mal hepatocyte nuclear factor-1 binding site of the AFP gene promoter in one *(2)*. AFP is a widely used tumor marker. Although HPAFP is a rare disorder, it needs to be considered and differentiated from tumors to avoid inappropriate explorations and treatment decisions.

We present a simple test based on restriction fragment length polymorphism (RFLP) analysis for screening of HPAFP mutations. This test can be used in patients with increased AFP not accompanied by any obvious tumor or liver disease, and may avoid invasive studies. We have previously reported two unrelated families with HPAFP related to −119G>A and −55C>A substitutions *(2)*. The −119G>A mutation creates a *Psh*AI restriction site. A gain of this site in HPAFP patients with the −119G>A mutation produced two fragments on agarose gel electrophoresis compared with the one obtained from an individual without the substitution (Fig. 1A). DNA isolated from four individuals of family 1 was tested for the presence of the −119G>A mutation by RFLP analysis. All of these patients also had increased AFP and were heterozygous for the RFLP (Fig. 1B). The individual with a normal AFP concentration was homozygous for the wild-type (WT) allele. The −55C>A mutation abolished a *Spe*I restriction site. A loss of this site in HPAFP patients with the −55C>A mutation produced one fragment on agarose gel electrophoresis compared with the two fragments obtained from a control individual (Fig. 1C).

DNA isolated from seven individuals of family 2 was tested for the presence of the −55C>A mutation by RFLP analysis. All of the affected family members who had increased serum AFP were heterozygous for the RFLP (Fig. 1D). The five individuals with normal AFP concentrations were homozygous for the WT allele.

Our RFLP analysis is potentially useful for direct detection of both mutations known to be associated with HPAFP *(2–4)*. Detection of the −119G>A substitution by conformation-sensitive gel electrophoresis has recently been proposed *(5)*. However, this method was designed for detection of the −119G>A substitution and not of the second reported mutation (−55C>A). Our RFLP test is inexpensive and simple to perform.

We thank S. Wenk for careful English proofreading of the text. We are grateful to the patients, to their families, and to the physicians, especially Dr. G. Perlemuter and Prof. C. Buffet.

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DOI: 10.1373/clinchem.2004.034157

Multicenter Evaluation of the TOSOH AIA-Pack Second-Generation Cardiac Troponin I Assay

To the Editor:

The redefined biochemical criterion proposed to diagnose myocardial infarction (MI) necessitates the availability of highly sensitive and precise cardiac troponin assays *(1)*. In general, a substantial improvement in the analytical performance is offered by the newer assays. In this multicenter study, we evaluated one of these improved cardiac troponin I (cTnI) assays, performed on the AIA 21 immunoassay system (TOSOH Corp.), using criteria tailored in accordance with IFCC recommendations *(2)*.

The AIA-Pack second-generation cTnI assay uses a combination of two monoclonal antibodies, one directed to amino acids 41–49 of the cTnI molecule and another directed to amino acids 87–91. A human cardiac ternary troponin ITC complex is used as calibration antigen. The minimum detectable cTnI concentration was defined as the value corresponding to a signal 3 SD greater than the mean of 20 replicates of the zero calibrator in a single run. Buffered stock solutions of both free cTnI and troponin IC complex (Scripps Laboratories) were serially diluted in AIA-Pack diluent and tested to estimate the degree of equimolarity. Four cTnI-rich serum pools (native concentrations, ~2.5 to ~100 μg/L) were serially diluted with serum pools having cTnI below the detection limit or with the manufacturer's diluent. The undiluted sample and four dilutions were assayed in duplicate, and the curve obtained was tested for linearity *(3)*.

To evaluate imprecision, Liquichek™ control sera (three concentrations; Bio-Rad Laboratories) were tested in duplicate in one run per day for 20 days, using two reagent lots and two calibrations. Seven serum pools (cTnI concentrations, 0.02, 0.05, 0.10, 0.15, 0.25, 0.40, and 0.65 μg/L) were also tested with the same protocol, and the reported total CVs

were used to construct the imprecision profile for the method.

We used Deming regression to compare the AIA cTnI assay with the Access AccuTnI™ (Beckman Coulter), AxSYM™ (Abbott), Dimension RxL™ (Dade Behring), and Vitros ECi™ (Ortho-Clinical Diagnostics) cTnI assays.

To evaluate anticoagulant interference, we collected paired samples from 53 MI patients to prepare serum, heparin (lithium), and EDTA-plasma specimens and assayed them in duplicate in the same run. To study sample stability, we divided three serum samples (native cTnI concentrations, 0.22, 1.32, and 10.5 μg/L) into aliquots kept at different temperatures until assayed in duplicate. To establish reference values, we assayed samples from 120 apparently healthy Caucasian individuals (60 women and 60 men; age range, 22–83 years) *(4)*. To study the cTnI release kinetics, we obtained serum samples from 67 MI patients every 6 h throughout the first 48 h after hospital admission. cTnI was measured with the AIA and the routinely used assays for comparison [ACS:180® (Bayer Diagnostics) and Dimension RxL]. The procedures followed were in accordance with the current revision of the Helsinki Declaration.

The detection limit of the AIA-Pack assay was 0.038 μg/L (range, 0.035–0.041 μg/L; n = 6). The IC complex was fully detected by the assay [mean (SE) recovery, 103.8 (2.2)% for the four dilutions]. However, the assay produced a response to free cTnI that was ~50% less than that to the IC complex [mean (SE) ratio of IC complex to free cTnI, 1.85 (0.12); n = 4]. The hypothesis of a linear fit was accepted for all samples tested for linearity ($P > 0.10$). The correlation coefficients (r) were >0.9998 (dilution with negative pool) and >0.9995 (dilution with diluent), respectively.

The within-run and total CVs for the controls (cTnI concentrations, 0.32, 2.67, and 8.18 μg/L) were 3.5–6.5% and 4.4–8.2%, respectively. Shown in Fig. 1A is the imprecision profile for the AIA-Pack method. The cTnI concentration corresponding to a total CV of 10% was 0.13 μg/L. Method comparisons produced a wide range of slopes [0.15 vs AxSYM (n = 41); 1.18 vs RxL (n = 68); 1.35 vs ECi (n = 58); and 1.86 vs AccuTnI (n = 73), respectively]. Nevertheless, the correlations were quite good (r >0.96).

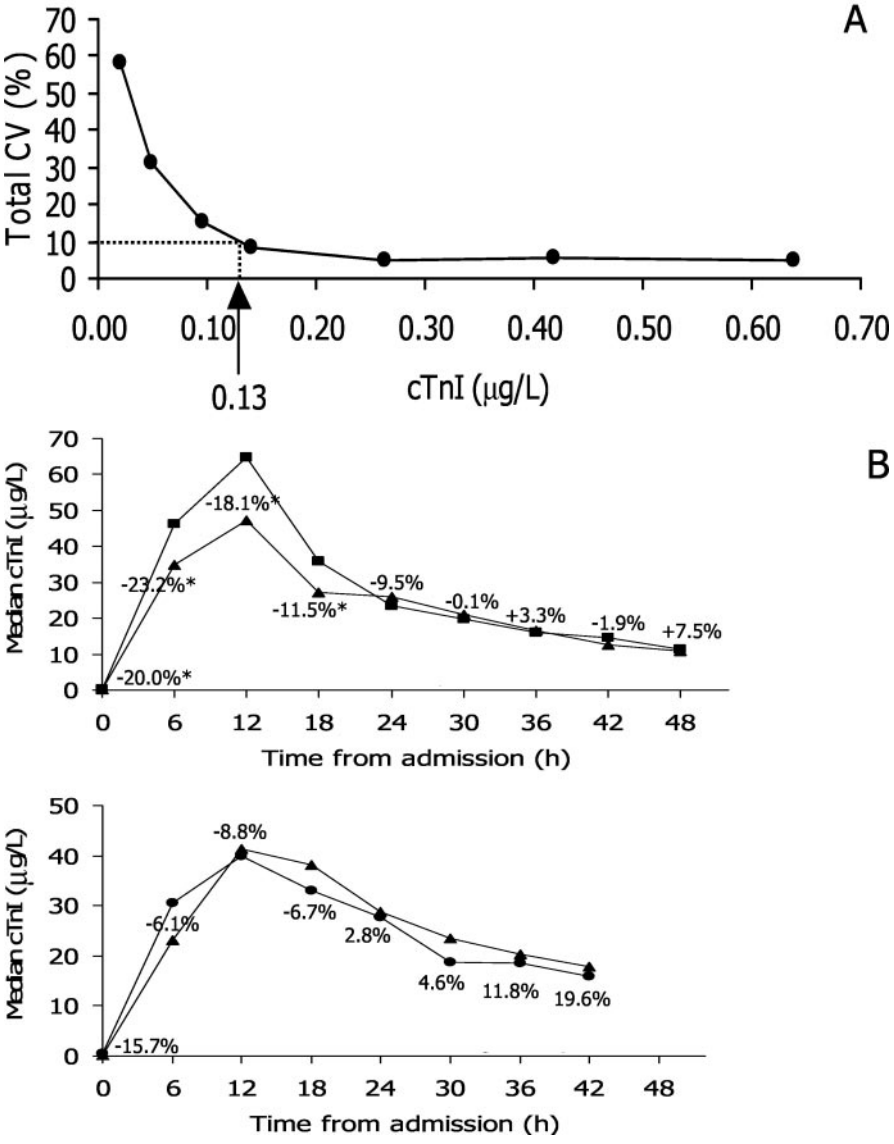

Fig. 1. Imprecision results for human serum pools measured with the AIA-Pack cTnI assay (*A*), and comparison of release kinetics of cTnI, as measured by the AIA-Pack assay and two other methods (ACS:180 and Dimension RxL), after acute MI (*B*).

(*A*), the cTnI concentration corresponding to the 10% total CV is shown by the *arrow*. (*B*), results are median cTnI values (▲, AIA-Pack; ■, ACS:180; ●, Dimension) and the median difference between methods (*percentages*) at each time point. Center 1 (*upper panel*; AIA-Pack vs ACS:180), n = 50 patients studied; center 2 (*lower panel*; AIA-Pack vs Dimension), n = 17 patients studied. ∗, the difference between the AIA-Pack and the ACS:180 method was significant at these time points ($P < 0.05$). The difference between the AIA-Pack and the Dimension method was not significantly different from 0% at any time point.

On average, the difference (95% confidence interval) for heparin samples was 14.5% higher (11.1–17.8%; $P < 0.001$) and the difference for EDTA-plasma was 13.2% higher (7.9–18.6%; $P = 0.0001$) compared with the paired sera. cTnI was stable for 48 h at room temperature [mean (SE) measured value, 98.8 (0.9)% of the expected], for 4 days at 4 °C [98.5 (1.5)% of the expected], for 3 months at −20 °C [94.3 (2.0)% of the expected], and for 6 months at −80 °C

[91.4 (1.8)% of the expected]. All cTnI values in healthy volunteers were below the assay detection limit. The release kinetics measured by the AIA and Dimension after MI were comparable, whereas AIA and ACS:180 results showed a time-dependent significant difference ($P < 0.05$) in the first 18 h after admission (median difference between the AIA and ACS results, −20.0% at admission and −11.5% after 18 h; Fig. 1B).

Previous publications have shown that cTnI assays from different manufacturers recognize complexed and free cTnI forms differently *(5)*. In particular, whereas for the ACS:180 cTnI assay the ability to recognize free cTnI and the IC complex was similar, the Dade assays behaved quite differently to free cTnI than to the IC complex, producing responses to free cTnI that were significantly less than those for the IC complex, as was the case for the AIA assay *(5)*. Because free cTnI in the cytoplasm could be released more rapidly from damaged cardiac myocytes than would the structural troponin complex, cTnI concentrations in earlier phases after MI may contain a higher percentage of free cTnI. This could explain the time-dependent difference we observed in our study of the cTnI release kinetics after MI, in which the AIA and ACS assays, which have different reactivities to various troponin forms, were used. On the other hand, the agreement noted in the same study between AIA and Dimension results can be explained by the similar reactivity of these two assays toward various cTnI forms.

In the current study, the AIA assay did not detect cTnI in healthy individuals. However, in a separate study, the same assay, performed on a different platform (AIA 600 II), was able to detect cTnI in a small percentage of healthy individuals and indicated a significant difference in cTnI concentrations between blacks and whites *(6)*. Aspects such as the criteria for individual selection and sample size should be considered to explain this difference. However, an additional analytical aspect should also be noted in the mentioned study: the use of heparin-plasma samples, which potentially give higher cTnI results with the present method.

In conclusion, the AIA cTnI assay showed acceptable analytical performance when compared with results obtained with other commercial systems. It should be noted, however, that the 10% CV value obtained in the present study exceeds that reported in the original IFCC-coordinated study (0.09 μg/L), in which the same imprecision protocol was used *(7)*; in that study, however, the experiments were performed directly by the manufacturers.

The present study was funded by TOSOH.

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DOI: 10.1373/clinchem.2004.035451

Labor Increases Maternal DNA Contamination in Cord Blood

To the Editor:

Both maternal cells and maternal DNA are often present in the umbilical cord blood (UCB) *(1)*. When UCB is used for bone marrow transplantation *(2)*, the presence of maternal cells in UCB plasma is a theoretical risk factor for graft-vs-host disease and may lead to vertical transfer of infectious agents to a fetus. Previous studies showed that elective cesarean section (C/S) re-