The Effect of Sample Hemolysis on Cardiac Troponin I and T Assays

To the Editor:

Cardiac troponin (cTnI and cTnT) assays are used for the diagnosis of acute myocardial infarction (AMI) (1). Recent improvements in these assays have lowered the imprecision and detection limit so that the assays meet guidelines for using the 99th percentile cutoff concentration. These new cTn assays have the potential to improve clinical practice by earlier diagnosis, improved risk stratification, and improved monitoring of patients. They also, however, increase the number of true- and false-positive results for patients suspected of AMI (2). Hemolysis is the most common preanalytical interference encountered in the routine laboratory. I tested the effect of sample hemolysis on 1 contemporary and 1 high-sensitivity (hs) cTn assay.

I prepared hemolysate from erythrocytes that had been washed 3 times with 0.9% saline. After the final centrifugation, the cells were diluted with an equal volume of distilled water, thoroughly mixed, and lysed by freezing. I measured cTnI using the Ortho Clinical Diagnostics TnI ES assay (contemporary) on the Vitros® 5600 Integrated System and cTnT using the Roche TnT hs assay on the Elecsys E170 immunoassay system, which was part of a modular integrated system. To measure the effect of hemolysate on cTnI and hs cTnT, thawed hemolysate was added to lithium heparin plasma samples that contained concentrations of cTn selected to be around the 99th percentile cutoffs for the respective assays, namely 34 ng/L for cTnI (24, 36, 49 ng/L) and 13 ng/L for cTnT (6, 12, 23 ng/L). Indices were measured as recommended by the manufacturers. The Vitros 5600 measures the indices using the residual sample left in the sample tip. The Modular measures indices on the chemistry module by taking an aliquot of the patient specimen and diluting it in 0.9% NaCl. For both instruments, algorithms convert the absorbance measured at wavelength pairs into qualitative values that correlate with estimated concentrations of the sample interferent.

According to the recommendations of the National Academy of Clinical Biochemistry, a 20% change in cTn value is suggestive of an acute myocardial infarction that is either evolving (cTn increasing) or resolving (cTn decreasing) (3). For both assays, a hemolysis index of around 150 caused a >20% change in cTn (Fig. 1), which equates to a hemoglobin concentration of 1.9 g/L. It has been suggested that at baseline concentrations of cTn, δ changes of >20% are needed for improved clinical specificity and, thus, laboratories must consider carefully what constitutes a clinically significant change in cTn (4).

One important aspect of these experiments is that they were carried out at cTn concentrations close to the 99th percentile for each assay. When the same experiments were done at higher cTn concentrations, a clinically significant effect (for example, ±20%) was not observed. This is understandable because a change of 10 ng/L at a concentration of 10 ng/L represents a 100% change, whereas at 100 ng/L it represents a 10% change, demonstrating the need to do these types of experiments at critical concentrations for any analysis.

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1 Nonstandard abbreviations: cTn, cardiac troponin; AMI, acute myocardial infarction; hs, high sensitivity.
lyte. Many reagent package inserts contain limited information on interferences and often only on what concentration of interfering material interferes with the assay, with no information on what concentrations of analyte were tested. If the analyte concentration was relatively high, the effect of the interfering material may not be observed. There is no indication of what concentrations of cTnT were tested. In the cTnI brochure, there is a table indicating the effect of increasing sample Hb, but this was tested at 0.006 μg/L, which is half the stated limit of detection for the assay.

Hemolysis has been reported to be as high as 8.8% for samples collected in an emergency department (5). In my own hospital, the number of cTnT requests from the emergency department rejected because of hemolysis interference is 3.9%. Studies indicate that the contemporary cTnI and high-sensitivity cTnT assays I tested are sufficiently affected at relatively low degrees of hemolysis to indicate that interference must be monitored for every specimen. With the advent of integrated analyzers that incorporate both chemistry and immunoassays, I advocate that laboratories investigate how interferences such as hemolysis affect key assays, and that indexes be measured on all samples for which cTn has been requested. If this is not possible, at least a visual examination of the sample quality should be done before analysis.

Author Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

Authors’ Disclosures of Potential Conflicts of Interest: Upon manuscript submission, all authors completed the Disclosures of Potential Conflict of Interest form. Potential conflicts of interest:

Employment or Leadership: None declared.
Consultant or Advisory Role: None declared.
Stock Ownership: None declared.

Honoraria: R. Bais, for presentation at workshop for Ortho Clinical Diagnostics.
Research Funding: R. Bais, Roche Diagnostics Australia, support for a research project on NTproBNP; Ortho Clinical Diagnostics (Australia) and Roche Diagnostics (Australia), troponin kits.
Expert Testimony: None declared.
Other Remuneration: R. Bais, Roche, supported to attend AACC meeting, Chicago 2009, and AACB meeting, Brisbane 2009.

Role of Sponsor: The funding organizations played no role in the design of study, choice of enrolled patients, review and interpretation of data, or preparation or approval of manuscript.

Acknowledgments: The author acknowledges Ortho Clinical Diagnostics (Australia) and Roche Diagnostics (Australia) for their support of troponin kits for these studies.

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Previously published online at DOI: 10.1373/clinchem.2010.144139