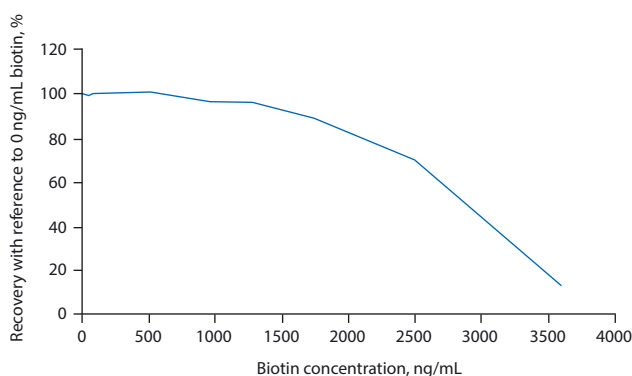

Figure 1: cTnT-hs* assay recovery with increasing biotin concentrations, using the 18 min application on the cobas e 601 analyzer. A native serum sample pool spiked with biotin was serially diluted with a biotin-free serum pool to achieve 11 biotin concentrations. The troponin T concentration of the serum pool was 16.1 ng/L. cTnT-hs*, updated Elecsys Troponin T-high sensitive assay designed to reduce biotin interference.

(Tables 1 and 2). Repeatability and reproducibility CVs were highest (12.33–13.79% and 21.67–25.53%, respectively) for the lowest cTnT concentration sample pool (8.344–10.21 ng/L) on the cobas e 411 analyzer (Tables 1 and 2). Results in the serum sample pools were similar to those in the plasma sample pools (Supplemental Tables 1 and 2).

Method comparisons

High correlation (Pearson's r=1.000) was demonstrated between the cTnT-hs and cTnT-hs* assays (18 min application; cobas e 601 analyzer; Figure 2), and between the 18 and 9 min applications for the cTnT-hs* assay (cobas e 601 analyzer; Figure 3). High correlation (Pearson's r=1.000) was also demonstrated for the cTnT-hs* assay (18 min application) when run on the cobas e 601 vs. cobas e 411 analyzers (Figure 4A), and cobas e 601 vs. cobas e 801 analyzers (Figure 4B).

Concordance at the 99th percentile decision limit

Concordance at the 99th percentile decision limit was high between the cTnT-hs* and cTnT-hs assays (9 min application; cobas e 601 analyzer) when using a 14 ng/L cutoff: negative agreement=95.3%; positive agreement=100.0% (Table 3).

Table 1: Repeatability of the cTnT-hs* assay measured in plasma samples, using 18 and 9 min applications on cobas e 411, cobas e 601 and cobas e 801 analyzers. Testing was conducted over five days at three different study sites; one run with five replicates was performed per day (n=75 results per sample pool). Repeatability corresponds to the between-replicate variance component that was extracted by ANOVA from the reproducibility experiment shown in Table 2.

Plasma sample pool (n=75 each)	Mean cTnT concentration, ng/L	Coefficient of variation, %					
		Cobas e 411		Cobas e 601		Cobas e 801	
		18 min	9 min	18 min	9 min	18 min	9 min
1	8.344–10.21	12.33	13.79	3.74	3.51	4.94	3.21
2	19.69–22.48	4.50	4.36	2.48	2.26	2.64	1.74
3	147.2–171.0	2.62	2.42	1.65	1.48	2.85	2.63
4	4325–5218	1.10	1.29	1.77	1.35	1.12	1.49
5	8730–9490	1.50	1.75	1.47	1.39	1.11	1.66

cTnT, cardiac troponin T; cTnT-hs*, updated Elecsys Troponin T-high sensitive assay designed to reduce biotin interference.

Table 2: Reproducibility of the cTnT-hs* assay measured in plasma samples, using 18 and 9 min applications on cobas e 411, cobas e 601 and cobas e 801 analyzers. Testing was conducted over five days at three different study sites; one run with five replicates was performed per day (n=75 results per sample pool). Reproducibility includes the variance components between-replicate, between-day and between-site.

Plasma sample pool (n=75 each)	Mean cTnT concentration, ng/L	Coefficient of variation, %					
		Cobas e 411		Cobas e 601		Cobas e 801	
		18 min	9 min	18 min	9 min	18 min	9 min
1	8.344–10.21	21.67	25.53	4.51	6.57	7.66	8.81
2	19.69–22.48	8.95	8.28	2.85	2.99	3.81	3.80
3	147.2–171.0	3.18	2.75	2.42	2.70	4.19	3.50
4	4325–5218	2.30	2.00	2.67	2.81	3.25	3.62
5	8730–9490	2.42	2.08	2.56	2.45	2.59	2.97

cTnT, cardiac troponin T; cTnT-hs*, updated Elecsys Troponin T-high sensitive assay designed to reduce biotin interference.

Discussion

We evaluated the performance of an update for the Elecsys cTnT-hs assay, referred to here as cTnT-hs*, which was designed to reduce biotin interference. We demonstrated that the updated cTnT-hs* assay provides substantially greater tolerance to biotin interference vs. the previous cTnT-hs assay: recovery with the cTnT-hs* assay was ≥99% for biotin concentrations up to 500 ng/mL and 96% for a biotin concentration of 1250 ng/mL (acknowledging that this is a representative example of the biotin interference curve). These results are in contrast to the previous cTnT-hs assay, which was only unaffected by biotin concentrations <20 ng/mL [25]. Importantly, the updated cTnT-hs* assay increases the biotin ±10% interference threshold above 1200 ng/mL. This is the concentration to which manufacturers are required by the US Food and Drug Administration (FDA) to investigate biotin interference in assays that utilize biotin technology [18], and is higher than the maximum plasma biotin concentrations following high-dose biotin administration reported in previous pharmacokinetic studies [13,

16]. In addition, the analytical performance of the updated cTnT-hs* assay was equivalent to the previous cTnT-hs assay when using both 18 and 9 min applications on three different analyzers (cobas e 411, cobas e 601 and cobas e 801), and a high level of concordance at the 99th percentile decision limit was observed between the cTnT-hs* and cTnT-hs assays, including 100% positive agreement and 95.3% negative agreement.

Biotin interference has come under increasing scrutiny due to the societal trend for high-dose biotin in cosmetic supplements and investigational use of very high doses of biotin for the treatment of multiple sclerosis [13–19]. While it remains unclear whether high-dose biotin treatments will prove effective for use in multiple sclerosis (one clinical study was recently stopped, although testing continues at some sites), potential biotin interference with cTn assays is of particular concern. As a cTn result above the 99th percentile upper reference limit is a key criterion in the diagnosis of AMI [26], a false-negative result may have important implications for patient safety. However, it is important to stress that AMI diagnosis must be made in
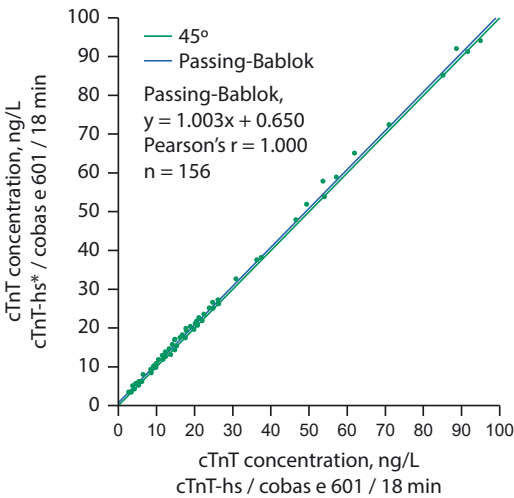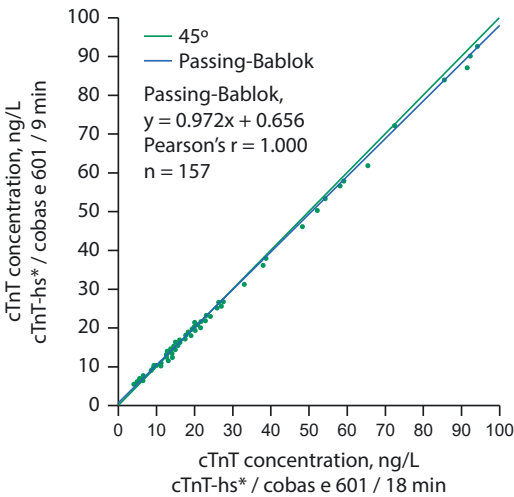
Figure 2: Method comparison of the cTnT-hs* vs. cTnT-hs assays, using the 18 min application on the cobas e 601 analyzer.
n=148 native serum samples and eight serum samples spiked with recombinant cTnT. Data are presented for the clinically relevant range up to cTnT 100 ng/L (note that Passing-Bablok regression and Pearson's correlation estimates are given for the entire dataset). cTnT, cardiac troponin T; cTnT-hs, Elecsys Troponin T-high sensitive assay; cTnT-hs*, updated Elecsys Troponin T-high sensitive assay designed to reduce biotin interference.

Figure 3: Method comparison of 9 vs. 18 min applications of the cTnT-hs* assay on the cobas e 601 analyzer.
n=149 native serum samples and eight serum samples spiked with recombinant cTnT. Data are presented for the clinically relevant range up to cTnT 100 ng/L (note that Passing-Bablok regression and Pearson's correlation estimates are given for the entire dataset). cTnT, cardiac troponin T; cTnT-hs*, updated Elecsys Troponin T-high sensitive assay designed to reduce biotin interference.

conjunction with clinical signs and symptoms, and cannot be based on cTn results alone [26]. Furthermore, the prevalence of elevated biotin in the intended-use population for cTn testing and the likelihood of false-negative
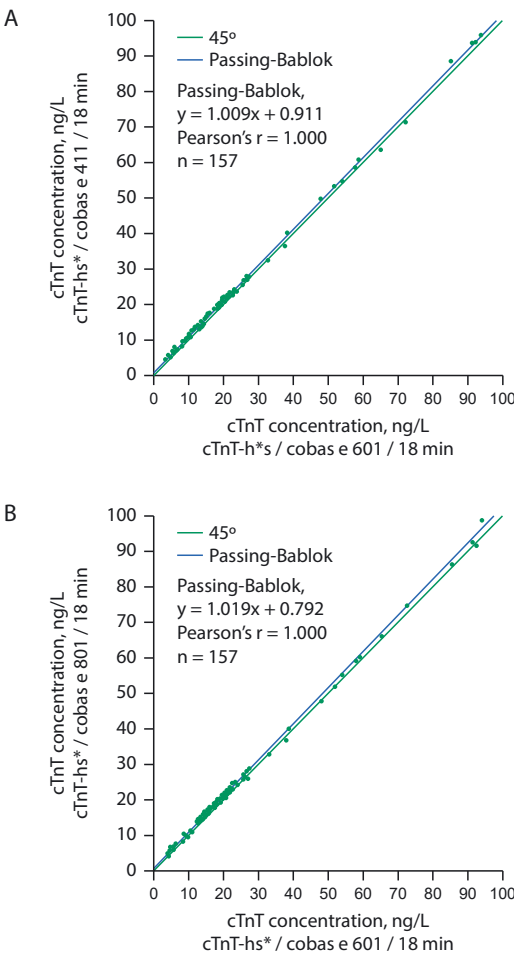

Figure 4: Method comparison of the cTnT-hs* assay, using the 18 min application, on (A) cobas e 411 vs. cobas e 601 analyzers, and (B) cobas e 801 vs. cobas e 601 analyzers.
For (A) and (B): n=149 native serum samples and eight serum samples spiked with recombinant cTnT. Data are presented for the clinically relevant range up to cTnT 100 ng/L (note that Passing-Bablok regression and Pearson's correlation estimates are given for the entire dataset). cTnT, cardiac troponin T; cTnT-hs*, updated Elecsys Troponin T-high sensitive assay designed to reduce biotin interference.

Table 3: Concordance at the 99th percentile decision limit between the updated cTnT-hs* and previous cTnT-hs assays, using the 9 min application on the cobas e 601 analyzer and an assay cutoff of 14 ng/L.

		cTnT-hs assay	
		<14 ng/L	**≥14 ng/L**
cTnT-hs* assay	<14 ng/L	101	0
	≥14 ng/L	5	194

cTnT-hs, Elecsys Troponin T-high sensitive assay; cTnT-hs*, updated Elecsys Troponin T-high sensitive assay designed to reduce biotin interference (n=300 native lithium-heparin plasma samples).

AMI prediction with the cTnT-hs assay have both been shown to be very low [11, 21, 27, 28]. A recent study of 3071 patient samples sent for cTn testing from an Australian emergency department found that the vast majority (97.7%) contained biotin concentrations ≤1 ng/mL [27]. Four samples (0.1%) contained biotin concentrations sufficient to cause analytical bias affecting the cTnT-hs assay (i.e. above the 10% analytical bias threshold); however, the rate of diagnostic classification change was 0% [28]. Our findings suggest that the updated cTnT-hs* assay could reduce the risk of biotin interference with cTn testing even further by providing a much higher tolerance to biotin interference vs. the previous cTnT-hs assay, while still providing equivalent analytical performance and concordance at the 99th percentile decision limit.

A strength of the present study is that the performance of the updated cTnT-hs* assay was tested using both applications (18 and 9 min) and on three different analyzers (cobas e 411, cobas e 601 and cobas e 801 analyzers). The observed differences in analytical performance between the two reagents tested, cTnT-hs and cTnT-hs* assays, are not larger than typical lot-to-lot variations in cTn testing in similar settings [29], and are smaller than observed biological variations in cTn [30]. This reflects the fact that design changes to the updated cTnT-hs* assay were minimal with a clear aim to not change the antibody epitopes. A study limitation is the current lack of real-world performance data for the cTnT-hs* measured in clinical laboratories under routine conditions, although studies are currently ongoing to establish this. Although the updated cTnT-hs* assay has undergone extensive analytical performance testing, including potential interference from drugs, endogenous metabolites, and heterophilic antibodies, establishing the clinical robustness of a new assay requires longer-term testing. For example, the misclassification risk due to biotin interference with the existing cTnT-hs assay has been estimated to be 0.026% (based on current biotin regimens in the intended-use population) [11]. Therefore, on average, 3800 samples would need to be tested to find one instance of biotin interference, and even more samples would need to be tested to make powered conclusions.

In conclusion, the updated cTnT-hs* assay may provide greater tolerance to biotin interference across all platforms evaluated and shows very good agreement/concordance with the previous cTnT-hs assay.

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