To the Editor:

Cardiac troponin has been designated as the preferred biomarker for diagnosis of myocardial infarction (MI) (1). Previously published data, however, confirm the large diversity among cardiac troponin assays with respect to important analytical characteristics, including assay standardization, antibody specificity, interferences, and assay imprecision, and underscore the need for improved cardiac troponin assays (2, 3). Results obtained with more recently released next-generation assays show that the newer assays indeed have substantially improved analytical performance (4, 5). The aim of this study was to evaluate one of these next-generation cardiac troponin I (cTnI) assays, performed on the Aio¹™ immunoanalyzer (Innotrac Diagnostics Oy), by defining key performance characteristics, including detection limit, linearity on dilution, imprecision, reference interval, and cutoff for MI diagnosis.

The assay is based on “all-in-one” dry chemistry technology, in which all of the reagents are precoated in assay cups, and time-resolved fluorometric detection, with a total analysis time of <20 min (6). An eight-point factory-constructed calibration curve is provided on a bar code with each reagent lot, and the instrument-specific calibration adjustment is performed by running the cups of the appropriate calibration pen. A purified preparation of human cardiac ternary troponin I–troponin T–troponin C complex (HyTest Ltd.) is used as calibration antigen. The antibody configuration of the assay, adding a monoclonal antibody with an epitope in the N-terminal region of cTnI (amino acid residues 20–35) and one with an epitope in the C-terminal region (amino acid residues 185–200) to the mid-fragment cTnI antibodies (epitopes in the region of amino acid residues 35–55 and 80–95), has recently been described, and the potential ramifications of this have been discussed (7, 8).

The Aio¹ analyzer was handled strictly according to the manufacturer’s instructions. Unless otherwise stated, fresh serum was used as sample. The minimum detectable cTnI concentration was assessed by 20 replicate measurements of the cTnI-rich serum specimen (native cTnI concentration of 4.3, 9.2, 27.6, 46.9, and 79.5 μg/L) were serially diluted with serum pools having undetectable cTnI concentrations, i.e., lower than the detection limit of the Aio¹ assay, or with the instrument buffer solution. The undiluted sample and four separate dilutions (3:4, 1:2, 1:4, and 1:8) were assayed in duplicate in the same analytical run. The curve obtained was tested for linearity as suggested by Burnett (10). After demonstration of linearity, linear regression analysis of the data was performed, and correlation coefficients (r) were calculated. A recovery study was also performed. For the imprecision study, seven serum pools were prepared and stored at −80 °C until used. Two replicates/specimens were analyzed per run, and one run per day for 20 days was performed, using two reagent lots and calibrations (3). Using ANOVA method, we calculated the total CV at different concentrations and used the CV values reported for the seven pools to construct the imprecision profile for the method (3).

To establish reference values, we a priori selected 150 apparently healthy individuals (75 women and 75 men; median age, 60 years; range, 23–89 years), using exclusion criteria as suggested in IFCC recommendations on the theory of reference values (11). Serum cTnI was measured, and the 99th percentile of the value distribution was calculated by non-parametric determination of percentiles. The procedures followed were

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Fig. 1. Imprecision results for human serum pools measured with the Aio¹ second-generation cTnI assay. The cTnI concentration corresponding to a 10% total CV is indicated by the arrow.
in accordance with the current revision of the Helsinki Declaration.

The detection limit for the assay was 0.006 μg/L. In the linearity studies, the hypothesis of a linear fit was accepted for all examined samples (P > 0.11), and r values were >0.9989 for dilution with a negative serum pool and >0.9978 for dilution with buffer. All mean recoveries were within ±10% of the native concentrations. The following results for assay imprecision were obtained (total CV and mean cTnI concentration): CV = 14% at 0.019 μg/L; CV = 8.2% at 0.044 μg/L; CV = 6.3% at 0.065 μg/L; CV = 6.3% at 0.086 μg/L; CV = 8.1% at 0.14 μg/L; CV = 6.0% at 0.22 μg/L; and CV = 4.3% at 0.39 μg/L. Because a total CV ≤10% at the MI decision limit is recommended, the cTnI concentration corresponding to this analytical imprecision was determined from the intercept of the total CV (y axis) equal to 10% on the imprecision profile curve (Fig. 1). The lowest cTnI concentration yielding a CV of 10% was 0.036 μg/L. This value is therefore recommended as a de facto cutoff for detection of myocardial necrosis.

In the group of apparently healthy individuals, 97% of the cTnI values were <0.010 μg/L, whereas only four individuals (two males and two females) had measurable cTnI concentrations, i.e., 0.010, 0.014, 0.016, and 0.029 μg/L. The calculated 99th percentile of the cTnI value distribution was 0.015 μg/L. The evaluated assay was close to meeting the 10% CV at the 99th percentile value, with 10% CV/99th percentile ratio of 2.4.

In conclusion, the Aio! second-generation cTnI assay has improved performance compared with data published earlier for the corresponding first-generation assay (12).

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References

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Early Detection of the Selectivity Loss of the Cobas Integra Analyzer
Chloride Ion-Selective Electrode

To the Editor:
Electrolyte measurements predominantly use ion-selective electrodes (ISEs) for sodium, potassium, and chloride. In contrast to the reliability of the sodium and potassium electrodes, erroneous chloride ISE values have occasionally been reported (1–3). We recently observed an increasing number of falsely high chloride concentrations in patient plasma samples. These results appeared at unpredictable intervals after replacement of the electrode on a Cobas Integra analyzer (Roche Diagnostics). Repeat analyses with a coulometric method yielded significantly lower results.

The falsely high values appeared not to have been caused by a general low selectivity of the chloride electrode, which responds to other ions such as iodide, thiocyanate, nitrate, and bromide (1–3). More likely was a time-dependent deterioration of the chloride electrode, for which the manufacturer claims a lifetime of 3 months but which needed to be changed more frequently. To attain reliable chloride ISE results, electrodes could be changed on a more frequent schedule (e.g., weekly), but that approach is expensive. Hence we aimed to introduce a reliable and inexpensive indicator of deterioration of the electrode.

Routine blood specimens were collected in tubes containing ammonium heparin (Sarstedt) and immediately centrifuged after arrival in our laboratory. Selectivity of the ISE was checked by comparison with chloride concentrations measured by coulometric titration on a Model 925 chloride analyzer (Corning). For quality control we used Duotrol™ abnormal (Biomed; target value, 127 mmol/L; control A) and Precinorm™ U (Roche Diagnostics; target value, 111 mmol/L; control B). As a selectivity control we used ISE solution 1 (Roche Diagnostics) supplemented by an aqueous KSCN solution. Briefly, we added 25 μL of 5

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