Successfully meeting analytical expectations for the fast 0/1-h algorithm for NSTEMI by internal control procedures for cardiac troponin T

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Letter to the Editor

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To the Editor,

In 2015, as a result of major technological advances, the European Society of Cardiology (ESC) recommended criteria for patients with acute chest pain based on 0/1-h assessments of high sensitivity cardiac troponins (hs-cTn) [1]. In our hospital, we use the fifth generation Roche Elecsys hs-cTnT STAT assay on two Cobas 8000 e602 analysers. We locally implemented the fast 0/1-h algorithm for NSTEMI using cut-offs for rule-in and rule-out for hs-cTnT in serum for this assay based on the APACE [2] and TRAPID-AMI [3] studies in December 2016 (Appendix 1). To meet clinical expectations, it is of critical importance that serum cTnT test results are interchangeable when samples are randomly allocated to the two analysers.

Using data from the Dutch EQAS organisation (SKML), Van der Hagen already showed that most laboratories using Roche Elecsys in the Netherlands meet the strict analytical performance specifications including limited inter-instrument bias for the 0/1-h algorithm [4]. However, Haagensen and colleagues recently demonstrated large lot-to-lot variations for the Elecsys assay [5]. Therefore, to structurally monitor analytical performance and instrument exchangeability in our laboratory, we implemented long-term stable commutable cTnT harmonizer control samples (IQC), at four clinically relevant levels (L1 target 3.4 ng/L (SD=1.0), L2 target 8.1 ng/L (SD=1.0), L3 target 16.8 ng/L (SD=1.0) and L4 target 52.9 ng/L (SD=1.5)). These harmonizer control samples are made at the Department of Clinical Chemistry of the Queen Beatrix Hospital in Winterswijk (MCA Laboratory, ISO 13485 accredited EQA-material production center, coordinating laboratory for the SKML in the Netherlands) by pooling and aliquoting human sera, and subsequent storage at −70 °C. These samples are stable for 5 years when stored at <−70 °C, and stable for 48 h at 2–8 °C. Every day, two IQC levels are measured on both analysers (uneven days: L1 (afternoon) + L3 (after maintenance); even days: L2 (afternoon) + L4 (after maintenance)). The absolute difference between both analysers is not allowed to exceed two SD at all concentration levels.

We here evaluate the effectiveness of our human pool-based IQC strategy to monitor instrument exchangeability and lot-to-lot variation in reagents and calibrators by (1) comparing IQC results for both analysers at all IQC levels and (2) comparing means and SDs for subsequent reagents and calibrator lot combinations, per IQC level, per analyser.

For this purpose, we first extracted IQC results from our laboratory information system between 3 November 2017 and 9 October 2018. Test results for L1 <3 ng/L (limit of blank) were analysed as being 2 ng/L. Outliers due to obvious tube misplacements were discarded (n=4). Overall, coefficients of variation were 10.9% (analyser 1, mean 3.3 ng/L, n=203) and 12.0% (analyser 2, mean 3.2 ng/L, n=183) for L1, 8.0% (analyser 1, mean 8.1 ng/L, n=206) and 8.5% (analyser 2, mean 7.9 ng/L, n=189) for L2, 3.9% (analyser 1, mean 16.7 ng/L, n=276) and 4.1% (analyser 2, mean 16.4 ng/L, n=317) for L3, and 2.5% (analyser 1, mean 53.7 ng/L, n=273) and 2.0% (analyser 2, mean 53.2 ng/L, n=291) for L4.

To evaluate instrument exchangeability, IQC results for L1 and L2 were included when the maximum time

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between sample analysis between both analysers did not exceed 1 h (to mimic the fast 0/1-h algorithm). For L3 and L4, the IQC samples had to be measured on the same day on both analysers. Normality was evaluated by visual inspection of the histogram. For normally distributed variables (L2, L3 and L4), means and standard deviations were calculated; and p-values were obtained by paired t-tests (IBM SPSS Statistics v25). For non-normally distributed variables (L1), medians and interquartile ranges were calculated and p-values were obtained by Wilcoxon Signed Rank tests. Reassuringly, we did not observe any statistically significant differences in cTnT between analyser 1 and 2 for L2, L3 and L4:

- L1 (n=124): median 3.2 ng/L (IQR 2.0–3.5) vs. median 2.0 ng/L (IQR 2.0–3.4), p=0.004.
- L2 (n=112): 8.0 ng/L (SD 0.5) vs. 7.9 ng/L (SD 0.6), p=0.532
- L3 (n=23): 16.5 ng/L (SD 0.6) vs. 16.5 ng/L (SD 0.6), p=0.931
- L4 (n=27): 53.5 ng/L (SD 1.5) vs. 53.3 ng/L (SD 1.3), p=0.384

The statistically significant differences for L1 are likely to be caused by the large number of IQC test results of exactly 2 ng/L. Appendix 2 shows the proportion of paired samples with differences between analyser 1 and analyser 2 of >1 ng/L, of >2 ng/L or of >3 ng/L. For L1 through L3, no differences >2 ng/L were found between both analysers. At four separate days we observed a difference in L4 >3 ng/L (2 SD) between both analysers. In three instances, the technicians had responded adequately according to protocol by re-analysis of the IQC samples (n=1), calibration (n=2) and re-analysis of patient samples, if necessary. Based on these results, the analysers can be considered to produce exchangeable cTnT results on a daily basis, 24/7.

Secondly, we extracted IQC data between November 3rd 2017 and June 13th 2019 from our laboratory information system and information from the analyser software about lot numbers and dates of lot changes. We calculated means and SDs for all combinations of reagents and calibrator lot for all levels, separately for both analysers, and evaluated the differences by comparing mean values for reagent and calibrator lot combinations with the overall mean. The results are presented in Table 1 and Appendix 3. We did not observe any differences in case of lot changes, except for analyser 1, IQC level 4, in the period of October 2018 through November 2018 (combination reagents lot 34588800 and calibrator lot 309398). We were not able to find the exact cause of this difference, but the difference was still smaller than 3 ng/L (i.e. the clinically significant cut-off).

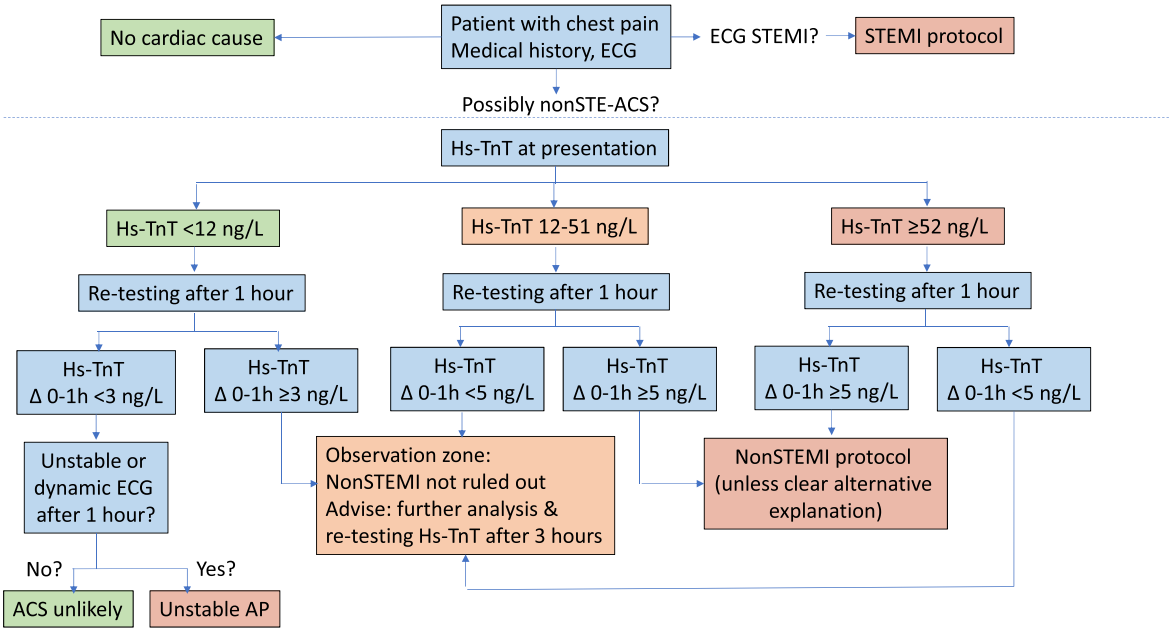
In conclusion, stable reagent and calibrator lot-to-lot performance for cTnT and exchangeability of test results between analysers is possible when appropriate IQC procedures are applied, using commutable IQC samples at appropriate clinically relevant levels. This greatly reduces lot-to-lot variability observed earlier by others [5]. This is of vital importance to meet the ESC guideline requirements for the fast 0/1-h algorithm for detection of NSTEMI and the needs of clinicians to reliably rule-out or rule-in patients with acute chest pain [1].

Table 1: Mean and standard deviations hsTnT concentrations (ng/L) per reagent and calibrator lot combination, per IQC level, per analyser.

	Level 1		Level 2		Level 3		Level 4	
	Analyser 1	Analyser 2	Analyser 1	Analyser 2	Analyser 1	Analyser 2	Analyser 1	Analyser 2
Reagent 26765400 and calibrator 255926	3.4 (0.4) n=101	3.4 (0.5) n=93	8.1 (0.5) n=101	8.1 (0.5) n=90	16.8 (0.7) n=123	16.6 (0.5) n=144	53.8 (1.5) n=128	53.2 (1.1) n=141
Reagent 30564600 and calibrator 255926	3.2 (0.3) n=58	3.2 (0.4) n=63	7.9 (0.5) n=59	7.7 (0.9) n=64	16.7 (0.6) n=86	16.3 (0.9) n=121	54.1 (1.1) n=82	53.3 (1.0) n=97
Reagent 30564600 and calibrator 309398	3.2 (0.2) n=49	3.0 (0.1) n=31	7.9 (0.3) n=51	7.6 (0.3) n=39	16.4 (0.4) n=72	16.3 (0.4) n=55	52.9 (0.9) n=71	53.3 (1.1) n=55
Reagent 34588800 and calibrator 309398	4.2 (0.7) n=31	3.3 (0.3) n=24	8.4 (0.8) n=31	7.7 (0.3) n=22	17.0 (0.7) n=36	16.1 (0.4) n=40	52.2 (2.0) n=49	51.5 (1.2) n=45
Reagent 34588800 and calibrator 340896	3.9 (0.5) n=93	3.8 (0.6) n=84	8.6 (0.5) n=96	8.3 (0.5) n=79	16.8 (0.6) n=122	16.8 (0.6) n=144	52.5 (1.2) n=126	53.0 (1.0) n=130
Reagent 38153900 and calibrator 340896	4.1 (0.3) n=28	3.9 (0.3) n=25	8.3 (0.3) n=32	8.2 (0.3) n=30	16.5 (0.4) n=38	16.5 (0.4) n=43	52.4 (1.0) n=40	52.5 (0.9) n=45
Overall mean (SD)	3.6 (0.6) n=360	3.5 (0.5) n=320	8.2 (0.6) n=370	8.0 (0.6) n=324	16.7 (0.6) n=477	16.5 (0.6) n=547	53.1 (1.5) n=496	53.0 (1.2) n=513

Appendix 1

Rule in/rule out criteria for NSTEMI in the Leiden University Medical Centre.



This algorithm was developed in the LUMC by the Department of Clinical Chemistry and Laboratory Medicine and the department of Cardiology, based on the APACE [2] and TRAPID-AMI [3] studies.

Appendix 2

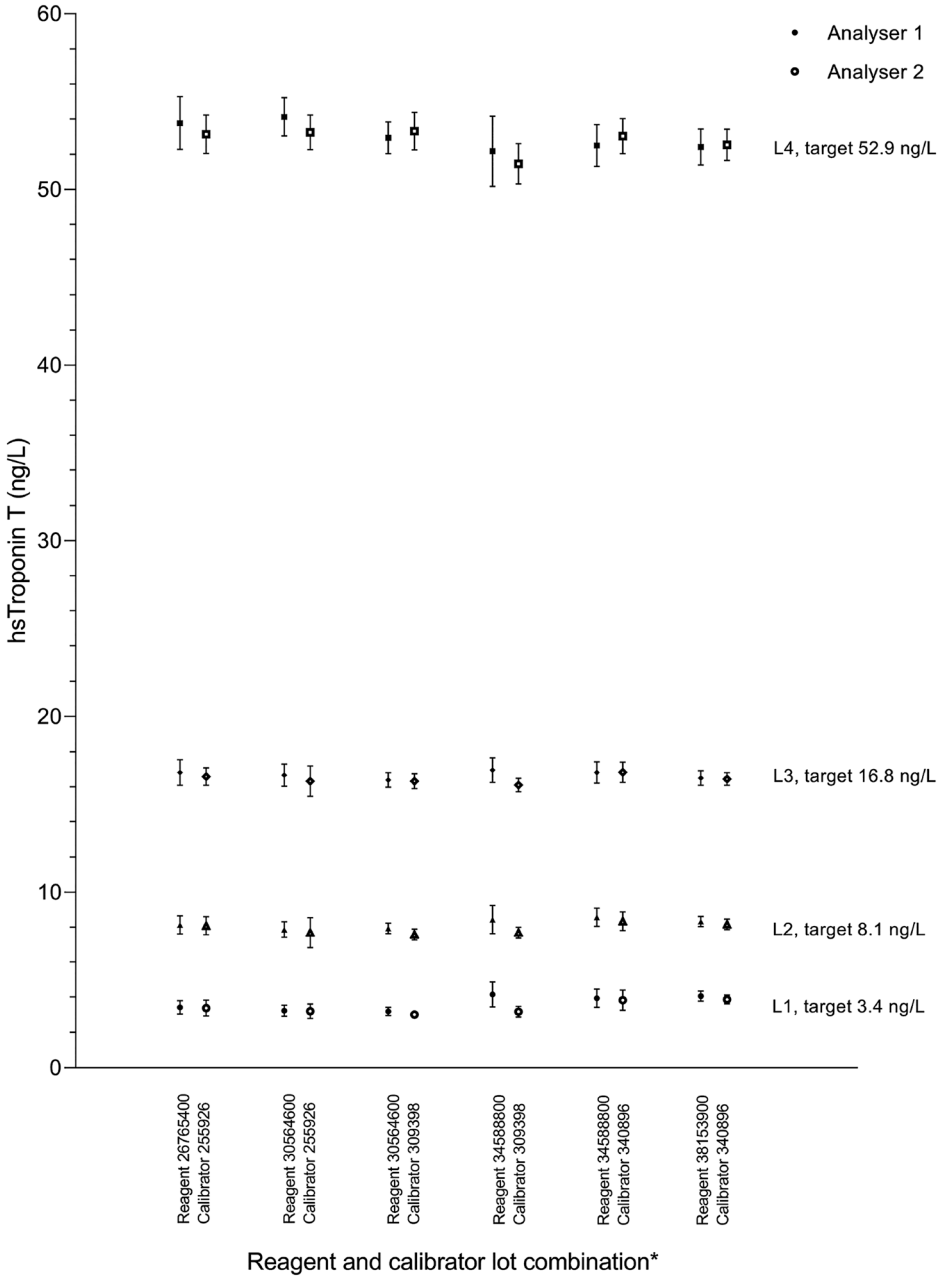
Proportion of paired samples with differences between analyser 1 and analyser 2 >1 ng/L, >2 ng/L or >3 ng/L.

	Differences between analyser 1 and analyser 2	n (%)
L1	>1 ng/L (1 SD)	5 (4%)
(n=124)	>2 ng/L (2 SD)	0 (0%)
Within 1 h	>3 ng/L (1 hr algorithm)	0 (0%)
L2	>1 ng/L (1 SD)	18 (16%)
(n=112)	>2 ng/L (2 SD)	0 (0%)
Within 1 h	>3 ng/L (1 hr algorithm)	0 (0%)
L3	>1 ng/L (1 SD)	4 (17%)
(n=23)	>2 ng/L (2 SD)	0 (0%)
Within 1 day	>3 ng/L (1 hr algorithm)	0 (0%)
L4	>1 ng/L (1 SD)	15 (56%)
(n=27)	>2 ng/L (2 SD)	4 (15%)*
Within 1 day	>3 ng/L (1 hr algorithm)	4 (15%)

*3 November 2017: IQC samples measured again, after which results were within limits.
9 April 2018: recalibration at 10 April 2018.
20 June 2018: recalibration at 22 June 2018.
5 October 2018: no explanation found.

Appendix 3

Mean and standard deviations hs-TnT concentrations per reagent and calibrator lot combination, per IQC level, per analyser.



*Dates in use (starting date):
Reagents:
Lot 26765400: Analyser 1 3 November 2017, Analyser 2 3 November 2017
Lot 30564600: Analyser 1 18 April 2018 20:25, Analyser 2 18 April 2018 01:45
Lot 34588800: Analyser 1 17 October 2018 18:28, Analyser 2 17 October 201801:12
Lot 38153900: Analyser 1 28 April 2019 12:01, Analyser 2 28 April 2019 01:05
Calibrator:
Lot 255926: Analyser 1 3 November 2017, Analyser 2 3 November 2017
Lot 309398: Analyser 1 25 July 2018 19:20: Analyser 2 15 August 2018 01:37
Lot 340896: Analyser 1 1 December 2018 11:24, Analyser 2 26 November 2018 03:00

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