Global Provider of Quality in Diagnostic Medicine

UKAS PROFICIENCY TESTING 4301

Serum Cardiac marker Scheme Guide

Director: Annette Thomas

WEQAS Unit 6
Parc Ty Glas
Llanishen
Cardiff,
UK CF14 5DU
Tel.: +44 2920 314750
Fax.: +44 2920 314760
Email: office@weqas.com
www.weqas.com

Contents

1. Scheme details and repertoire

1.1 Source Material and Serum Integrity

The base material is human serum, tested negative for HIV and Hepatitis B and C at donor level. The pools are spiked with a source of recombinant CK MB and a preparation of Troponin I/T/C complex and myoglobin from cardiac tissue.

The pools are filtered aseptically down to a 0.2μm, and gentamicin added to maintain sterility. **Preservatives such as sodium azide are not added as these are known to inhibit certain immuno enzymatic methods**. Great care is taken to ensure that aseptic techniques are used throughout all procedures to maintain sterility. The serum is dispensed aseptically into 1ml aliquots and stored at -20°C until dispatched. The samples are dispatched by first class mail as frozen samples packaged in containers conforming to Post Office guidelines.

A Plasma cardiac marker scheme is also available with material specifically formulated to suit the Triage meter.These samples are stored at -20°C and distributed on dry ice by TNT as batched samples.

1.2 List of Analytes and Frequency of Distribution

Three 1.0 ml samples covering the physiological and pathological ranges are distributed every month. Six analytes are included in each distribution (TnT results can be submitted as quantitative or qualitative results. There are a range of samples

Table 1 – Analyte and Range covered.

Analyte	Range Covered	
Troponin T	3 - 500	ng/L
Qualitative TnT	3 - 500	ng/L
Troponin I	1 - 700	ng/L
CK MB (mass)	2 - 200	µg/L
CK MB (activity)	5 - 140	IU/L
Myoglobin	40 - 500	µg/L

Please note the ranges for the Troponin I target range values are based on the Berckman AcuuTnI assay.

Comparibility factors are taken into account and are used for multimodal data where a wide variation is observed for the overall consensus mean due to the widely different methods used.
A method specific comparability factor (CF) is calculated for each method by analysing the method data using linear regression analysis against a peer reference method (Beckman AccuTnI for Troponin I). The result sfor each laboratory are then adjusted using the CF. Each laboratory's results can therefore be compared with their own method group, the peer reference method and directly compared with the overall mean of allgroups. The CF's for each scheme are available on request.
For TnI the comparability factor (CF) is the ratio of the slope of the method to the slope of the AccuTnI data and gives a measure of the magnitude of the bias.

2. Instructions for use

The samples are dispatched frozen, and will thaw in transit. Please ensure that the samples are well mixed before analysis. Please analyse on the day of arrival. If there is a delay in analysis please store at 4°C.

Although every effort is made to ensure that the material is free from any known infectious agent, the samples should be handled as for clinical specimens.

3. Statistical Analysis

Please refer to the WEQAS Participants Manual for full details on statistical analysis and interpretation of results for both quantitative and qualitative analytes.

3.1 Performance criteria

Precision profiles for these analytes have been calculated over a number of years and reflects the "state of the art" of Cardiac Marker methods. The precision profile is used to detrmine the WEQAS SD for each analyte across the pathological range. The performance criteria used is Target value +/- 2*WEQAS SD. The precision profile for TnT and TnI is provided in the following figures.

Figure 1 a-e Precision Profiles

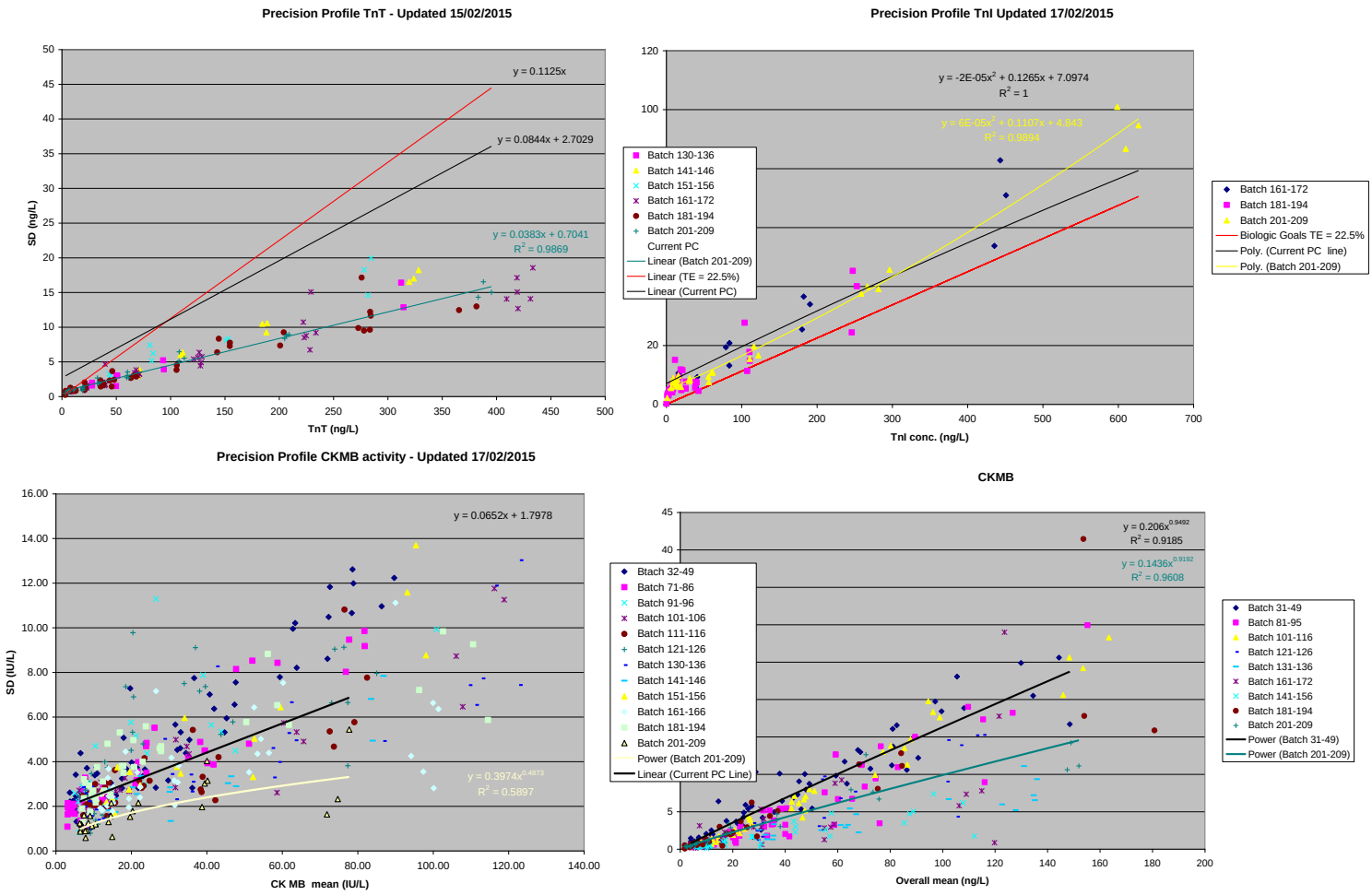
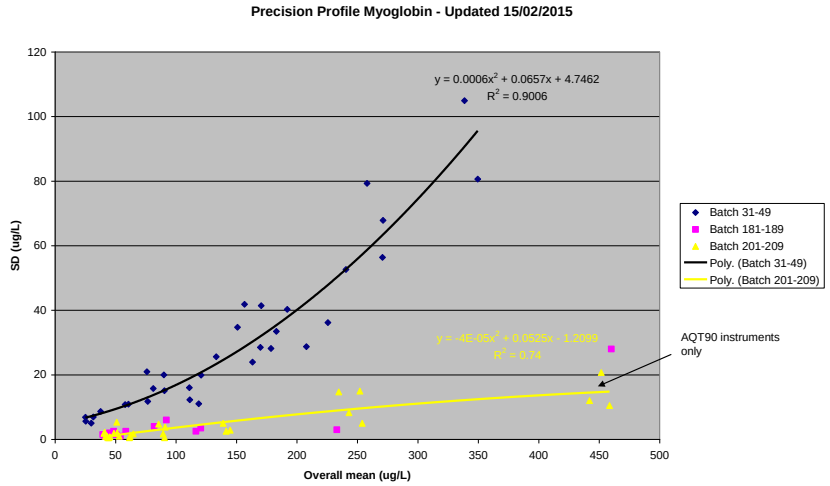


Figure 1e



3.2 Method Performance

A Summary of Method and Analyser performance is included with each distribution. This is given in tabular form, as the mean, standard deviation and estimate of uncertainty. An example for Troponin I is given in the following Table. Analysers groups are only included where there are 2 or more participants.

Figure 2 Method Summary Report

Weqas

Distribution:	N174
Distribution Date:	21-Jan-15
Analyte:	Troponin T (ng/l)

Method	Instrument		1	2	3	4
		Overall Mean	60.19	3.33	206.83	18.96
		Overall SD	3.04	0.47	8.88	0.87
		Est. Uncertainty of Consensus	0.389	0.272	1.156	0.117
		Overall Number	61	3	59	55
Roche High Sensitivity		Method Mean	60.64	3.33	206.94	18.98
		Method SD	2.65	0.47	8.61	0.87
		Est. Uncertainty of Consensus	0.357	0.272	1.160	0.120
		Number	55	3	55	53
	Elecsys E170	Instrument Mean	60.79	3.00	207.65	18.87
		Instrument SD	2.31	0.00	7.28	0.86
		Number	16	2	16	15
		Sample 2: 2 labs reported <14, 4 <12, 1 <5 and 5 <3				
	Cobas E Module	Instrument Mean	60.61	2 labs	206.98	18.97
		Instrument SD	2.80	reported	8.96	0.83
		Number	38	<14, 4 <5 and 21 <3	38	37
STAT hsTnT	Elecsys 2010	Method Mean	50.87	<3	169.65	15.93
		Method SD	0.14		0.65	0.08
		Est. Uncertainty of Consensus	0.095		0.460	0.053
		Number	2	2	2	2
AQT90	AQT90	Method Mean	30.60	<10	134.00	<10
		Method SD	2.73		8.00	
		Est. Uncertainty of Consensus	1.22		3.578	
		Number	5	5	5	5

3.3 Qualitative Results

Qualitative or semi-quantitative results for POCT devices can also be submitted.
An example of a typical method Summary report and Laboratory reports are included in Figures 3 and 4.

Figure 3 Qualitative Summary Report

Qualitative Report

Please note that Roche have stated that serum samples will have a lower recovery than whole blood

Distribution N174

Qual Reader Trop T Results

Lab Code	Section	Instrument	Sample Number				Sample Score				Average Score (Average)
			1	2	3	4	1	2	3	4	
AAM	Lab	Cardiac Reader	<(30) 50	<(30) 50	(30) 50-100	<(30) 50	0	0	0	0	0
AFK	RACPC	Cardiac Reader	<(30) 50	<(30) 50	(30) 50-100	<(30) 50	0	0	0	0	0
AGZ	Dunoon	Cobas h232									
AGZ	Mid Argyll	Cobas h232	<(30) 50	<(30) 50	(30) 50-100	<(30) 50	0	0	0	0	0
FZ	Pathology	Cobas h232	<(30) 50	<(30) 50	(30) 50-100	<(30) 50	0	0	0	0	0
HK	Endocrine	Cobas h232	<(30) 50	<(30) 50	(30) 50-100	<(30) 50	0	0	0	0	0
SH	Alexandra	Cobas h232	<(30) 50		(30) 50-100	<(30) 50	0		0	0	0
XR	Pathology	Cobas h232									

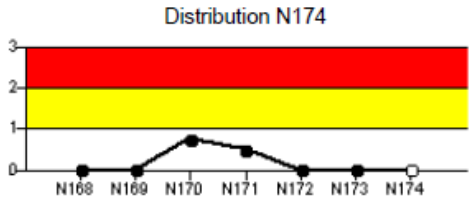
Interpretation	<(30) 50	<(30) 50	(30) 50-100	<(30) 50

From Troponin T Results				
Mean	60.2	3.3	206.8	19.0
SD	3.04	0.47	8.88	0.87
CV %	5%	14%	4%	5%

Cardiac Reader / h232 Targets				
Sample	1	2	3	4
Target	24.26	1.33	82.78	7.59

Cardiac Marker Summary Sheet Distribution N174

Figure 4 –Typical Laboratory Report for Qualitative TnT

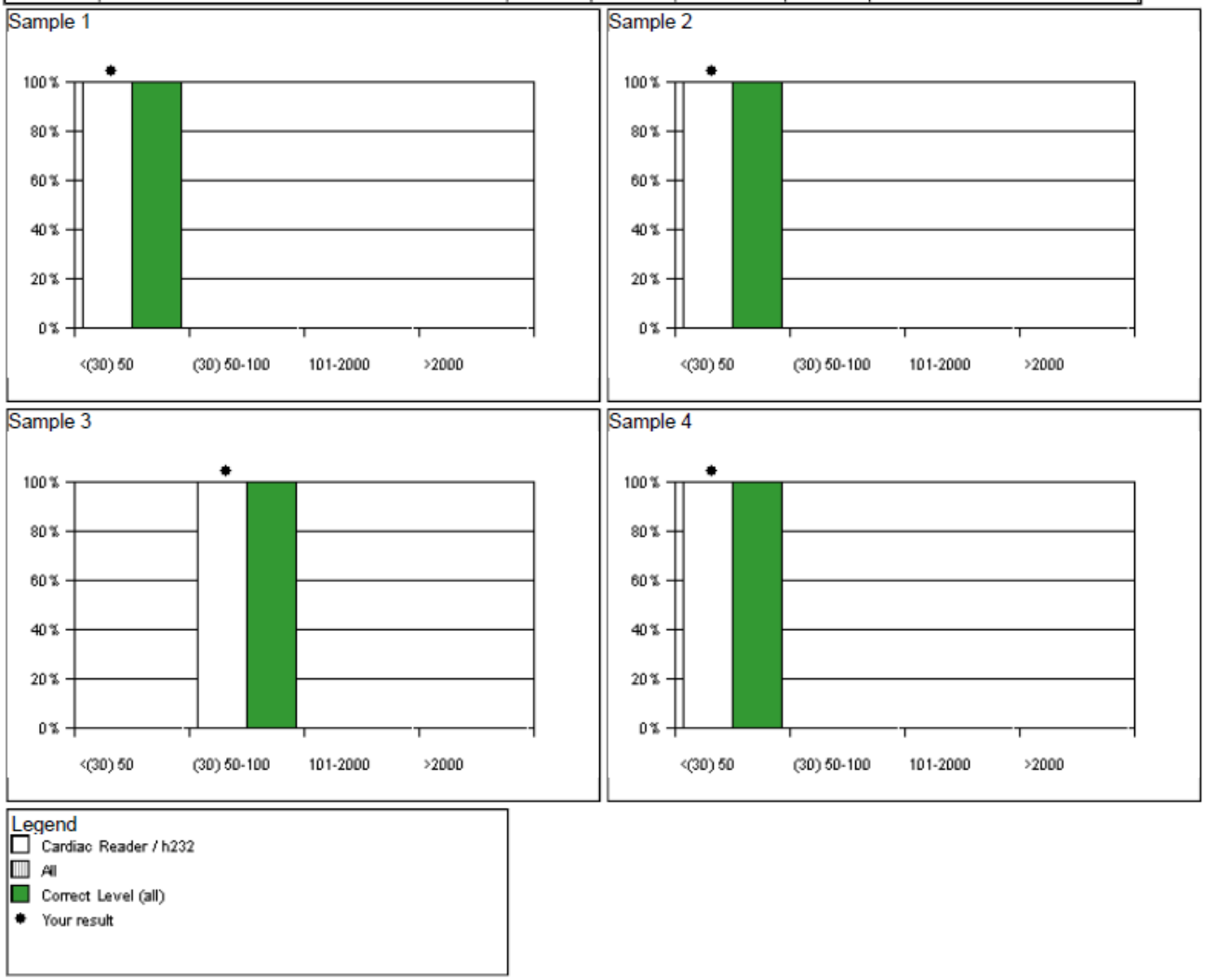


Qualitative Report

Distribution N174

Qual Reader Trop T Results

Lab Code	Section	Method	Instrument	Sample Number				Sample Score				Average Score
				1	2	3	4	1	2	3	4	(Average)
AAM	Lab	Cardiac Reader / h232	Cardiac Reader	<(30) 50	<(30) 50	(30) 50-100	<(30) 50	0	0	0	0	0.00
Interpretation				<(30) 50	<(30) 50	(30) 50-100	<(30) 50					
From Troponin T Results												
Mean				60.2	3.3	206.8	19.0					
SD				3.04	0.47	8.88	0.87					
CV %				5%	14%	4%	5%					



4 Assessment of Intralaboratory Variation and Sensitivity of current assays.

This study was a repeat of the work conducted in 2006 and 2004 to establish the "state of the art" of Troponin assays. The aim of the study was to determine the intralaboratory variation (both within and between batch) at a range of Troponin concentration for group of laboratories and to establish the coefficient of variation at or near the limit of detection of the assay.

4.1 Pool preparation

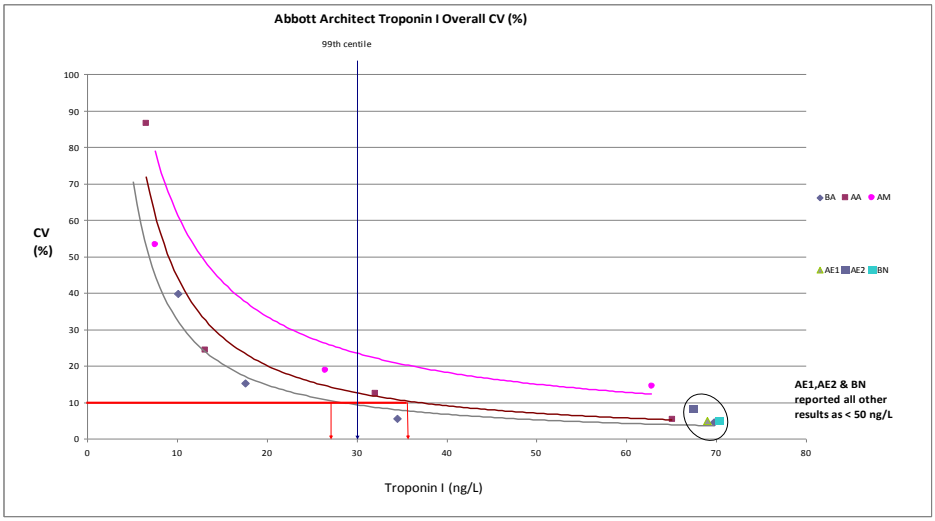
A base pool of human serum from a healthy donor was spiked with ternary Troponin complex (ITC) to an approximate concentration of 60ng/L TnT and TnI as measured on the Beckman AccuTnI method. Three doubling dilutions using the base serum were prepared to give final concentrations close to the cut off points of all the methods. The samples were aliquotted and stored at -70°C until dispatch. Ten sets of 4 pools were dispatched to a cohort of 16 laboratories and asked to freeze the samples on arrival.

4.2 Assay Protocol

The protocol consisted of two replicates per pool per run, and two runs per day for 5 days (10 runs). The laboratories were asked to analyse the samples as if they were patient samples, therefore calibration frequency and reagent lot numbers was laboratory dependent.
Each laboratory carried out 80 assays. The within run, between batch and interlaboratory coefficient of variation was calculated for all laboratories for all methods.

4.3 Results

Figure 5 a –b, Intralaboratory performance of Troponin I methods



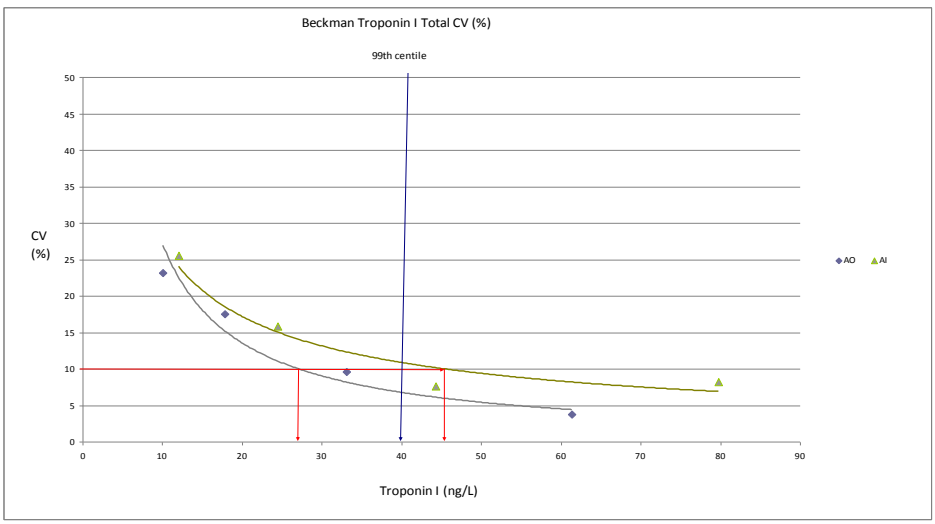


Figure 6, Intralaboratory performance of Troponin T method

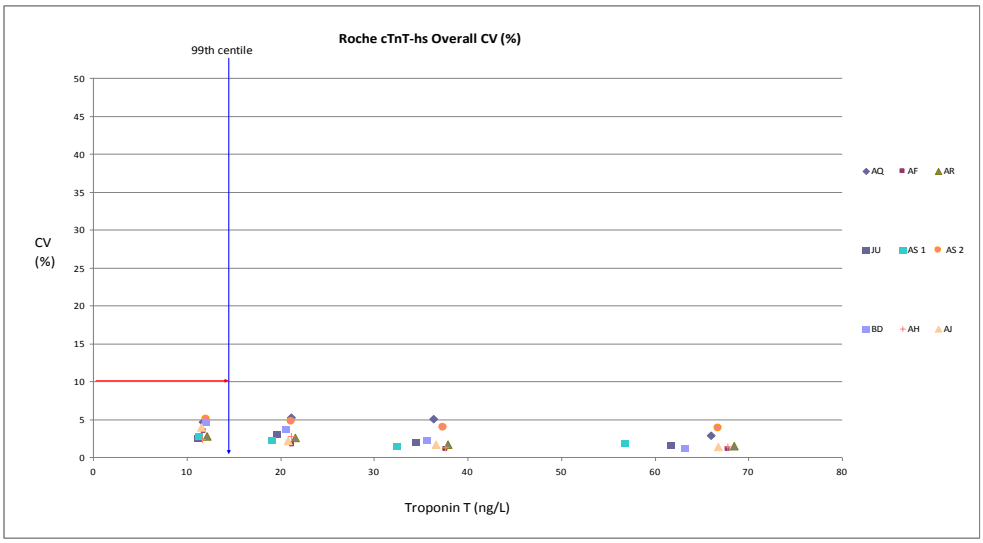


Table 2 Summary of Performance at or near "limit of detection" and 99th percentile of the methods.

Roche hs-cTnT	CV @14ng/L	Tn@10% CV	Guideline acceptable	Clinically Usable
Lab 1	4.6	5.4	√	
Lab 2	2.4	0.1	√	
Lab 3	5.2	1.1	√	
Lab 4	2.6	1.8	√	
Lab 5	2.7	0.6	√	
Lab 6	2.7	0.2	√	
Lab 7	2.5	0.5	√	
Lab 8	3.2	2.0	√	
Abbott	**CV @30ng/L**	**Tn@10% CV**		
Lab 9	9.3	28.1	√	
Lab 10	12.6	36.7	x	√
Lab 11	23.5	78.9	x	x
Lab 12 & 13	Could not calculate		x	
Beckman	**CV @40ng/L**	**Tn@10% CV**		
Lab 14	6.8	27.1	√	
Lab 15	10.9	45.4	?	√
Lab 16	No results submitted			

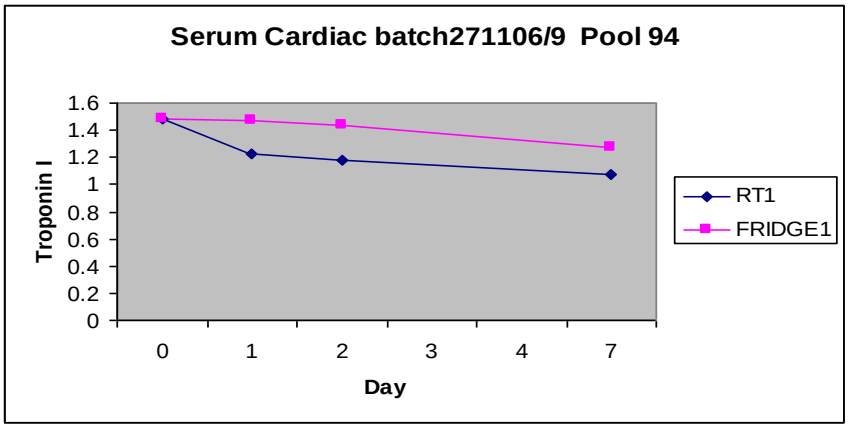
Using the Apple Storecard as a scoring matrix, all users of the Roche hs-cTnT method performed well, the Coefficient of Variation (CV) at the "cut off" of 14 ng/L was well within the 10% acceptance criteria for all laboratories and the method was deemed Guideline acceptable. For the Abbott Architect method, 2 of the 5 laboratories were unable to provide quantitative results for TnI < 50 ng/L, one laboratory had a CV of < 10% at the "cut off" of 30 ng/L , however the other 2 laboratories failed to meet this criteria. Overall the method did not fulfil the criterion of Guideline acceptable, and deemed to be Clinically useable in only 2 out of the 5 Laboratories. For the Beckman AccuTnI method, only 2 Laboratories submitted results, one of which was Guideline acceptable with a CV of 6.8% and one which was just outside the critera with a CV of 10.9% at the 40 ng/L "cut off".

5 Sample Stability

5.1 Short Term Stability

The following stability study was assayed using the Siemens Advia method on a pool prepared as part of a batch of material made in November 2006. Stability measured suggests samples are stable for seven days at room temperature. For this reason all samples are dispatched frozen to each participant and will thaw in transit. Participants are advised if unable to assay on day of receipt to store ay 4^{o}C and assay within five days.

Figure 7



5.2 Long term stability at -20 °C

Six pools were distributed on a number of occasions over a 12 month period. The following figures illustrate the change in analyte concentration (WEQAS overall mean – as measured on the Beckman AccuTnI) for each of the pools distributed to all WEQAS participants over that period.

Figures 8a-c