

## Development of the Cardiac Troponin T Immunoassay

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**Featured Article:** Katus HA, Looser S, Hallermayer K, Remppis A, Scheffold T, Borgya A, et al. Development and in vitro characterization of a new immunoassay of cardiac troponin T. *Clin Chem* 1992;38:386–93.<sup>2</sup>

This paper was the first report on the development and analytical characterization of an immunoassay for cardiac troponin T (cTnT)<sup>3</sup> that was useful for large-scale clinical chemistry testing. Preceding methods we had generated for measurement of cTnT using a pilot immunoassay yielded promising clinical data in patients with chest pain, indicating a higher diagnostic sensitivity and specificity than measurement of cardiac enzymes for detection of myocardial injury. Whereas the pilot assay used polyclonal antisera, the novel sandwich assay employed 2 preselected monoclonal antibodies with high cardiospecificity, thereby decreasing cross-reactivity with skeletal muscle troponin T to <0.5%. This cTnT assay allowed measurement of cTnT in only 90 min with a between-run precision below 5%. The assay was manufactured by Boehringer Mannheim (Enzymun-Test System) and provided sufficient analytical sensitivity and precision to be employed in large multicenter trials, which ultimately led to the redefinition of acute myocardial infarction (AMI).

This report was the result of more than 15 years of scientific work stimulated by 2 circumstances. The first opportunity was my medical thesis work on myocardial function and muscle physiology, which provided knowledge of marked differences of contractile properties of isolated cardiac and skeletal muscle myofibrils and preliminary data on the existence of a tissue-specific isoform composition of sarcomeric proteins. The second was my research fellowship in the laboratory of E. Haber at Massachusetts General Hospital from 1978–1980. The research program (led by Ban An

Khaw) was initially designed to use radioactively labeled antibodies against myosin heavy-chains for imaging of AMI. I rapidly switched to the development of blood assays for sarcomeric proteins, however, because I assumed that if infarct imaging with antimyosin antibodies is possible over such extended time intervals, a prolonged release of the large pool of sarcomeric proteins into blood must also occur. An in vitro assay would then provide a much more rapid and inexpensive method for the diagnosis of AMI compared to imaging modalities. At that time we focused on cardiac myosin light-chains, but back in Heidelberg we stopped this work because we observed significant cross-reactivity with skeletal muscle myosin light-chains. In 1982, while analyzing the specificity of polyclonal goat antihuman cardiac myosin–light-chains antisera, we detected incidentally a cardiospecific antibody fraction directed against cTnT contaminations of the myosin light-chains antigen. This observation led to the purification of cTnT, the generation of monoclonal antibodies, the development of a pilot enzyme immunoassay for circulating troponin T (1), and the improved assay highlighted here.

After the initial publication on cTnT, more than 11 years of continuous work was required before measurement of cTn as a cardiac marker was finally established in the clinical community. Key to its success was the proof for troponin T—and importantly also troponin I—of absolute cardiospecificity and improved risk prediction in chest pain patients in many prospective multicenter trials. Most convincing was the finding that a positive test result had therapeutic consequences, because troponin-positive (but creatine kinase-MB-negative) patients benefited from more aggressive platelet inhibition and early coronary intervention. In 2000 a working group of the European Society of Cardiology/American College of Cardiology/American Heart Association introduced the redefinition of AMI, which replaced the WHO definition from 1979 (2, 3). This redefinition endorsed the diagnostic use of cTns instead of cardiac enzymes or creatine kinase-MB mass. In light of additional trial results supporting the usefulness of cTn for risk stratification and guidance of therapy in non-ST-segment elevation–acute coronary syndrome, in 2007 the joint European Society of Cardiology/American College of Cardiology Foundation/American Heart Association/World Heart Federation

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<sup>2</sup> This paper has been cited 330 times since publication.

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<sup>3</sup> Nonstandard abbreviations: cTnT, cardiac troponin T; AMI, acute myocardial infarction.

Task Force launched an updated universal definition of MI supported by the European and North American Societies of Cardiology and the World Heart Federation (4).

CTNs will remain the gold standard for diagnosis of AMI because no superior cardiospecific biomarkers are on the horizon. The development of highly sensitive and precise assays, however, will eventually reveal reference interval values and allow the detection of even lesser degrees of cardiac injury, challenging the definition of MI. Thus it will become paramount to investigate the predictive power and therapeutic implications of troponin concentrations below the currently used discriminator values for MI. The use of highly sensitive assays will open a fascinating perspective for investigating the distressed heart but may also cause confusion regarding AMI definition and its impact in clinical medicine and public health.

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### Editor's Comment

The test principle shown here is from the 1992 featured article. The monoclonal antibodies, enzyme, and substrate used in the original assay provided results in 90 minutes. The principle behind the assay was a one-step sandwich colorimetric ELISA protocol. Sandwich immunoassays are still the primary methods for quantifying troponins T and I in plasma. However, improvements in the affinity of the capture and signal antibodies, plus the use of detection systems such as fluorescence, chemiluminescence, or electrochemiluminescence, have allowed manufacturers to produce assays with reduced specimen volume requirements, lower limits of quantification, and reduced turnaround times of 20 minutes or less.

