**Inter-Analyzer Analytical Variation of the Roche High-Sensitivity Cardiac Troponin T Assay Can Exceed the Cut-Off of the ESC One-Hour Algorithm for Ruling Out NSTEMI.**

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**Abbreviations**

ESC, European Society of Cardiology; NSTEMI, non-ST elevation myocardial infarction; hs-cTnT, high sensitivity cardiac troponin T; IQC, Internal quality control.

The European Society of Cardiology (ESC) guidelines *(1)* recommend that patients presenting with chest pain can be ruled out for non-ST elevation myocardial infarction (NSTEMI) if their baseline Roche Diagnostics high-sensitivity cardiac troponin T (hs-cTnT) result is either (i) <5 ng/L (if chest pain started at least three hours prior) or (ii) <12 ng/L and the change one hour later is <3 ng/L. Due to the rounding of results, the analytical variation of the Roche hs-cTnT assay should not exceed 2 ng/L at concentrations <12 ng/L for use in the zero to one-hour algorithm.

A large clinical biochemistry diagnostic laboratory usually has multiple analyzers performing a hs-cTnT assay in order to meet service demand. Zero and one-hour hs-cTnT samples from the same patient are likely to be processed on different analyzers. Although the lot-to-lot analytical variation of the Roche hs-cTnT assay has already been investigated *(2)*, the analytical variation between separate Cobas modules over the likely duration of a patients initial investigation is less understood. The aim of this study was to determine if the inter-analyzer analytical variation of the Roche hs-cTnT assay over an eight-hour period is satisfactory for the adoption of the ESC zero to one-hour algorithm.

A serum pool with a hs-cTnT concentration of approximately 6 ng/L was made from anonymous and previously separated redundant serum obtained from the blood sciences department at St George’s Hospital. Intra and inter-analyzer precision was assessed on the Roche Cobas modules in use in the central laboratory (two Cobas e602 and one Cobas e801) and the Emergency Department STAT-laboratory (one Cobas e411) at St George’s hospital. The serum pool was not re-centrifuged before analysis on any of the Cobas modules.

Intra and inter-analyzer repeatability (short term precision) was assessed by analysis of the serum pool 25 times on each analytical cell on each Cobas module within one hour (n=150 on one Cobas e411, two Cobas e801 and three Cobas e602 cells).

Inter-analyzer analytical variation over an eight-hour period was assessed by replicating realistic sample flow. Ten aliquots of the serum pool were analyzed in the STAT-laboratory on the Cobas e411 to represent a zero-hour hs-cTnT baseline. Five aliquots were then processed on our pre-analytical modular Roche Cobas 8000 in the central laboratory at each two-hour interval which sent the samples for analysis on a Cobas e602 or e801 (depending on system demand). The mean, SD and 95% confidence intervals were calculated using the Analyse-it software *(3)* for each Cobas module and time point.

The Cobas e411 module demonstrated the poorest intra-analyzer repeatability with a CV of 9.6% and a range of 3 ng/L. The Cobas e602 and e801 modules demonstrated improved intra-analyzer repeatability with CVs <5.7% and ranges ≤2 ng/L. After exclusion of the Cobas e411, the inter-analyzer repeatability within one hour gave a CV of 7.7% and a range of 2 ng/L (n=125).

Results of the eight-hour inter-analyzer analytical variation experiment are shown in table 1. When zero-hour baseline samples were processed on the Cobas e411 and subsequent samples were analyzed on the Cobas e602/e801 (n=30), results increased by up to 3 ng/L. After exclusion of the zero-hour measurements on the Cobas e411, no two results over the two, four, six and eight-hour period on the Cobas e602/e801 modules (n=20) differed by >1 ng/L.

A previous study reported that Roche hs-cTnT assay lot variations between 2013 to 2018 are large *(2)*. This could potentially render the use of the zero to one-hour algorithm inappropriate if the lot numbers between the different Cobas modules vary. If hospitals adopt these ESC rule out algorithms, it is important that laboratories monitor the performance of the Roche hs-cTnT assay by using third-party internal quality control (IQC) material with appropriate concentrations at the clinical cut-off levels (5 ng/L and 12 ng/L).

In conclusion, the inter-analyzer analytical variation between separate Cobas e601 and Cobas e802 modules is satisfactory for the implementation of the ESC zero to one-hour NSTEMI rule out algorithm. However, the independent Cobas e411 module should not be used to provide the baseline or repeat hs-cTnT measurement for use in this algorithm as the imprecision might lead to reporting a false increase of >2 ng/L, failing to correctly rule-out NSTEMI. We therefore recommend that all hs-cTnT requests intended for the use in the ESC zero to one-hour algorithm are processed on the more precise Cobas e602 and Cobas e801 modules. This is still subject to the monitoring of significant reagent lot-to-lot variation and the appropriate use of low concentration IQC.

**References**

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**Table 1**: Inter-analyzer analytical variation of the Roche hs-cTnT assay over an eight-hour period.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Hours** | **Mean (ng/L)** | **Range (ng/L)** | **CV (%)** | **SD** | **95% CI of SD** |
| 0 (e411) (n=10) | 5.46 | 4-6 | 11.5 | 0.63 | 0.43 – 1.14 |
| 2 (e601/e802) (n=5) | 6.34 | 6-7 | 5.4 | 0.34 | 0.21 – 0.99 |
| 4 (e601/e802) (n=5) | 6.42 | 6-7 | 6.6 | 0.42 | 0.25 – 1.22 |
| 6 (e601/e802) (n=5) | 6.03 | 6-6 | 4.6 | 0.28 | 0.17 – 0.80  |
| 8 (e601/e802) (n=5) | 6.43 | 6-7 | 3.4 | 0.22 | 0.13 – 0.62 |
| Mean (n=30) | 6.02 | 4-7 | 10.0 | 0.60 | 0.48 – 0.81 |
| Mean Excluding 0-Hour Baseline (n=20) | 6.30 | 6-7 | 5.4 | 0.34 | 0.26 – 0.50  |

*hs-cTnT, high-sensitivity cardiac troponin T; CV, Coefficient of Variation; SD, Standard Deviation; CI, Confidence Interval*