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Method Comparison Evaluation of Two Difference Assays for the Measurement of TT3 and TT4

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Abstract

Introduction: Different systems for serum total triiodothyronine (TT3) and total thyroxine (TT4) measuring are performed in clinical laboratories with evaluated consistency. Method comparison and bias estimation are important components of this study. The operation and analytical methods are conducted according to the Clinical and Laboratory Standard Institute (CLSI) new guideline EP09-A3. **Methods:** The two measurands were analyzed and evaluated on the Roche Cobas e602 and Sysmex HISCL 5000 analyzers. Analytical performance and method comparisons were performed according to EP09-A3. Outliers were computed by statistical methods and classified regression techniques were performed to evaluate and interpret biases. **Results:** No outliers were detected in this study. Linearity assessment demonstrated a coefficient of correlation as greater than 0.95 for the analytes. The comparison study and 95%CI computed by different regression methods showed acceptable biases except for the weighted Deming regression for TT4 measurement. **Conclusion:** The study demonstrated excellent analytical performance and acceptable biases for the two systems measuring TT3 and TT4. The new EP09 guide is suitable for the evaluation of methods comparison.

Keywords

Method comparison, EP09-A3, triiodothyronine, thyroxine, Cobas e602, HISCL 5000

1. Introduction

Thyroid hormones are any hormones produced and released by the thyroid gland, mainly named triiodothyronine and thyroxine. Adequate amounts of iodine are needed for the thyroid to be able to make these hormones [1]. T3 and T4 are tyrosine-based hormones that are primarily responsible for the regulation of metabolism. Low levels of these hormones act to cause metabolic disorders in nearly every cell in the body. They act to increase the basal metabolic rate, affect protein synthesis, help regulate long bone growth and neural maturation, and increase the body's sensitivity to catecholamines (such as adrenaline) by permissiveness [2]. The thyroid hormones are essential to the proper development and differentiation of all cells of the human body [3]. At present, they can be measured in serum as a protein-bound form, namely total triiodothyronine (TT3) and total thyroxine (TT4), by applying a variety of assays.

This study was carried out in a clinical laboratory in China (Affiliated Hospital of Jiangsu Institute of Nuclear Medicine, Wuxi). Two analyzers named Roche Cobas e602 and Sysmex HISCL 5000 were utilized to measure the levels of TT3 and TT4. Electrochemiluminescence immunoassay (ECLI), which is applied in Cobas e602, is a specific luminescence reaction triggered by electrochemistry on the electrode surface, including electrochemistry and

chemiluminescence. Electrochemical luminescence is the combination of the chemiluminescence method and the electrochemical method. It refers to the generation of some special substances (Ruthenium complex) by electrochemical method, and then the further reaction between these electrically generated substances or the electric biomass and other substances produces a luminescence phenomenon [4]. HISCL 5000 is based on chemiluminescence enzyme immunoassay (CLEIA). Alkaline phosphatase (ALP) labeling is used to detect chemiluminescence. CDP-Star, the fastest and most sensitive chemiluminescence substrate, is utilized in this assay.

For TT3 and TT4 measurements, the Cobas assay is widely used in clinical laboratories while the HISCL assay is a relatively new one in China. The comparison of the methods was evaluated in this study according to CLSI's new guideline (EP09-A3) which was approved in 2013 [5].

2. Materials and Methods

This study was performed between July 2021 and January 2022. A total of 111 and 110 serum samples were collected and investigated in this clinical evaluation for TT3 and TT4 measurement respectively, including interfering samples (hemolysis, chylous, jaundice, and rheumatoid factors), samples of toxic diffuse goiter, hyperthyroidism, hypothyroidism, thyroiditis, other thyroid diseases, other diseases, and healthy cases. All the human samples in this experiment were approved by the Chinese Ethical Committee.

The results of HISCL 5000 were compared with that of Cobas e602 according to the CLSI EP09-A3. The comparison devices were previously approved for the corresponding specimen type. Each sample was tested with all instruments serially in random order to avoid potential bias caused by the tested sequence. The total duration of time taken for a sample to be tested with both instruments was less than 5 min.

Medical decision points (MDP) were required for comparison to show proportional or systematic differences or bias. Statistical analyses were performed to figure out the data which may indicate the relationship between the assays. In addition to some basic data management, such as the calculation of mean, difference, and proportional differences, there are some specific analyses required in EP09-A3.

The extreme studentized deviate (ESD) technique, which assumes that the distribution of the vast majority of data points is normal, was performed to detect aberrant results (outliers). The significance level (α) was set to 0.05. Potential outliners from graphical or other reviews of the dataset were determined. The upper bound on number (h) could be no more than 5% (For both TT3 111 samples and TT4 110 samples, h equaled 5). The average ($\bar{d}$) and standard deviation (SD) were computed, including the suspected outliers. The maximum observed deviate ($ESDi$) and critical values λ_i were statistically calculated according to equations demonstrated in the EP document. The number of outliers could be determined by finding the largest i such that $ESDi > \lambda_i$.

Regression methods, including ordinary linear regression (OLR), weighted least squares (WLS) regression, Deming regression, weighted Deming regression, and Passing-Bablok (P&B) regression, were utilized in the measurement comparison. Bias results were estimated and interpreted using the procedure described in CLSI document EP09-A3 as a pilot study.

Microsoft Excel plugin Analyse-it and MedCalc were utilized for statistical operation.

3. Results

The performed ESD technique showed no outlier identified since all the maximum deviate ESD_i were below the critical level λ_i, which is listed in Table 1.

Table 1. Results of ESD technique for TT3 and TT4 outlier determination

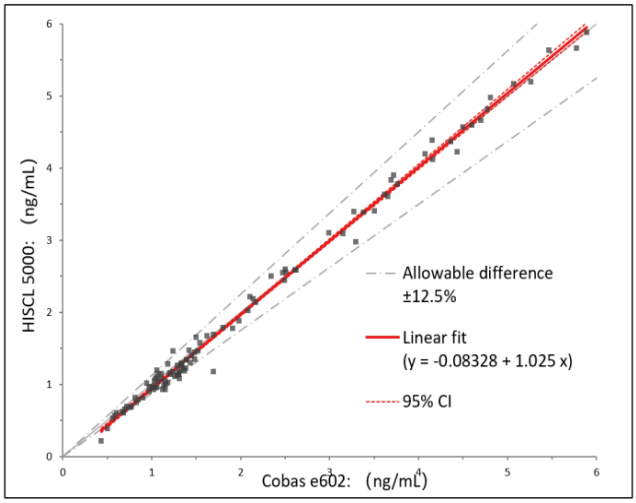
	Parameter	Potential outlier				
		i =1	i =2	i =3	i =4	i =5=h
TT3	$ESDi$	2.5655	2.5307	2.5076	2.4925	2.4598
	λ_i	3.6025	3.5987	3.5947	3.5907	3.5865
TT4	$ESDi$	2.9736	2.9343	2.8922	2.8781	2.8715
	λ_i	3.6016	3.5977	3.5937	3.5896	3.5854

Notes: Abbreviation: *ESD*, extreme studentized deviate; λ_i, critical value.

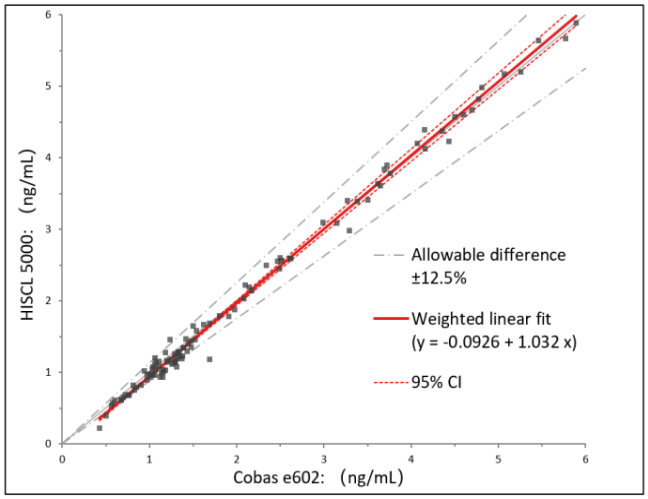
Scatter plots of 5 different regression methods for TT3 and TT4 analytical methods comparison were demonstrated in Figure 1 and Figure 2, respectively. According to EP09-A3 document, no suspected obvious aberrant results were observed in the scatter plots.

There is a constant SD, an even distribution of points over the measured interval, and an r^2 of 0.994, OLR method, which is demonstrated in Figure 1A and Figure 2A could be selected as a suitable technique to evaluate the bias. The equations of OLR measuring TT3 and TT4 are Y=-0.08328+1.025X and Y=-1.653+0.9952X, respectively. Refer to the new EP guideline, the constant SD Deming technique was also listed in the following figures (C), with equations as Y=-0.08937+1.028X and Y=1.437+0.9973X, which gave relatively imprecision considered options. For constant Coefficient of Variation (CV), regression equations are Y=-0.0926+1.032X and Y=-0.7854+1.026X for WLS (Figure 1B & 2B), Y=-0.1221+1.050X and Y=-0.9677+1.028X for weighted Deming regression (Figures 1D &2D). Besides, the results in Figures 1E and 2E demonstrate Passing-Bablock regression equations as Y=-0.09578+1.031X for TT3 and Y=2.097+0.9919X for TT4. All the correlation factors for the regression techniques are greater than 0.95. Expect for several individual points, all the regression results are within the allowable difference limits.

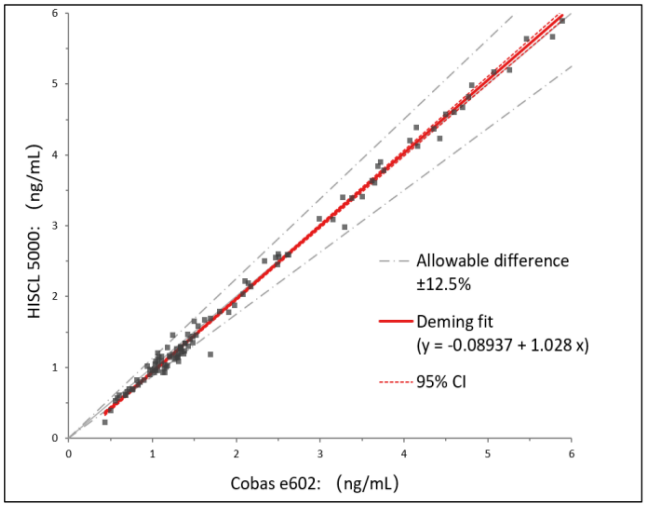
All statistically analyzed data for method comparison of the Cobas ECLI assay and HISCL CLEIA assay are given in Table 2. The MDPs of TT3 are 0.75ng/mL and 1.91ng/mL. Biases of the points are calculated and evaluated as acceptable situations (type A and B) except for weighted Deming outcomes. 95%CI of bias computed by weighted Deming is between -17.8% to -4.7%, which can be classified as type C outcome. For TT4, with MDPs referenced as 51ng/mL and 141ng/mL, the biases are completely classified as acceptable outcomes (type A and B). All calculations, interpretation and classification follow the new EP09 document.



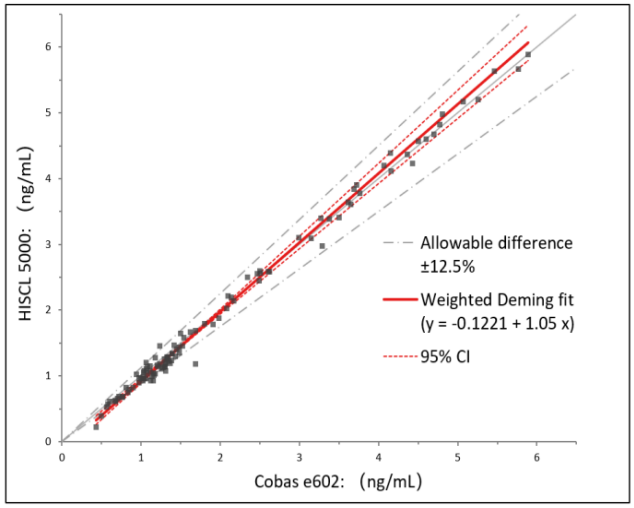
A



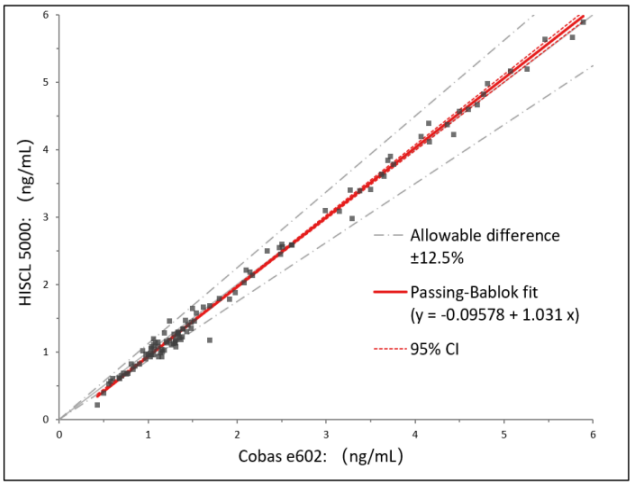
B



C

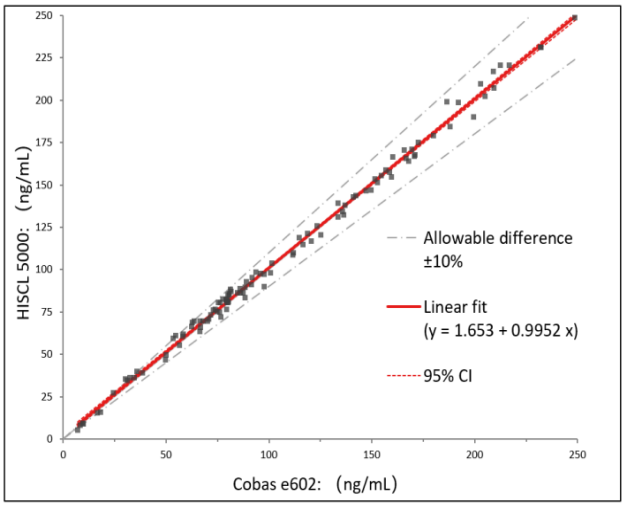


D

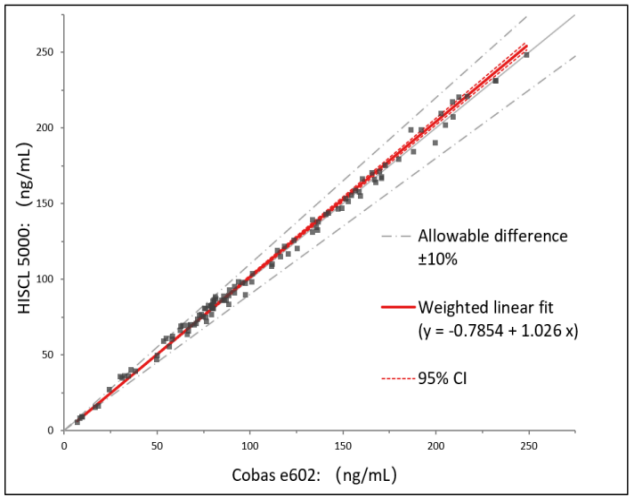


E

Figure 1. Regression plots of method comparison between Cobas e602 and HISCL 5000 assays measuring TT3. A: OLR; B: WLS regression; C: Deming regression; D: weighted Deming regression; E: P&B regression.



A



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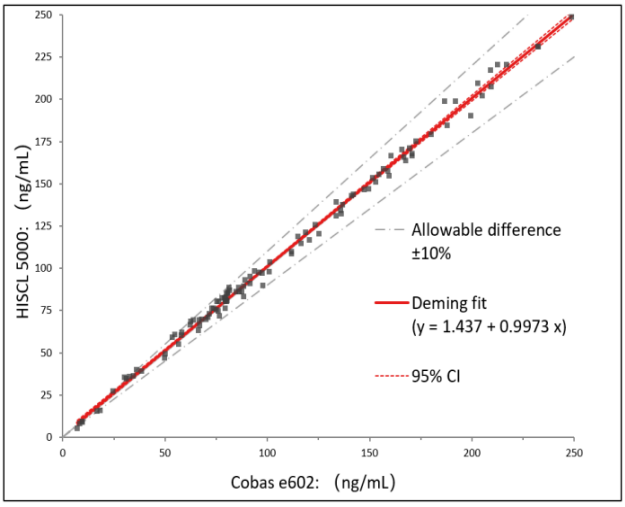
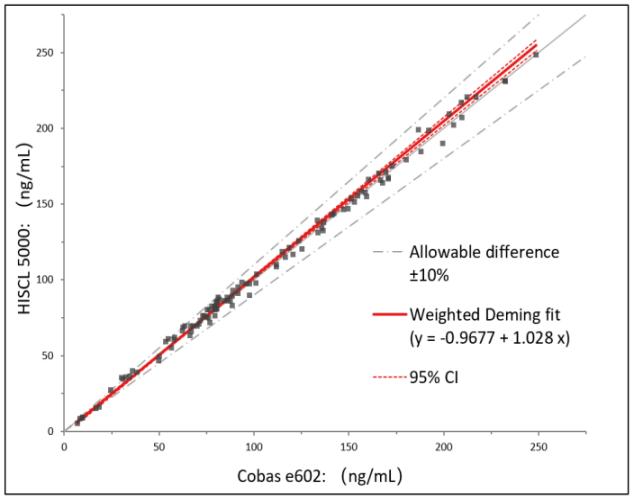
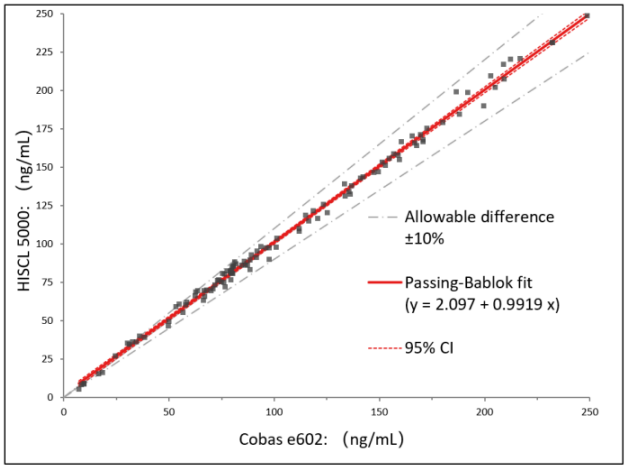


C



D



E

Figure 2. Regression plots of method comparison between Cobas e602 and HISCL 5000 assays measuring TT4. A: OLR; B: WLS regression; C: Deming regression; D: weighted Deming regression; E: P&B regression.

Table 2. Comparison summary of Cobas e602 and HISCL 5000 measuring TT3 and TT4

Analyte	Regression	Equation	MDP (Xc)	Predicted value (Yc)	%Bias	95%CI	Allowable difference	Outcome type	Acceptability
TT3	OLR	Y=-0.08328+1.025X	0.75	0.69	-8.6%	-12.2% to -4.9%	±12.5%	B	Acceptable
			1.91	1.88	-1.8%	-2.9% to -0.7%	±12.5%	B	Acceptable
	WLS	Y=-0.0926+1.032X	0.75	0.68	-9.2%	-11.7% to -6.7%	±12.5%	B	Acceptable
			1.91	1.88	-1.7%	-3.3% to -0.1%	±12.5%	B	Acceptable
	Deming	Y=-0.08937+1.028X	0.75	0.68	-9.1%	-12.5% to -5.7%	±12.5%	B	Acceptable
			1.91	1.87	-1.8%	-2.9% to -0.8%	±12.5%	B	Acceptable
	W-Deming	Y=-0.1221+1.050X	0.75	0.67	-12%	**-17.8% to -4.7%**	**±12.5%**	C	Acceptable/ Unacceptable
			1.91	1.88	-1.4%	-3.1% to 0.4%	±12.5%	A	Acceptable
	P&B	Y=-0.09578+1.031X	0.75	0.68	-9.7%	-11.6% to -6.5%	±12.5%	B	Acceptable
			1.91	1.87	-1.9%	-2.7% to -0.9%	±12.5%	B	Acceptable
TT4	OLR	Y=-1.653+0.9952X	51	52.41	2.8%	0.9% to 4.6%	±10%	B	Acceptable
			141	141.98	0.7%	0.1% to 1.3%	±10%	B	Acceptable
	WLS	Y=-0.7854+1.026X	51	51.56	1.1%	0.1% to 2.1%	±10%	B	Acceptable
			141	143.92	2.1%	1.0% to 3.1%	±10%	B	Acceptable
	Deming	Y=1.437+0.9973X	51	52.30	2.5%	0.9% to 4.1%	±10%	B	Acceptable
			141	142.05	0.7%	0.0% to 1.4%	±10%	A	Acceptable
	W-Deming	Y=-0.9677+1.028X	51	51.46	0.9%	-0.4% to 2.2%	±10%	A	Acceptable
			141	143.98	2.1%	1.0% to 3.3%	±10%	B	Acceptable
	P&B	Y=2.097+0.9919X	51	52.68	3.3%	0.5% to 5.6%	±10%	B	Acceptable
			141	141.96	0.7%	-0.3% to 1.5%	±10%	A	Acceptable

Notes: 95%CI bias values and Tea (%) are in bold if the difference is beyond the total allowable error.

4. Discussion

The assays for detecting the two thyroid hormones are universally utilized in hospitals. However, the results sometimes do not as resembling to each other, which may make medical diagnoses uncertain or pathologic decisions complicated. The CLSI new document EP09-A3 for methods comparison is a professional and scientific guide that

can be used to standardize the performance and evaluate the differences and biases of a great variety of systems. Nevertheless, since it was officially replaced with EP09-A2 in August 2013 [6], EP09-A3 has still not been widely used in medical laboratories [7].

This study completely followed the requirements described in EP09-A3 for method comparison evaluation (Cobas system & HISCL system). For validation or evaluation of newly introduced procedures or systems in clinical laboratories, it is recommended that clinical laboratories should analyze at least 40 samples to establish the bias between measurement procedures. Unlike raw-number-ordered sequencing measurement required in EP09-A2, EP09-A3 stipulates that samples can be measured once and in a random order [8-9]. In terms of the detection of outliers, a scatter plot and visual judgement are required and then followed by the ESD statistical analysis. If some outliers are observed and excluded, the same number of points shall be added to continue the ESD analysis until there are no outliers at all. The maximum number of potential outliers of 111 and 110 samples in this study is 5 which is required as a proportion of less than 20% [10, 11]. No outliers were confirmed by this analysis.

The optimal technique used to determine the bias between the candidate (HISCL) and comparative (Cobas) measurement procedures is highly dependent upon whether the data meet specific underlying assumptions. Whether the variability of differences between the two measurement procedures is constant or proportional to concentration should be determined [12-14]. There is also a visual judgement first performed to demonstrate the distribution and frequency of difference. Secondly, different regression techniques can be performed to conform to the assumption, and the equations are calculated to evaluate the results. It can be noticed that regression techniques are properly classified as they may be applied in two different situations identified as constant SD and constant CV in the EP document [15-18]. 95%CI of intercepts and slopes derived from the equations may be required to be below the total error allowable. Remarkably, all the correlation factors for the regression techniques are greater than 0.95, showing a relatively high correlation between the Cobas system and the HISCL system. In our study, 5 regression techniques results are listed and compared. Eventually, the TT4 results, which showed constant standard deviation, obtain acceptable biases from the regression analyses; and the 95%CI results of the bias marked at MDPs are all within the error limits as type A&B outcomes. However, when the TT3 results are analyzed, except the other four techniques, the weighted Deming results showed estimated bias (-12%) within the predefined limits of acceptance (±12.5%), but the CI (-17.8% to -4.7%) is not (outcome C). It cannot be judged that acceptance criteria were met with 95% confidence. According to EP09-A3, the CI includes the highest acceptable bias limit may be concluded that the bias is acceptable. However, Outcome C has less confidence than Outcome A and Outcome B and may be defined as having bias outside the limits.

5. Conclusion

In conclusion, this clinical laboratory-based study demonstrated excellent analytical performance and results for the two systems measuring TT3 and TT4. The biases are acceptable. The new guide EP09-A3, with some changes in operations, classifications, and interpretations, makes the evaluation more reasonable and scientific.

Data availability

The authors declare that the data supporting the findings of this study are available within the paper and its Supplementary Information files. Should any raw data files be needed in another format they are available from the corresponding author upon reasonable request.

Conflict of interest

Yongwei Chen, Jing Wang, Xiaolong Yang, Fangyu Hu, and Fang Lin declare no potential conflicts of interest.

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