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The importance of low level QC for high sensitivity troponin assays

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ABSTRACT

BACKGROUND:

With the advent of the new high-sensitivity troponin assays, it is becoming critical to measure troponin accurately to low concentrations. To ensure assay performance is acceptable, appropriate QC must be run.

METHODS:

In addition to the routine use of commercial QC material, we prepared pools of human QC material with low troponin concentrations close to the limit of quantitation, and ran these regularly on our laboratory analysers

RESULTS:

Over 3 years we found no drift or shift in our hs-cTnI assay. We found that only the very low concentration human QC material gave warning of precision problems with the hs-cTnI assay. At the time of the documented poor assay precision, the higher concentration QC material indicated satisfactory performance.

CONCLUSIONS:

Choice of QC material with an appropriate concentration is important for any assay. For hs-cTn assays, it is of particular importance to use control material with a concentration near to the limit of quantitation.

KEYWORDS:

Troponin, limit of quantitation, QC

Introduction

Troponin is an important element in defining myocardial injury. The Universal Definition of Myocardial Infarction requires a troponin rise to above the 99th percentile of a healthy population [1]. However, it has been shown that even concentrations below the 99th percentile are associated with worse outcomes [2,3,4,5] and that because of the low biological variation in troponin, important changes can occur without the 99th percentile being reached [6].

Further, with the advent of the new hs-cTn assays, it has become apparent that the majority of healthy persons have low concentrations of cTn in their blood [7,8,9], and that concentrations of hs-cTnI below 10 ng/L are predictive of future cardiac events in asymptomatic populations [10,11]. It is apparent that being able to measure troponin with precision and accuracy down to low concentrations is of increasing importance.

The Roche hs-cTnT assay was the first hs-cTn assay to become commercially available. Early population studies with this assay demonstrated that approximately 30% of healthy subjects had detectable hs-cTnT in their blood (>LoD) [12]. Problems arose when a recalibration of this assay caused a marked change in the proportion of persons with detectable hs-cTnT in their blood [13,14] and further that this changed the 99th percentile of a healthy population [15,16].

This emphasised that running QC material of very low concentrations was necessary to identify and handle subtle changes in assay performance [17]. However, QC material provided by diagnostic companies is often not optimal in terms of the concentration ranges provided.

With these considerations in mind, when we introduced the Abbott hs-cTnI into routine clinical practice, besides commercially available QC material, we also elected to use a low concentration human control as QC, both to identify any possible drift in the assay, and also to monitor assay performance near the assay Limit of Quantitation (LoQ) [8].

Materials and Methods

We used the Abbott hs-cTnI assay which has a Limit of Detection (LoD) of 1.9 ng/L, a 20% CV at a concentration of 1.8 ng/L and a 10% CV at a concentration of 4.7 ng/L [18]. With this assay 98.6% of healthy adults had hs-cTnI concentrations above the LoD [8]. While we report 99th percentiles of 26 ng/L for men and 16 ng/L for women, we have shown the 99th percentiles can vary markedly depending upon the rigour with which the reference population is screened [19].

For routine QC we ran as a low control Thermofisher MAS Omni Cardio (lot OCRD1906U 11.7 ng/L and lot OCRD1701U <10 ng/L) and as Medium and High controls we ran Biorad Liquicheck Cardiac Markers Plus Control Levels 1 and 2.

Identifying the LoQ is important for the reliable measurement of low concentrations of any analyte [20]. To confirm the validity of measurements at these low concentrations it is necessary to run QC of a comparable concentration. We have shown that the LoQ for hs-cTnI is <10 ng/L [8].

A healthy adult volunteer provided serum at a concentration of approximately 2 ng/L and this was diluted with post-AMI serum which was screened by PEG precipitation to ensure no macro-complexes were present [21]. The final concentration was approximately 4-5 ng/L,

well below the 99th percentile and approximating to the LoQ but at a concentration where the assay had precision with a CV near 10%. Sufficient blood was collected to run weekly QC on our 3 instruments (2 x Abbott ci16200 and 1 x Abbott ci 4100) for approximately 1 year. Blood recollection was performed in time so old and new batches of human control could be run in parallel for at least 4 weeks.

We used unpaired Student *t*-tests and ANOVA (www.socscistatistics.com accessed 2018-03-18) for comparisons of results obtained between the different analyzers, locations and different lot numbers of reagents over the 30 month time period. Passing–Bablok linear regression analysis (Analyse-it Software Ltd, UK) was used for comparison of slope and intercept for assays results from the different analyzers and locations.

Results

The 3 pools of very low human control material had mean concentrations and CVs of 4.46 (13.0%), 4.60 (11.0%) and 3.60 ng/L (14.0%) respectively.

Over the 30 months that we have been using the human low QC material, numbers generated using the Abbott hs-cTnI assay have remained steady. Using ANOVA statistics and linear regression analysis showed no differences between the concentrations obtained using these low concentration human control materials on all three instruments. ANOVA showed no differences between Analyzers 1 and 2 for CMP2 but showed differences between these results and those obtained on Analyzer 3 ($p=0.03$). Regression analysis showed a difference in intercept between Analyzer 3 and Analyzers 1 and 2 (Analyzer 1 4.83, Analyzer 2 4.83, and Analyzer 3 4.46) but no difference in slope (0.00 for all instruments).

Despite being of extremely low concentration, assay CV's have been excellent with CVs similar to the quoted CVs in the Abbott product information. In Figures 1 and 2 we show the weekly QC over approximately 1 year, using 2 separate batches of the human QC material.

For CMP2 it is apparent that there was one major occurrence of assay instability for Analyzer 3. Table 1 shows the assay performance for the 4 levels of QC over this period, and also for a period when, using human QC material CMP3 the assay was stable on all instruments. The precision is markedly worse with the very low human control, moderately worse with the low commercial QC and quite satisfactory with the higher concentration QC samples.

Discussion

In our study reported here we have shown 2 noteworthy items. Firstly, the hs-cTnI assay has been stable with no evidence of drift or change in concentration over nearly 3 years of using very low concentration human control material. For each batch of very low concentration human QC material, the slope of the line was zero, indicating no variation in sample concentration. Secondly we have shown that assay problems will only become apparent if QC material of an appropriate concentration is used. The very low human QC material showed a large rise in CV around the time of assay instability while even the low QC control showed only moderate loss of control. The higher QC samples showed no evidence of assay performance problems at all.

With the older troponin assays, the 99th percentile was very near to the limit of detection of the assays and the presence of any troponin was regarded as of clinical concern. However,

since the introduction of the hs-cTn assays, it is possible to measure precisely to well below the 99th percentile, and it is apparent that most healthy persons have detectable concentrations of troponin in their blood [7,8,9]. Measuring precisely at concentrations well below any possible decision limit for cTn be it the 99th percentile, 97.5th percentile or other, is of considerable importance as cTn has a low biological variation. Further, several studies have shown that in asymptomatic populations, concentrations of hs-cTnI <10 ng/L are strongly predictive of future cardiovascular events [10,11].

Any suggestion of assay drift or significant change on recalibration, as occurred with the Roche hs-cTnT assay at lower concentrations, is problematic as it has the potential to move results both around the decision point of the 99th percentile, and at lower concentrations that are important for prognostic assessment of patients.

Hammarsten et al [15] adopted a similar approach with their hs-cTnT assay using a serum pool of 15.8 ng/L and 2 higher commercial QC materials. With both the serum and the lower control (34.5 ng/L) they observed shifts when the assay was re-calibrated. These appeared to be of particular importance as they showed changes above the reported 99th percentile for hs-cTnT [12]. Similarly, Parsonage et al [16] found that after the recalibration the number of persons with detectable troponin in their study [22] increased from 29% to 66%, and Franzini et al found that the number of subjects with results above the limit of blank increased from 34% to 66% [14].

Different analytes have different requirements in terms of the concentrations used to assess their performance. It is widely accepted that QC concentrations should be chosen so that they reflect decision points for the different analytes [17]. For troponin this would not only be near the 99th percentile but also at lower concentrations because troponin has a low

biological variation and critical changes can occur without the 99th percentile being breached [6]. It is suggested that assay control near the limit of quantitation is important (20) and a recent report from an expert panel recommends that QC should include a sample between the limit of detection and the lowest sex-specific 99th percentile (23). Our current study supports this contention.

It can be argued that for troponin QC material at concentrations much above the 99th percentile are less important on clinical grounds. If for example an assay reported the troponin concentration to be 100 ng/L when it reality it should have been 90 ng/L this would not have altered the clinical use of that data – the troponin is high and clinical action is required. We would argue that the key cTn concentrations for QC purposes are around the 99th percentile and at a lower concentration as we have used in this study, near the limit of quantitation, where assay instability will become apparent.

In summary, our study has shown that the Abbott cTnI assay has been stable over the 4 years we have been critically reviewing its performance with low level QC material. In particular, we have demonstrated that only the low level QC material was able to demonstrate the assay going out of control. All laboratories using hs-cTn assays should review the QC material that they use and ensure it is of an appropriate concentration.

DECLARATIONS OF INTEREST: None

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Table 1: Assay precision performance at 4 different QC concentrations. The first panel shows typical performance during a period of satisfactory assay performance, while the second panel shows assay performance at a time when precision for the low human QC material was unacceptably poor.

In control				
		Instrument		
		#1	#2	#3
		Mean (ng/L) (CV)		
V low human		3.6 (14.0%)	3.7 (10.6%)	3.6 (13.1%)
Low QC		11.1 (6.9%)	11.0 (6.9%)	11.4 (7.7%)
Medium QC		849.1 (3.0%)	852.6 (3.3%)	892.6 (4.2%)
High QC		2132.6 (3.5%)	2170.7 (3.7%)	2248.0 (3.7%)
Out of control				
		Instrument		
		#1	#2	#3
		Mean (ng/L) (CV)		
V low human		4.6 (11.0%)	4.7 (11.7%)	4.0 (31.2%)
Low QC		6.9 (10.3%)	6.6 (13.8%)	7.4 (19.2%)
Medium QC		858.7 (3.1%)	891.9 (2.5%)	831.5 (4.8%)
High QC		2190.0 (4.1%)	2262.5 (4.4%)	2179.4 (4.3%)
Low QC: in control lot number OCRD1906U; out-of-control lot number OCDR1701U				
Medium QC: in control lot number 29841; out-of-control lot number 29841				
High QC: in control lot number 29842; out-of-control lot number 29842				

Legends to figures

Figure 1: hs-cTnI assay precision on 3 analyzers using very low concentration human control material, demonstrating satisfactory assay performance

Figure 2: hs-cTnI assay precision on 3 analyzers using very low concentration human control material, demonstrating poor precision on Analyzer 3

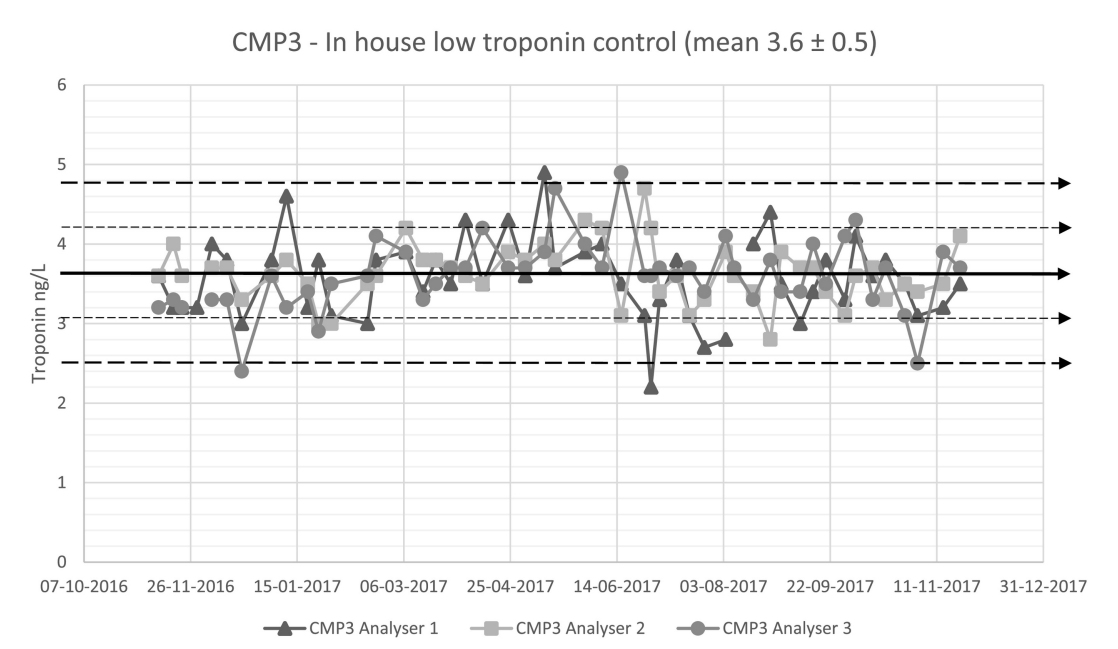
CMP3 - In house low troponin control (mean 3.6 ± 0.5)
Troponin ng/L
6
5
4
3
2
1
0
07-10-2016
26-11-2016
15-01-2017
06-03-2017
25-04-2017
14-06-2017
03-08-2017
22-09-2017
11-11-2017
31-12-2017
CMP3 Analyser 1
CMP3 Analyser 2
CMP3 Analyser 3

Figure 1

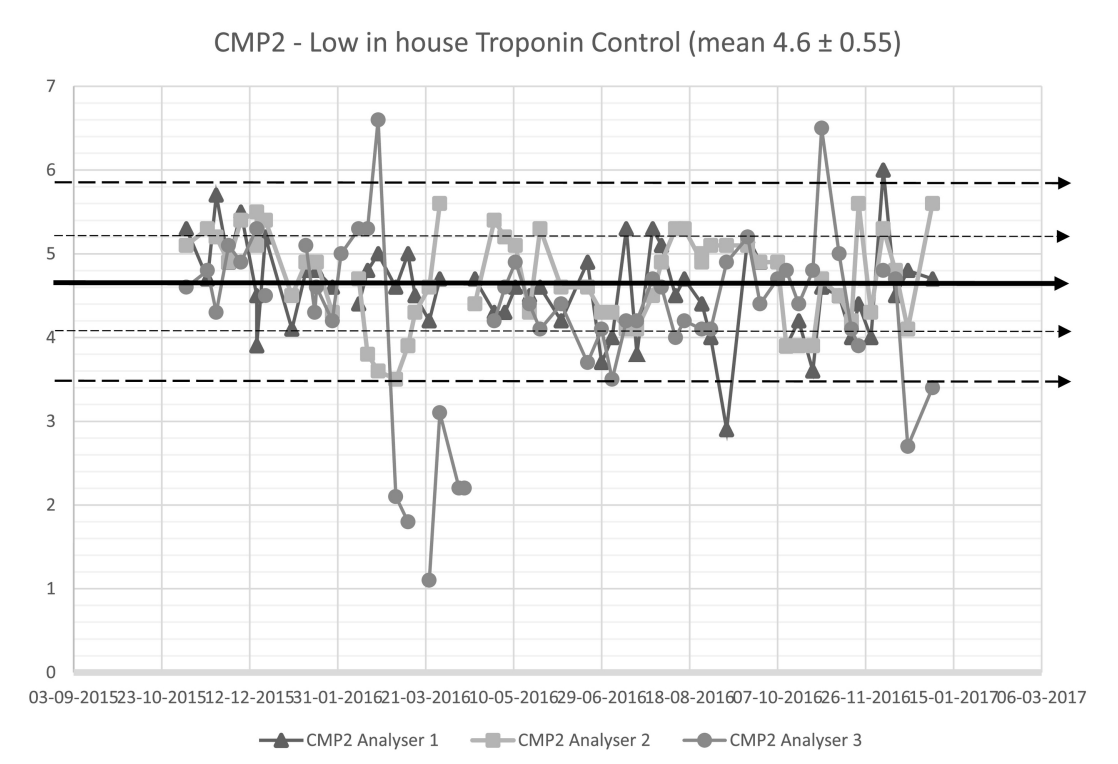
CMP2 - Low in house Troponin Control (mean 4.6 ± 0.55)
7
6
5
4
3
2
1
0
03-09-2015
23-10-2015
12-12-2015
31-01-2016
21-03-2016
10-05-2016
29-06-2016
18-08-2016
07-10-2016
26-11-2016
15-01-2017
06-03-2017
CMP2 Analyser 1
CMP2 Analyser 2
CMP2 Analyser 3

Figure 2