Implications of Adjustment of High-Sensitivity Cardiac Troponin T Assay

To the Editor:

We read with great interest the letter published in September by Apple and Jaffe regarding the recent adjustment to the calibration of a high-sensitivity cardiac troponin T (hs-cTnT) 1 assay (1). The authors raised several important questions regarding the clinical implications of this assay recalibration and pointed out that no correlation studies had been performed for the earlier and new reagent lots.

Although we received no information from Roche Diagnostics in France, we observed a shift in the distribution of troponin T concentrations in our university hospital laboratory when we used new reagent lot 167650 instead of lot 164776. Indeed, the overall percentage of the results below the limit of detection (LoD) (<5 ng/L) decreased from 22.7% (from May 1 to June 15, 2012; n = 3696) to 5.8% (from June 20 to July 30, 2012; n = 3097), and the median reported concentration increased from 25.3 ng/L to 29.8 ng/L.

We thus decided to measure hs-cTnT with the former and recalibrated lots (lot numbers 164776 and 167650, respectively) in samples available from a cohort of 311 elderly patients [median (interquartile range), 81.5 years (75.8–84.9 years)]. These samples had been frozen at −80 °C before analysis. The study was approved by the ethics committee of Montpellier, informed consent was obtained from all patients, and the collection was registered in the Ministère de la Recherche (DC-2008–417). Although this population cannot be considered a healthy reference population, the differences observed between the measurements with each reagent lot provided preliminary information on the consequences of this recalibration for concentrations ≤100 ng/L.

First, use of the former non-recalibrated lot produced hs-cTnT concentrations <5 ng/L in 94 patients (30.2%); however, only 6 patients had results below the LoD for the same samples measured with the recalibrated reagent lot [median (interquartile range), 8.9 ng/L (7.4–9.5 ng/L); maximum concentration, 11.8 ng/L]. We used Passing–Bablok regression to compare the results obtained with recalibrated and earlier lots (Fig. 1). We obtained the following relationship: y = 0.93x + 6.18 ng/L (Pearson r = 0.992), where y is the hs-cTnT value obtained with lot 167650 and x is the hs-cTnT value obtained with lot 164773. The median between-lot difference in concentration was 5.8 ng/L at 5–8 ng/L, 5.2 ng/L at 8–20 ng/L, and 4.2 ng/L at 20–50 ng/L.

These results are consistent with the information provided by Roche Diagnostics and seem to indicate a bias of approximately 6 ng/L for low concentrations that decreases slightly as the troponin T concentration increases. Bias was also observed for concentrations >20 ng/L up to 70 ng/L. Because the bias was quite large for concentrations of 5–20 ng/L, the distribution of hs-cTnT concentrations measured in healthy populations after recalibration is likely to shift upward. This shift carries many implications, which Apple and Jaffe have listed. The percentage of concentrations below the LoD will likely decrease dramatically. As Apple and Jaffe have pointed out, a progressive increase in the percentage of undetectable results has been observed over the past few years, and the Roche calibration correction likely will yield many more samples with detectable concentrations. The design of our study, however, does not allow us to speculate about a potential shift of the 99th percentile value, because troponin concentrations will be higher in older patients. Apple et al. recently reevaluated the 99th percentile with a new lot (lot 167345) and reported a 99th percentile value very close to 14 ng/L (2).

Applying the formula obtained from our correlation analysis to routine results measured in our laboratory with the earlier lot gave a corrected distribution with a median of 29.6 ng/L. Although use of this conversion formula allows correction of bias due to the recalibration, this process does not allow recalculation of concentrations that are below the LoD. Furthermore, recalculation of concentrations with the new calibration curve is not straightforward. hs-cTnT is calibrated with a stored 5-point master curve (3), which is not available to users, and a 2-point on-site calibration. Recalculation from calibration curves requires access to raw measurement data, which are not easily available. Although the results of recalibration have been suggested to be independent of reagent lot (4), whether the calibration issues remained constant across the incriminated lots is unknown.

Because recalibration of troponin assays could have major implications for patient care (including the classification of acute coronary syndrome) going forward, manufacturers need to provide accurate information to users proactively and transparently. In addition, determination of a lot-to-lot reference change value for use with an external quality control around the 99th percentile could help users appreciate the biological relevance of changes in reagent lots.

1 Nonstandard abbreviations: hs-cTnT, high-sensitivity cardiac troponin T (assay); LoD, limit of detection.
**Fig. 1.** Correlation analysis of earlier and recalibrated lots.

Black line represents the line of identity. Red line indicates the Passing–Bablok regression line ($y = 0.93x + 6.18$ ng/L) used to evaluate the relationship between the reagent lots. Gray zone represents the 95% CI for regression, determined via bootstrapping (1000 bootstraps).

**References**


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To the Editor:

Apple and Jaffe recently discussed potential implications of low-end adjustment of the Roche Diagnostics Elecsys® Troponin T high-sensitivity assay (TnT hs)1 (1), because we represent the product’s manufacturer, we reply to their questions. This fifth-generation assay was launched outside the US in 2009 and is not yet commercially available in the US.

In recent studies of the reference interval, we observed a downward shift in the TnT hs assay at low concentrations of the measurement interval (3–10,000 ng/L; Fig. 1A). This value was confirmed to be 2.7 ng/L in internal lot-to-lot comparisons (Fig. 1B) and reported as 3–5 ng/L in recent customer reports. This shift caused up to 88% of the samples from a healthy cohort to be measured with values below the limit of the blank (3 ng/L), rather than ≤50% of samples, as was observed in the initial multicenter evaluation (MCE) study (2). Apple and Jaffe detected the shift by using maximum values of individual samples rather than mean values. A review of Fig. 1A may help clarify this aspect.

We retrospectively identified the following 6 affected lots: 157120, 160197, and 163704 for the TnT hs 18 min assay and lots 158268, 161334, 164773 for the TnT hs STAT assay produced between 2009 and 2011. The criteria for lot release were fulfilled for all lots produced since the introduction of the assay. A subsequent analysis of the raw data (signal counts) confirmed that this shift was not caused by a reagent abnormality but by the acceptance criteria of standardization at the very low end of the calibration curve, which were too broad. To prevent such shifts in the future, we adjusted the standardization procedure by instituting more-stringent release criteria (see point 4 below). This optimization was first introduced in 2012 with lots 167345 (TnT hs 18 min assay) and 167650 (TnT hs STAT assay) to ensure that the low-end recovery was similar to that of the original MCE, in which 57% of the samples from the healthy population had measured values ≥3 ng/L (limit of the blank) and 32% had values ≥5 ng/L, the limit of detection. Specifically, we obtained a 99th percentile value of 14.4 ng/L in a single-center study that used lot 167345 (n = 739; median age, 49 years). This standardization also ensures that the 99th percentile decision threshold remains at 14 ng/L, as originally specified in the MCE (2).

The identification of approximately 50% of the healthy individuals with TnT values ≥3 ng/L has been confirmed in other studies (3, 4). Apple and Jaffe might have overlooked the facts that some of the cited studies had used the precommercial assay that produced preliminary performance data and that stable cardiovascular disease was the basis for selecting the study population in the PEACE (Prevention of Events with Angiotensin Converting Enzyme Inhibition) study (5).

Our answers to the specific questions are as follows:

1. The well-established 99th percentile value of approximately 14 ng/L is preserved for all lots released since 2012. For the 6 lots with the downward shift, the 99th percentile was lower by 3–5 ng/L (for additional information, please refer to our recent product management letter to customers no. 57/2012).

2. The readjustment of the standardization does not lead to a percentage increase in the detectable results (i.e., a limit of detection greater than that obtained in the MCE), but only compared with the affected lots. All lots introduced since 2012 present the original recovery concentration and the MCE study’s 99th percentile value of approximately 14 ng/L. Compared with the affected lots, the restandardization caused a percentage increase in the detectable results that was greater than the limit of detection (5 ng/L) for 27.6% (n = 1033) of the patients presenting to the emergency department with symptoms of suspected acute coronary syndrome.

3. The use of troponin to aid in the diagnosis of acute myocardial infarction (AMI) requires troponin results to be assessed in conjunction with the patient’s medical history and a clinical examination. Especially at low troponin concentrations, guidelines require that the diagnosis of AMI not be based on a single troponin measurement but rather on the detection of an increase/decrease in the troponin concentration (i.e., a change, Δ) so that AMI can be distinguished from chronic troponin increases.

References

1 Nonstandard abbreviations: TnT hs, Roche Diagnostics Elecsys® Troponin T high-sensitivity assay; MCE, multicenter valuation; PEACE, Prevention of Events with Angiotensin Converting Enzyme Inhibition (study); AMI, acute myocardial infarction.