Giovanni Baviera*
Saveria Chimicata
Roberta De Domenico
Roberta Granese
Camillo Carbone
Nella Dugo
Rosario D’Anna

University of Messina
Azienda Ospedaliera Universitaria Viale
del Policlinico 1
Messina, Italy

* Address correspondence to this author at:
Department of Obstetrics and Gynecology
Azienda Ospedaliera Universitaria Viale
del Policlinico 1
Messina 98125, NA, Italy
Fax +39-090-221201
E-mail bavierag@unime.it

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

To the Editor:

Cardiac troponin I and T (cTnI and cTnT)1 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 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 interference.

According to the recommendations of the National Academy of Clinical Biochemistry, a 20% change in cardiac troponin value is suggestive of an acute myocardial infarction that is either evolving (cardiac troponin increasing) or resolving (cardiac troponin 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 cardiac troponin (4).

One important aspect of these experiments is that they were carried out at cardiac troponin concentrations close to the 99th percentile for each assay. When the same experiments were done at higher cardiac troponin 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 analyte. 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. The hs cTnT brochure states that samples are unaffected by Hb <0.1 g/dL, and samples showing visible signs of hemolysis may be interfered. 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

1 Nonstandard abbreviations: cTnI and cTnT, cardiac troponin I and T; AMI, acute myocardial infarction; hs, high sensitivity.
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 indices be measured on all samples for which cardiac troponin has been requested. If this is not possible, at least a visual examination of the sample quality should be done before analysis.

**Fig. 1.** Effect of increasing added hemolysis on the Ortho Clinical Diagnostics TnI ES assay (open symbols) and the Roche TnT hs assay (closed symbols).

A 20% change was considered clinically significant. The 3 cTnI concentrations were 24 ng/L (◇), 36 ng/L (□), and 49 ng/L (△), and the 3 cTnT concentrations were 6 ng/L (◇), 12 ng/L (□), and 23 ng/L (△). (Note that the negative and positive scales are not equal.)


Renz Bais
Pacific Laboratory Medicine Services (PaLMS)
Northern Sydney Central Coast
Pathology North
Royal North Shore Hospital
and Sydney Medical School
Sydney University, Sydney, Australia

*Address correspondence to the author at:
Executive Unit
Level 5
Royal North Shore Hospital
St Leonards NSW Australia 2065
Fax +61-2-9926-6395
E-mail rbais@med.usyd.edu.au

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Assessing the Performance of Point-of-Care Hemoglobin A1c Systems

To the Editor:

Lenters-Westra and Slingerland (1) recently reported results of a comparative performance study of several point-of-care (POC)1 tests for hemoglobin A1c (Hb A1c), and we commend them for providing useful new information on instrument performance. We also believe that the results should be interpreted strictly in terms of the facts, including consideration of discrepancies between the study design and the instructions provided by the manufacturers.

Bayer’s A1CNow+® Multi-Test A1C System was included in the initial phase of this study, but testing was not continued for the main phase of the study after the local distributor of that product concluded that the preliminary CLSI EP-10 results did not warrant further testing. The authors reported that these results were probably due to EDTA interference. As a POC test, the A1CNow+ test is primarily intended for capillary blood samples, and the instructions for use specify use of heparin-containing collection tubes when using venous blood.

The authors’ title, “Six of Eight Hemoglobin A1c Point-of-Care Instruments Do Not Meet the General Accepted Analytical Performance Criteria,” and its conclusion are misleading when 7 instruments at most were used according to the manufacturers’ operating instructions; thus, performance conclusions are warranted only for these 7 instruments. No conclusion should be drawn regarding the performance of the A1CNow+ test because it was not used according to manufacturer’s labeling in the preliminary stage and thus was not included in the final study. In the Results section of the Abstract, the authors state that 2 of 8 manufacturers decided not to continue the study because of the disappointing EP-10 results, a simplification that omits the relevant fact that inappropriately obtained blood samples were used for the A1CNow+ test.

In the same issue of this journal, Bruns and Boyd (2) contributed an editorial offering further interpretation and an opinion of the Lenters-Westra and Slingerland report. They paraphrase the same misinterpretation by stating, “Two of the 8 manufacturers withdrew from the study after initial unpromising results with their POC methods.” This statement again misrepresents the reason for the withdrawal in the case of the A1CNow+ test and further supports our belief that there will be misunderstandings because of the conclusions and overall impression provided by the report.

Furthermore, the Results section and the Acknowledgments at the end of the Lenters-Westra and Slingerland report indicate that the study authors communicated directly with a local unaffiliated distributor for the Bayer A1CNow+ device in lieu of direct communication with the manufacturer (Bayer). We point out that Bayer was not asked to comment on the study protocol before its execution and thus did not have the opportunity to comment on the resulting negative bias when the test was used with EDTA-containing blood.

A challenge to future researchers (and their reviewers) examining the performance of POC Hb A1c devices would be to include an analysis of all the relevant information to provide a broader context for interpretation. In Lenters-Westra and Slingerland’s report, it is apparent that there was variation among the laboratory reference methods, although they were all controlled and calibrated in the authors’ laboratory. Reference 17 in their report is cited as a source of concern regarding the accuracy of

1 Nonstandard abbreviations: POC, point-of-care; Hb A1c, hemoglobin A1c.