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# Mass spectrometric ITEM-FOUR analysis reveals coding single nucleotide polymorphisms in human cardiac troponin T that evade detection by sandwich ELISAs which use monoclonal antibodies M7 and M11.7 from the Elecsys Troponin T® assay

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Immunoassays for cardiac troponin, such as the Elecsys® hs-TnT, have become the gold standard for myocardial infarction diagnostics. While various protein/chemical factors affecting the troponin complex and, thus, its diagnostic accuracy have been investigated the role of coding single nucleotide polymorphisms remains underexplored. To evaluate potential cSNP-induced interference with antibody binding in the Elecsys® hs-TnT immunoassay, we applied ITEM-FOUR, a mass spectrometry-based method that quantifies changes in antibody binding upon amino acid substitutions in epitope pep-tides. Candidate cSNPs were selected from the dbSNP database and were mapped to human cardiac troponin T by molecular modeling. Consuming micromolar antibody concentrations and microliter sample volumes, two wild-type and 17 cSNP-derived variant epitope peptides—six for monoclonal antibody M7 and eleven for monoclonal antibody M11.7—were investigated to reveal the binding motifs 'V131-K134-E138-A142' for M7 and 'E146-I150-R154-E157' for M11.7. Loss of binding to M11.7 was observed for substitutions Q148R (rs730880232), R154W (rs483352832), and R154Q (rs745632066), whereas the E138K (rs730881100) exchange disrupted binding of M7. Except for cSNP Q148R they are associated with cardiomyopathies, placing affected individuals at risk for both, underlying heart disease and false-negative hs-TnT assay results in case of myocardial infarction. Our results highlight the need to account for cSNP-related interferences in antibody-based diagnostics. ITEM-FOUR offers a powerful approach for tackling this challenge, fostering next-generation assay development.

## User consent

yes

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