Validation of an Electrochemiluminescence (ECL) method for the quantitation of cardiac markers in rat serum

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1 ABSTRACT

Cardiac Troponin I (cTnI) and Troponin T (cTnT) are subunits forming the Troponin heterotrimer that regulates muscle contraction in cardiac muscle. The Troponin controls the interaction of actin and myosin filaments in striated muscles fibers. Fatty Acid binding protein 3 (FABP3) is a monomeric protein that modulates the uptake of fatty acids in cells. It is released into circulation after myocardial ischemia and necrosis. Myosin light chain 3 (Myl3) is an essential light chain of the myosin (hexamer ATPase motor protein) molecule found in cardiac muscle. After damage to cardiac muscle tissue, myosin breaks down and Myl3 is released in the blood. These biomarkers can be useful to confirm cardiac muscle injury during safety assessment pre-clinical studies. Therefore, the validation of an Electrochemiluminescense (ECL) method for the simultaneous quantitation of cTnI, cTnT, FABP3, and Myl3 is a useful tool to support non-clinical studies where the endogenous level of these biomarkers will aid in study interpretation.

2 MATERIAL AND METHODS

A study was undertaken to qualify and validate the multi-spot cardiac panel assay using the MSD® (Meso Scale Discovery) platform for the quantitation of cTnI, cTnT, FABP3, and Myl3 in rat serum. Analytical qualification and validation parameters, including precision, accuracy, selectivity and parallelism of the method were assessed. Stability of cTnI, cTnT, FABP3, and Myl3 in rat serum was also validated under different conditions such as long term storage, freeze and thaw cycles as well as storage at ambient room temperature and 4°C.

Rats were treated with isoproterenol (structurally similar to adrenalin) by subcutaneous injection to induce higher levels of theses markers. Samples were collected 4 hrs following injection.

3 RESULTS

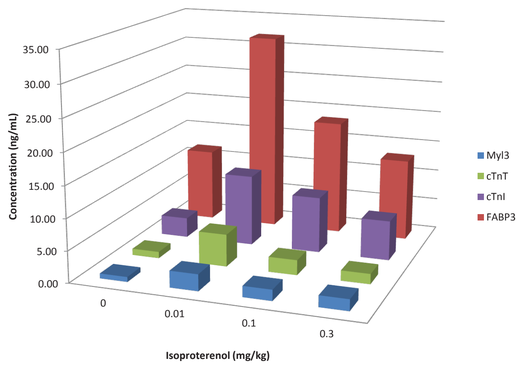
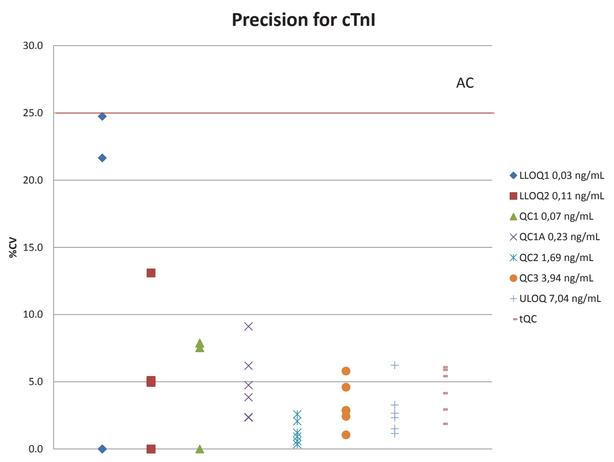


Figure 1 Levels of cardiac markers following isoproterenol injection

STD ID	Target Concentration (ng/mL)			
	cTnI	cTnT	FABP3	Myl3
Calibrator K	28,15	70,50	110,50	70,00
Cal J (ULOQ)	**7,04**	**17,63**	**27,63**	**17,50**
Cal I	2,50	6,26	9,81	6,21
Cal H	1,75	4,38	6,86	4,35
Cal G	1,17	2,92	4,58	2,90
Cal F	0,44	1,09	1,72	1,09
Cal E	0,29	0,73	1,14	0,72
Cal D (LLOQ2)	0,11	**0,27**	**0,43**	0,27
Cal C (LLOQ1)	**0,03**	0,07**	0,11**	**0,07**
Cal B	0,01**	0,02**	0,03**	0,02**
QC3	3,94	9,87	15,47	9,80
QC2	1,69	4,23	6,63	4,20
QC1A	0,23	**0,58**	**0,92**	0,58
QC1	**0,07**	0,18	0,27	**0,17**

*** Accessory standard*
Bold concentrations reflect the appropriate levels for each cytokine

Table 1 Range of Curve



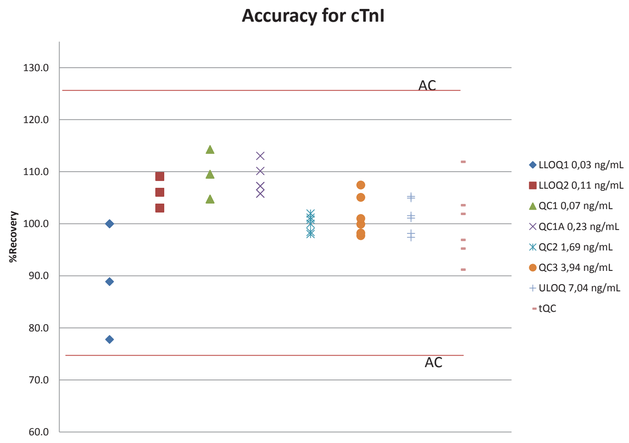


Figure 2 Precision and Accuracy for cTnI. The acceptance criteria were set at ±25% for the % Theoretical and ≤25% for %CV for at least 67% of the occasions performed. For the LLOQ and ULOQ, a %CV ≤30% and a % Theoretical within ±30% were acceptable.

4 DISCUSSION

Endogenous level

Sera from untreated rat were analyzed and detectable endogenous levels were observed for all cardiac markers. Higher levels were observed when rats were treated with isoproterenol, a compound used to induce infarct-like lesions. **(Figure 1)**

Precision and Accuracy

In order to define the lower and upper limit of quantitation of the assay, precision and accuracy assessments of QC samples (LLOQ, low, mid, high and ULOQ) were conducted. The lower and upper limit of quantitation (cTnI: 0,3 - 7.04 ng/mL; cTnT: 0,07-17,63 ng/mL; FAB3: 0,43-27,63 ng/ml; Myl3: 0,07- 17,50 ng/ml) met acceptance criteria, thus showing a wide dynamic range of standard curves.
(Table 1 and Figure 2)

Parallelism

Parallelism assessments for cTnI, cTnT and Myl3 analytes proved successful between undiluted samples and samples diluted 32-fold, 4-fold and 16-fold, respectively. Parallelism assessments for FABP3 analyte proved successful between 2 and 32-fold. **(Figure 3)**

Selectivity

The selectivity assessment demonstrated no interference effect of the matrix for cTnI, cTnT and FABP3 by spiking rat serum with the Cardiac Injury Panel 3 (rat) Calibrator (from the kit). No interference effect for Myl3 was noted when rat serum was spiked with a lot of rat serum having a high endogenous level. **(Figure 4)**

Stability

Short-term stability of cTnI, cTnT and FABP3 in rat serum was proven for up to 6 hours and 27 minutes at ambient RT and in a refrigerator set to maintain 4°C for up to 25 hours and 49 minutes. Short-term stability of Myl3 was proven for up to 6 hours and 40 minutes at ambient RT and in a refrigerator set to maintain 4°C for up to 25 hours and 49 minutes. Study samples can be subjected to a maximum of 5 freeze-thaw cycles in freezers set to maintain -20°C and -80°C for cTnI, cTnT and FABP3. For Myl3, the maximum number of freeze-thaw cycles were determined to be 5 and 4 in freezers set to maintain -20°C and -80°C , respectively. Also, study samples are stable for at least 97 days at both temperatures for all four analytes. **(Table 2)**

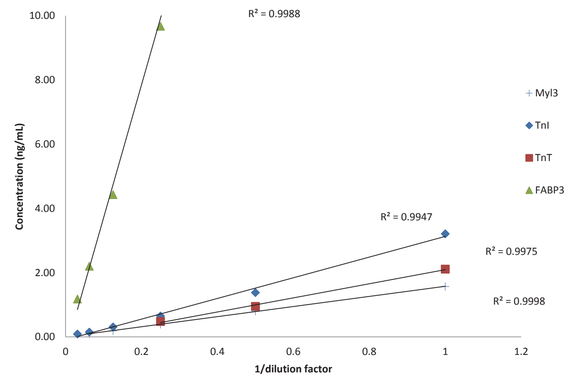


Figure 3 Parallelism performed with endogenous level of analyte. The acceptance criteria were set at ±25% for the % Recovery.

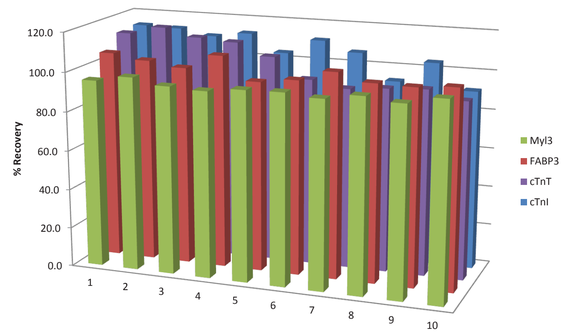


Figure 4 Selectivity performed by spiking rat serum lots with the reference material for cTnI, cTnT and FABP3 and with a rat serum with a higher endogenous level for Myl3. No interference was observed if at least 80% of spiked rat serum had a %recovery within ±25% .

Analyte	Bench top	4° C	Freeze-thaw cycles	Long term stability at -20° C and -80° C
cTnI	6 hrs 27 min	25 hrs 49 min	5 (-20° C and -80° C)	97 days
cTnT	6 hrs 27 min	25 hrs 49 min	5 (-20° C and -80° C)	97 days
FABP3	6 hrs 27 min	25 hrs 49 min	5 (-20° C and -80° C)	97 days
MYl3	6 hrs 40 min	25 hrs 49 min	5 for -20° C and 4 for -80° C	97 days

Table 2 Stabilities. The acceptance criteria were set at ±25% when the difference (%) was calculated between the global mean concentration of the stability sample and the global mean concentration of the control sample (for at least 2 out of 3 lots tested).

5 CONCLUSION

The validation data demonstrated that this ECL multiplexed method is suitable to measure cTnI, cTnT, FABP3, and Myl3 with less than 50 µL of rat serum for non-clinical studies. In addition, the results show that the method is sensitive enough to detect even subtle changes in these marker profiles and can be applied as a biomarker in non-clinical studies to detect cardiac injury.