

**Assay Imprecision and 99th-Percentile Reference Value of a
High-Sensitivity Cardiac Troponin I Assay**

To the Editor:

We undertook a performance evaluation and determination of the 99th-percentile reference value for the Siemens cTnl-Ultra with a fully characterized population that had undergone noninvasive cardiac imaging. Permission for the study was obtained from the local research ethics committee. Participants >45 years of age were randomly selected from the practice lists of 7 representative local community practices; 1392 individuals from the general population were invited. Demographic data were collected from the participants by questionnaire. Heart rate and blood pressure measurements (mean of 2 readings) and spirometry, electrocardiography, and echocardiography evaluations were performed. For measurements of fasting serum glucose, creatinine, and cardiac troponin, venous blood was collected into Becton Dickinson serum separator tubes and centrifuged. The serum was removed and stored at −70 °C. Frozen samples were thawed to room temperature, mixed, and centrifuged before analysis. The left ventricular ejection fraction was calculated quantitatively with Simpson’s apical biplane method (1). The left ventricular mass was calculated with the Devereux-modified American Society of Echocardiography equation (2). Valvular regurgitation and stenosis were assessed qualitatively on a 5-point scale. Diastolic heart failure was defined according to the European Study Group on Diastolic Heart Failure guidelines. Healthy individuals were defined as persons from the general population with the following: no history of vascular disease, diabetes mellitus, hypertension, or heavy alcohol intake; not receiving cardiac medication; blood pressure <160/90 mmHg (mean of 2 readings); fasting blood glucose <6 mmol/L; estimated creatinine clearance [calculated by the modification by diet of renal disease equation corrected to a reference creatinine method (3)] >60 mL·min⁻¹·(1.73 m²)⁻¹; and a nonpathologic echocardiogram [defined as follows: no significant valvular heart disease (grade 1–2), a left ventricular ejection fraction >50%, a left ventricular mass indexed <134 g/m² for men and <110 g/m² for women, no diastolic heart failure or regional wall-motion abnormalities, an isovolume relaxation time of <90 ms, an E/A ratio >1.0 at <50 years of age or >0.5 at >50 years, and an E-wave deceleration time of <220 ms at <50 years of age or <280 ms at >50 years].

All analyses were performed on the ADVIA Centaur (Siemens Healthcare Diagnostics) with the manufacturer’s recommended protocols. The stated detection limit is 0.006 μg/L, and upper limit is 50 μg/L. The claimed 10% CV is 0.03 μg/L, with a 99th percentile value of 0.04 μg/L. Total imprecision was assessed by following CLSI protocol EP15-A with serum pools prepared from sera of known high cardiac troponin concentrations that were adjusted by dilution with serum considered to be troponin free according to the cTnl-Ultra assay. The pools were then stored frozen at −20 °C until use. Seven concentrations were measured 4 times daily for 5 days. The samples were stored at 4 °C between runs. Statistical analyses were performed with the Analyse-it add-in for Microsoft Excel (version 2.12; Analyse-it, www.analyse-it.com).

Fig. 1 summarizes assay imprecision. By interpolation, we estimated that the 10% CV occurred at 0.045 μg/L. Of the 1392 individuals invited to participate, 699 (50.2%) reported for screening; 336 were male. The median age was 58.0 years (interquartile range, 53–71 years). After the exclusion of ineligible individuals, we collected samples from the reference population of 309 individuals (127 men, 182 women). The median age was 53 years (range, 45–80 years; interquartile range, 49–62 years). The age distributions of the male and female participants were not statistically different for either the screening set or the reference set. There was no association between

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**Fig. 1.** Mean CVs and 95% CIs for cardiac troponin I (cTnl) concentrations.
cardiac troponin I concentration and blood pressure. The troponin values of the male and female participants were not significantly different, and there was no correlation between troponin concentration and age for either the overall population or the reference set. Troponin was undetectable (no signal detectable above background) in 25 individuals and had a calculated value between 0 μg/L and 0.006 μg/L in 144 individuals; hence, 165 (53.4%) of the 309 individuals could be considered to have no measurable troponin. The 99th percentile was 0.039 μg/L.

This study used a randomly selected population of ostensibly healthy individuals, which was then screened to exclude any possibility of active cardiovascular disease. The one previous evaluation of the Siemens Ultra method showed both an age and sex dependence of the troponin values (4). That study did not use imaging and obtained a higher value for the 99th percentile. A comparable study that also measured cardiac troponin I by a high-sensitivity method demonstrated similar findings (5). In contrast, however, our study with a highly selected subset of individuals defined by nonpathologic results in cardiac-imaging analyses found no effect of sex on the 99th percentile and obtained a lower value for the 99th percentile.

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References


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