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Do NIST SRM 2921 and recombinant cTnI-based serum pools have potential to harmonize cTnI results?

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The history of cardiac troponin assays starts from the late 1980s. Today cardiac troponins (cTn) are "popular" cardiac markers which have largely replaced CK and CK-MB. Dozens of commercial cTnI assays are available nowadays while there is only one cTnT assay because of an existing patent (Roche Diagnostics). For qualitative or quantitative detection of different cTn antigens in body fluids, immunoassays are used in clinical practice. As the sought parameter is the cTn antigen, companies have developed strategies of antibody selection based on the knowledge of the antigen's structure and properties. Notwithstanding well-considered antibody selection, extreme microheterogeneity of the cTnI antigen and lack of a complete cTnI reference system (figure 1) complicate assay standardization, resulting in huge between-method variation for existing cTnI assays (1).

An important prerequisite for guaranteeing comparability of results among different cTnI methods is the availability of suitable reference materials. Nowadays, well characterized Standard Reference Material (SRM) 2921 and recombinant cTnI (rec cTnI) are available to manufacturers for cTnI harmonization and/or standardization (2, 3). From an EQAS viewpoint, the Chemistry Section of the Dutch EQAS (SKML) aims to monitor the effect of ongoing cTnI standardization efforts by in-vitro diagnostic industries. Consequently, we wanted to develop stable, well characterized, matrix-based cTnI pools. Secondly, we aimed to investigate the cTn harmonization potential of these matrix-based cTnI pools.

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Materials and Methods

SRM 2921, a human cardiac troponin complex-based reference material, was purchased from NIST (Certificate Issue Date: 25 August 2004). Free recombinant cTnI (Cat Nr RP-3400) was purchased from Spectral Diagnostics Inc. cTn-free serum pools from apparently healthy blood donors were made in the production facility at Beatrix Hospital Winterswijk, according to CLSI protocol C37A. The latter pools were spiked with SRM 2921 (Hytest, certified by NIST) and rec cTnI (Spectral Diagnostics). In addition, cTn-positive patient pools A (heparin plasma pool) and B (serum pool) were prepared from left-over serum or plasma collected fresh at two large patient hospitals. All matrix-based pools were lyophilized and had to be reconstituted with 1.0 mL of A.D. Precautions were taken to limit the entire pool preparation procedure to 6-8 hours. A national EQA-survey was scheduled in December 2006.

Ninety-three labs participated and analysed the matrix-based cTn pools as unknown samples under routine cTn calibration conditions. EQA participants reported their quantitative cTnI or cTnT data to the SKML office, according to the regular procedures for external quality control assessment and data retrieval. Central data analysis was performed at the SKML office in Nijmegen.

Commutability of the four matrix-based pools was assessed by comparison of their behaviour to that of patient pools across available cTnI assays. cTnI measurement procedures from Beckman (N =11), Abbott (N = 6), BioMerieux (N = 5), DPC (N = 16) and a miscellaneous group (N = 11) (49 labs) were involved. For comparative reasons Elecsys/Modular cTnT methods from Roche Diagnostics were also included (45 labs). For each pool cTn data were plotted into histograms and interlaboratory CVs were calculated, either overall and per method group. The harmonization potential of the candidate pools, when used as calibrator, was investigated by calculating the effect on interlaboratory CVs. Recoveries of cTn in the SRM 2921-based serum pool were also examined.

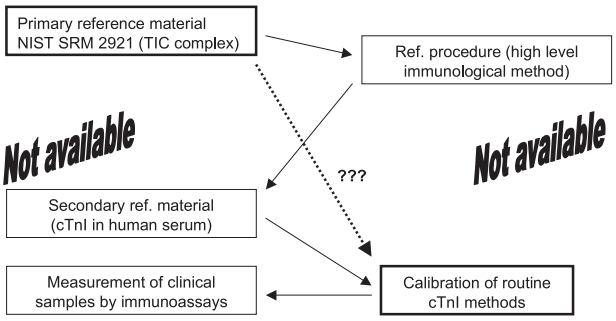

Figure 1. The proposed cTnI reference system (1).

In-vitro incubation studies on cTnI stability were performed in one centre by spiking SRM 2921 into four different media: i.e. in milli-Q water, in TRIS-buffer - comparable to the buffer used for the SRM 2921 reference material-, in donor serum and in donor heparin plasma. cTn-free donor serum and plasma was harvested from a healthy co-worker. cTn recoveries were measured at 0h, 2h, 6h and 48 hrs after spiking using cTnI kits from Abbott (Axsym) and DPC (Immulite 2000), and a cTnT kit from Roche (Elecsys). Dilutions were made in glass vials which were stored intermittently at 4 ºC.

In-vitro incubation experiments of cTnT were carried out in another centre. Two different cTnT standards were incubated at 37 ºC in serum of healthy controls during 96 hours: a highly purified (>98%) human cTnT standard (Advanced Immunochemical Inc, Long Beach, USA) and NIST SRM 2921 (Hytest Ltd., Turku, Finland). Time-dependent cTnT recoveries were measured at 0h, 24h and 96h with the cTnT assay from Roche (Elecsys). Fragmentation studies were done using immunoprecipitation of intact and fragmented cTnT using four different anti-troponin antibodies, followed by SDS-PAGE and Western blotting (4, 5). cTnT fragmentation occurred even at T= 0 hr, i.e. immediately after spiking.

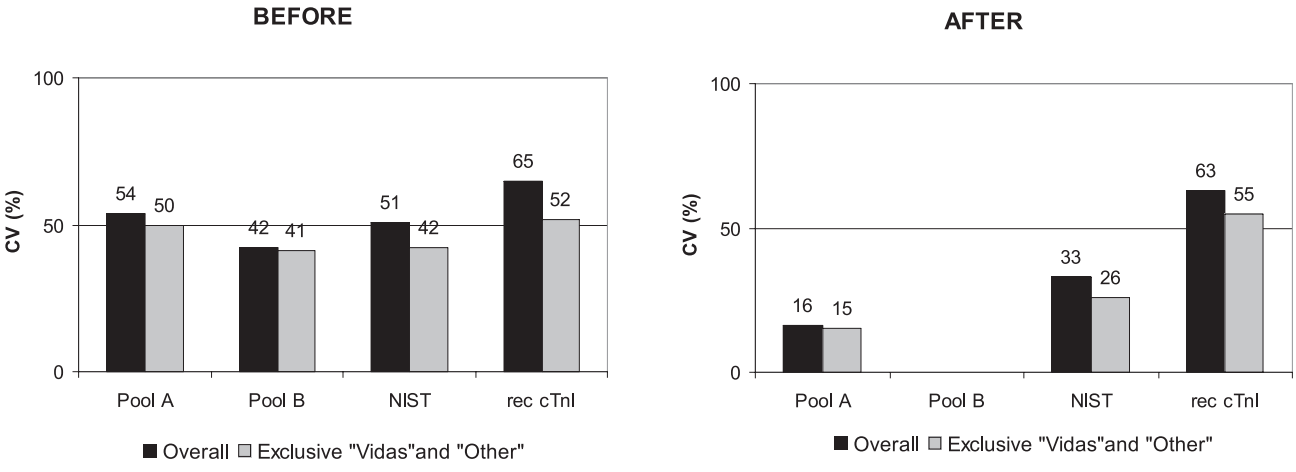

Figure 2. Interlaboratory CV's (%) in the Netherlands before and after harmonisation on serum pool B. This national survey was performed in December 2006.

Results

National EQA-survey

Overall interlaboratory CVs for cTnI ranged from 42 to 65% across the pools tested. In case of patient pool A the overall interlaboratory CV for cTnI is 54%, compared to 9% for cTnT. Using patient pool B as a "harmonizer"; theoretical interlaboratory CVs for serum pool A, NIST SRM and rec cTnI pools could be reduced to respectively 16%, 33% and 63% (figure 2). When excluding Bio-Merieux cTnI (VIDAS) and the miscellaneous group a similar reduction of the inter-laboratory CVs occurred across the pools tested. Figure 2 illustrates that especially the patient pool, and to a lesser extent the SRM 2921 based pool, have potential for harmonization, whereas the pool spiked with rec cTnI has no potential for cTnI harmonization. cTnI histograms in figure 3 reveal a comparable bimodal distribution for Beckman and Abbott versus DPC measurements across the patient pools and the pool spiked with NIST SRM 2921, suggesting commutability of the SRM 2921-based pool. The pool spiked with rec cTnI displays a different distribution with overlapping cTnI data for Beckman, Abbott and DPC.

Average recoveries in the SRM 2921-based pool differ per cTnI method group and range between 9-55%, compared to a recovery of 7% for cTnT. cTnI recoveries in the rec cTnI-based pools were only 3-11% (table 1).

Table 1. cTnI recoveries in SRM 2921- and rec cTnI- spiked, matrix-based serum pools. Average recoveries (in µg/L cTnI and in % of the nominal value) are presented per method group.

	SRM 2921 spiked pool µg/L	% recovery	Rec. cTnI spiked pool µg/L	% recovery
Beckman (n=11)	5.8	24	3.9	10
Abbott (n=6)	6.0	25	3.7	10
DPC (n=16)	13.2	55	4.3	11
Vidas (n=5)	2.2	9	1.0	3
Miscellaneous (n=11)	5.2	22	3.2	8
Roche cTnT / Elecsys (n=44)	2	7	-	-

In-vitro incubation experiments

The in-vitro incubation experiments of the cTn standards in figures 4a/b reveal a time- and medium-dependent cTn recovery, even during intermittent storage at 4 ºC. cTn recoveries are lowest in water < buffer < serum = plasma. Fragmentation studies in figure 5 reveal cTnT fragments even at T = 0 hr, i.e. immediately after spiking, as well as on-going fragmentation during incubation at 37 ºC in the absence of protease inhibitors.

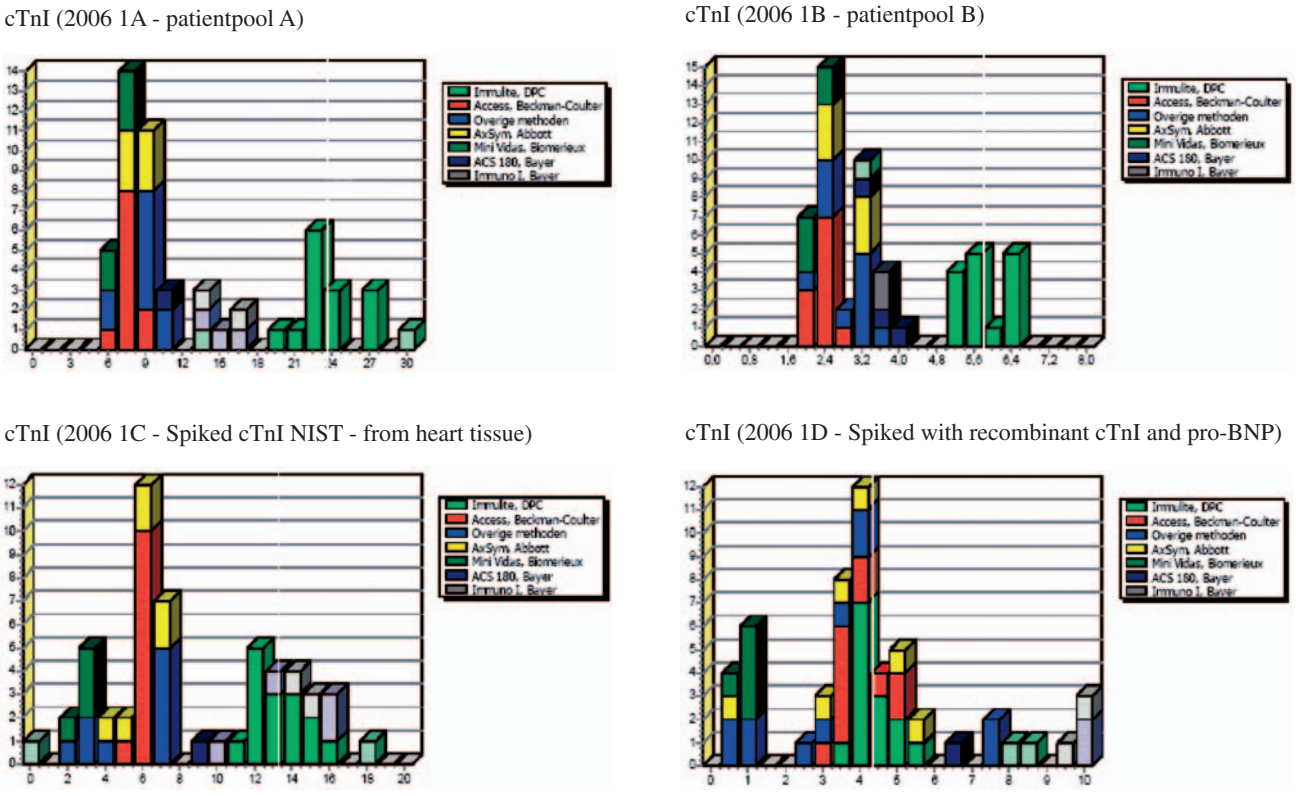

Figure 3. cTnI data from a Dutch EQA survey in December 2006. The histograms display cTnI distributions for patient pool A; patient pool B; NIST SRM 2921- spiked serum pool and rec cTnI-spiked serum pool across different method groups. Different colors are given to different method groups.

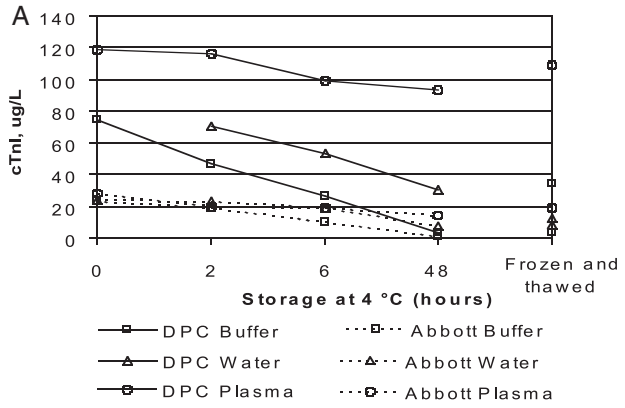

Figure 4A. In-vitro incubation of SRM 2921 in water, TRIS-buffer and heparin plasma. Dilutions were intermittently stored at 4 ºC. The nominal cTnI concentration brought into solution was 19.73 µg/L. cTnI recovery was measured with cTnI assays from DPC (Immulite 2000) and Abbott (Axsym).

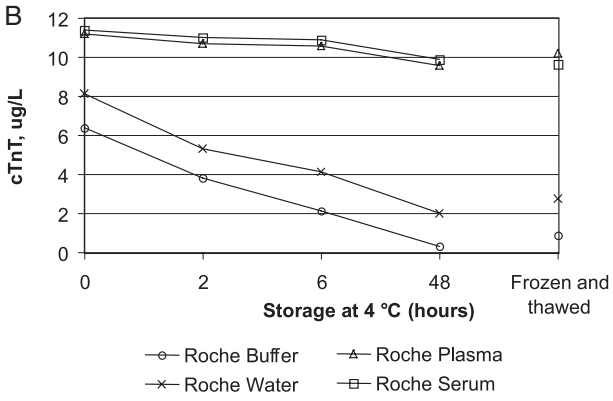

Figure 4B. In-vitro incubation of SRM 2921 in water, TRIS-buffer, serum and heparin plasma. Dilutions were intermittently stored at 4 ºC. The nominal cTnT concentration brought into dilution was 23.34 µg/L. cTnT recovery was measured with a Roche cTnT assay (Elecsys).

Discussion

Standardization of the majority of plasma protein determinations has been accomplished by preparation of s serum-based material (6). A similar way was chosen by the Committee on Standardization of Markers on Cardiac Damage (C-SMCD) for the standardization of myoglobin. For cTnI, it was also thought that a common reference material would be sufficient for reaching method standardization. Hence, the development of a cTnI reference material has not been supported by the simultaneous development of a reference measurement procedure which would have been in agreement with a metrologically sound approach for standardization and traceablity of cTnI measurements (figure 1). Unfortunately, the use of SRM 2921 as common calibrator in commercial systems did not improve result comparability.

In line with the above and in order to monitor the effect of cTn (re)standardization by manufacturers, the Chemistry Section of the SKML attempted to develop matrix-based pools with cTn harmonization potential. We found that especially the patient pools and to a lesser extent the SRM 2921-based serum pool can reduce cTnI interlaboratory CVs fivefold and have potential for harmonization of cTnI assays. The free rec cTnI serum pool has no potential for harmonization of cTnI assays. Second, Beckman, Abbott and DPC apparently use similar cTnI calibrators for calibration of their assay. Third, when preparing SRM 2921-based serum pools cTn recovery is incomplete and assay-, medium- and time-dependent. Fragmentation studies reveal degradation of the human cardiac troponin complex from the moment of manipulation of the reference preparations. We hypothesize that our cTnT fragmentation data can be extrapolated to cTnI.

Conclusion

The SRM 2921 reference material appears to be highly unstable upon utilization. The future potential of SRM 2921-spiked serum pools for cTn harmonization and trueness verification heavily depends on finding solutions for stabilizing cTn reference preparations and preventing cTn standard degradation.

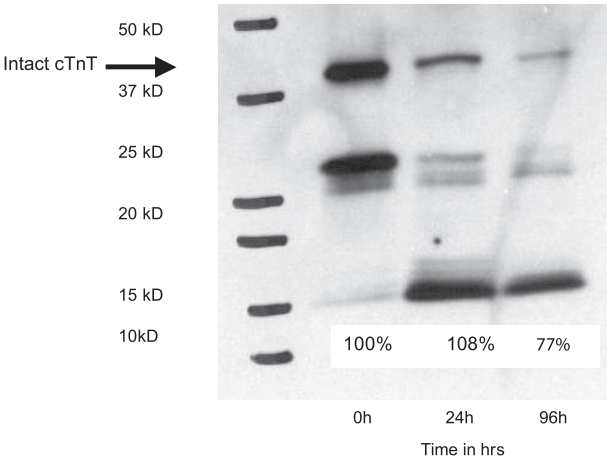

Figure 5. Time-dependent degradation of SRM 2921-based serum pool as measured with a sensitive immunoprecipitation technique, in combination with SDS-PAGE electrophoresis and Western blotting of intact and fragmented cTnT (4, 5). cTnT recoveries at 0h, 24 and 96h as quantified with Roche cTnT assay were 100%, 108% and 77% respectively. In lane 1 a molecular weight marker has been applied.

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