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Initial Evaluation of the Meritas Troponin I test for Measurement of Equine Cardiac Troponin I

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Initial Evaluation of the Meritas Troponin I test for Measurement of Equine Cardiac Troponin I

Initial utvärdering av Meritas Troponin I test för mätning av ekvint hjärtspecifikt troponin I

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Abstract

Cardiac troponin I (cTnI) is considered a specific and a sensitive biomarker of cardiac disease, and due to a high inter-species sequence homology; human cTnI assays can often be used in veterinary medicine. The main aim of this study was to perform an initial evaluation of a human cTnI test, the Meritas Troponin I test, for measurement of equine plasma cTnI, in order to investigate whether the test could be a possible subject for a more comprehensive validation. The hypothesis was that the test could measure equine cTnI, as there is a good homology between equine cTnI and human cTnI.

The evaluation included a dilution parallelism, an intra-assay precision study and measurement of plasma cTnI healthy horses. In the dilution parallelism and the intra-assay precision study, equine plasma with previously established high cTnI concentration, according to another cTnI assay, was used. In addition, cTnI concentration was measured in plasma, collected from 19 horses, without signs of disease at physical and ECG examinations.

The obtained curve in the dilution parallelism was linear, indicating that the Meritas Troponin I test can be used to measure different concentrations of equine cTnI in plasma. The mean CV% of the test was 6.7% when the concentration of cTnI in plasma was above the detection limit of 12 ng/L. However, the CV% varied extensively when the concentration of cTnI in plasma was < 12 ng/L. All healthy horses, except for one, had a mean cTnI concentration below or close to the detection limit of the test, which is consistent with previous studies. The results indicate that the Meritas Troponin I test may be used to measure equine cTnI, and may have a potential use in equine medicine. However, in order to determine its clinical value in horses, a more comprehensive validation is required.

Sammanfattning

Hjärtspecifikt troponin I (cTnI) anses vara en specifik och en känslig biomarkör för hjärtsjukdom. Aminosyrasekvensen mellan arter är väl konserverad vilket innebär att humana cTnI assays ofta kan användas inom veterinärmedicinen. Huvudsyftet med denna studie var att göra en initial utvärdering av ett human-baserat cTnI-test, Meritas Troponin I test, och undersöka om det skulle kunna mäta ekvint cTnI i plasma. Utvärderingen utfördes för att undersöka om testet skulle kunna bli föremål för en mer omfattande validering. Hypotesen var att ekvint cTnI i plasma kunde mätas med Meritas Troponin I test då homologin är god mellan humant och ekvint cTnI.

I utvärderingen ingick utförande av en spädningsserie, kontroll av testets exakthet samt mätning av plasma cTnI hos friska hästar. I spädningsserien och utvärderingen av testets exakthet användes ekvin plasma, tidigare analyserat med en annan cTnI-assay, med förhöjt cTnI-värde. I denna studie mättes också koncentration av cTnI i plasma hos 19 stycken hästar, som vid klinisk undersökning och EKG inte uppvisade tecken på sjukdom.

I spädningsserien erhölls en rätlinjig spädningskurva vilket indikerar att ekvint cTnI i olika koncentrationer kan mätas med Meritas Troponin I test. Vid plasmakoncentrationer över testets detektionsgräns (12 ng/l) låg CV-värdet på 6,7 % och vid plasmakoncentrationer < 12 ng/l varierade CV-värdet mycket. Alla friska hästar, utom en, hade en medelkoncentration av cTnI i plasma som låg nära eller under detektionsgränsen för testet vilket stämmer överens med tidigare utförda studier. Resultaten från denna studie indikerar att Meritas Troponin I test kan användas för att mäta ekvint cTnI och att testet eventuellt skulle kunna användas kliniskt inom hästmedicinen. Innan detta kan ske krävs dock en mer omfattande validering av denna metod.

DISTRIBUTION

INTRODUCTION

Myocardial disease

Disorders that primarily affect the myocardium by causing necrosis, degeneration or inflammation are called "myocardial diseases", which can be classified as either primary or secondary myocardial diseases (Mattingly 1965, Van Vleet & Ferrans 1986). Diseases that primary involve the myocardium, with none or little involvement of other structures of the heart, are referred to as primary myocardial diseases (Mattingly 1965). Fowler and Gueron (1965) defined primary myocardial disease in humans as "a variety of cardiac enlargement which is of unknown cause and frequently leads to cardiac dilatation, congestive heart failure and finally to death". The term "secondary myocardial disease" refers to diseases which, while not primarily affecting the myocardium, do cause secondary pathological changes in the myocardium (Mattingly 1965).

In horses, there is a variety of pathophysiological mechanisms behind myocardial diseases e.g. infection and trauma, and myocardial diseases are likely to be under-diagnosed (Reed, Bayley & Sellon 2010, p. 447). Clinical signs of myocardial disease are exercise intolerance, ataxia, sudden death, and reduced performance (Reed, Bayley & Sellon 2010, p. 448).

In case of myocardial injury, an intracellular regulatory protein called cardiac troponin, may leak out from injured myocytes out into the circulation (Hein, Scheffold & Schaper 1995, Bleier et al. 1998, Buergelt et al. 2003, Fishbein et al. 2003, Wilson et al. 2009). Troponin can therefore be measured to detect myocardial damage.

Troponin

Troponin is part of the muscle troponin-tropomyosin complex and consists of three proteins, troponin C (TnC), troponin I (TnI) and troponin T (TnT) (Greaser & Gergely 1971, Katz 2011). Together with tropomyosin, troponin forms a complex which is part of the thin filaments in the myofibrils present in skeletal muscle and in cardiac muscle. The troponin complex is involved in the calcium-dependent regulation of muscle contraction. (Ebashi, Kodama & Ebashi 1968, Katz 2011). Each of the three troponin proteins exists in tissue specific isoforms, in cardiac muscle and in skeletal muscle respectively (Syska, Perry & Trayer 1974, Dhoot, Gell & Perry 1978, Dhoot & Perry 1979, Dhoot & Perry 1980, Wilkinson 1980, Schreier, Kedes & Gahlman 1990, Pan & Potter 1992, Anderson et al. 1995).

There are three isoforms of cTnI, one for cardiac muscle, one for fast skeletal muscle fibers and one for slow skeletal muscle fibers (Dhoot, Gell & Perry 1978). Cardiac troponin I (cTnI) is considered cardio-specific since the level of cTnI is 1000 times higher in cardiac myocytes, compared to the level in skeletal muscle fibers, and in commercial tests 0.05 - 0.1% of skeletal muscle troponin cross reacts with cTnI (Mair et al. 1996, O'Brien, Landt & Ladenson 1997). In human medicine, cTnI has become the clinically most important of the isoforms due to its cardio-specificity, and also because it has the longest half-life in plasma (Bodor et al. 1992, Hjortshøj et al. 2008).

Cardiac troponin is located intracellularly, where approximately 8% of the total amount of troponin in the myocardium is unbound in the cytoplasm (Bleier et al. 1998). In case of myocardial injury, caused by ischemia or necrosis, the permeability of the cell membrane of the myocytes is altered, which causes troponin to leak out from the myocytes into the circulation (Hein, Scheffold & Schaper 1995, Buerguelt 2003, Fishbein et al. 2003, Wilson et al. 2009).

Cardiac troponin I in humans

Cardiac troponin I (cTnI) is one of the most studied biomarkers of cardiac disease in human medicine, and has proven to be a sensitive and a specific biomarker of cardiac disease (Adams et al. 1994a, Adams et al. 1994b, Jaffe et al. 1996, Mair et al. 1996). The increase of cTnI in plasma can be correlated to the size of myocardial infarction (Mair et al. 1995).

Cummins, Auckland & Cummins (1987) reported the first human cTnI assay, which had a lower limit of detection of 10,000 ng/L and had a cross reactivity of 2 % with skeletal muscle TnI. Since then, second and third generations human cTnI assays with higher sensitivity and lower limit of detection, have been developed (Panteghini et al. 2004, Venge et al. 2008, Wilson 2009). For example, the Nanosphere assay can measure cTnI concentrations in plasma down to 0.2 ng/L (Wilson et al. 2009).

Cardiac Troponin I in horses

In 1997, O'Brien, Landt & Ladenson confirmed the presence of cTnI in the equine myocardium and studies have shown that cTnI is a specific and a sensitive marker of myocardial injury in horses (O'Brien et al. 1997, Divers et al. 2009, Kraus et al. 2010). The sequence homology for cTnI across species is > 90 % (equine 89.1%[1]) and therefore human cTnI assays may be used for measurement of cTnI concentration in plasma in other species including horses (Rishniw & Simpson 2005). Most second and third generations human cTnI assays have antibodies targeted against epitopes of the cTnI molecule that are highly conserved between species (Rishniw & Simpson 2005). Therefore, these assays can often be used across species to measure cTnI (Rishniw & Simpson 2005). However, it is recommended to validate the assay, for the desired species, before using the assay in veterinary practice in order to control its specificity and sensitivity (Serra et al. 2010).

In horses without clinical signs of disease, plasma cTnI concentrations are often very low, and the concentration of cTnI does not seem to be influenced by age, sex or breed in horses (Phillips et al. 2003, Begg & Begg 2006, Kraus et al. 2010). However, no comprehensive study has been made to determine plasma cTnI concentrations in healthy horses, and therefore it is uncertain which factors influence the concentration. In horses with myocardial damage, plasma cTnI concentration is often increased (Schwarzwald et al. 2003, Kraus et al. 2010, Decloedt et al. 2012, Nath et al. 2012b). Myocardial damage/necrosis has been seen in horses with monensin toxicosis, and lasalocid poisoning, together with increased cTnI concentrations in plasma, indicating that increased cTnI concentration in plasma is associated with myocardial injury in horses (Divers et al. 2009, Kraus et al, 2010, Decloedt et. al. 2012).

[1] Uniprot. (2014-01-26). *Alignment*. http://www.uniprot.org/align/201401262BIDE1ZHUM [2014-01-26].

Increased plasma cTnI concentrations have been reported in horses with experimentally induced endotoxaemia, nutritional masseter myodegeneration, myocardial disease, rattlesnake envenomation, horses with colic, in some horses after transvenous electrical cardioversion, rhabdomyolysis secondary to Anaplasma infection, atypical myopathy, in septic neonatal foals, after strenuous exercise and in some horses after race (Slack et al. 2005, Holbrook et al. 2006, Hilton, Madigan & Aleman 2008, Nostell & Häggström 2008, Divers et al. 2009, Jesty et al. 2009, Schefer et al. 2011, Gilliam et al. 2012, Nath et al. 2012a, Nath et al. 2012b, Nostell et. al. 2012, Radcliff et al. 2012, Verheyen et. al. 2012).

The half-life of equine cTnI in plasma is 0.47 h. However, elevated concentrations of plasma cTnI have been reported in horses for a prolonged period of time (14 days) (Kraus et al. 2012, Nath et al. 2012b). This is likely related to a continuous release of cTnI from the myocardium caused by an ongoing myocardial injury (Nath et al. 2012b). Increased cTnI-concentrations in plasma can be observed four hours after infusion of endotoxin using a high sensitivity cTnI assay (Nostell et al. 2012). After intragastric administration of sodium monensin, increased plasma cTnI concentration was observed after 24 – 72 hours (Divers et al. 2009).

Various assays have been used in different studies for determination of cTnI concentration in equine plasma, but few of the assays have been validated. In 2006, Begg & Begg validated the ADVIA Centaur assay from Bayer, and found that it could detect equine cTnI. Kraus et al. (2010) validated a bedside assay, and found that the obtained cTnI concentrations in equine plasma, using the bedside assay, were similar to the concentrations obtained when using a bench-top analyzer. Slack et al. (2012) performed a validation of the Stratus CS immunoassay and determined the 95^{th} and 99^{th} percentile upper reference limits for healthy Standardbred horses.

The advantage of a beside test is that the clinician will obtain a result faster, and thus be able to decide and choose further diagnostics or treatment when presented to a horse with a suspected myocardial injury. In addition, the samples can be run immediately after collection which may give a more accurate value as the concentration of cTnI may decrease rapidly in room temperature. In dogs and humans cTnI concentrations in plasma decrease to various extent in room temperature (Venge, Lindahl & Wallentin 2001, Oyama & Solter 2004, Slack et al. 2012). However, no study of the stability of cTnI in equine plasma stored in room temperature has been made. There is also an economic aspect of having a bedside test because assaying the samples with a bedside test is often cheaper than sending the samples to a reference laboratory.

Aims of the study

Evaluation of the Meritas Troponin I test

The main aim of the study was to perform an initial evaluation of the human cTnI test, Meritas Troponin I test, manufactured by Trinity Biotech, for measurement of equine cTnI in plasma. The initial evaluation in the present study included a dilution parallelism, investigation of the intra-assay precision and measurement of plasma cTnI in healthy horses. This initial evaluation was performed in order to determine if the test could be subject for a further, more comprehensive validation. The hypothesis was that the test could measure equine cTnI as there is a good homology between equine and human cTnI.

Previous studies have shown that plasma cTnI concentrations in healthy horses are low, often near or below the detection limit of the assay. In the present study, it was hypothesized that the healthy horses would have low plasma cTnI concentrations, or concentrations below the detection limit of the Meritas Troponin I test.

MATERIALS AND METHODS

The present study was performed at the Swedish University of Agricultural Studies (SLU) in Uppsala, Sweden. Blood samples were collected from horses at a riding school in Uppsala, and at the Department of Clinical Sciences at the Swedish University of Agricultural Sciences in Uppsala. The study was sanctioned by the Ethical Committee for Animal Experiments, Uppsala, Sweden.

Assay[2]

The Meritas Troponin I test is used with the Meritas POC analyzer, a quantitative lateral flow immunoassay that uses fluorescence-based measurement for detection of cTnI. The assay is developed for human use, and can measure various biomarkers by using different analyse cartridges/tests. In the present study, the cartridge for measurement of cTnI was used, Meritas Troponin I test. The assay is manufactured by Trinity Biotech, and Fiomi Diagnostics manufactures the cartridges.

The test uses detection antibodies directed towards human epitope 24 – 40, 137 – 148 and 190 – 196 and capture antibodies directed against epitope 41 – 49 and 87 – 91. The epitopes are homologous between humans and horses except for human epitope 91 where human cTnI has an alanine and equine cTnI has a glutamic acid.

Whole blood, lithium heparin and EDTA plasma can be used for measurement of human cTnI with the Meritas POC analyzer. However, the manufacturer recommends EDTA-plasma for measurement of cTnI in human plasma. In the present study only lithium heparin plasma was used. The defined measuring range of the test is 12 – 30,000 ng/l for human EDTA plasma.[3] The amount of sample required per run is 200 µl.

Experimental Design

Evaluation of the Meritas Troponin I test

The total volume of each sample analysed was 200 µl, as stated in the guidelines from the manufacturer.

Dilution parallelism

A lithium heparin plasma sample collected from a horse previously treated at the Animal Hospital at SLU, with known cardiac injury and high concentrations of cTnI in plasma[4] was used for the dilution parallelism study. The sample had been stored at –80 ° C, and had not been thawed before the start of the present study. The plasma sample was diluted with lithium heparin plasma collected from two healthy Standardbred trotters, owned by the Department of Clinical

[2] As stated in the Meritas POC analyzer operation manual.
[3] According to the IFU provided by the manufacturer.
[4] Sample had been analysed at Akademiska Sjukhuset in Uppsala, Sweden, where the cTnI concentration had been measured by Beckman-Coulter assay.

Sciences at the Swedish University of Agricultural Sciences, with known low concentrations of plasma cTnI (< 2 ng/L[5]).

Due to low sample volume, the plasma with high cTnI concentration was first diluted 1:5 with the plasma mentioned above. Before the start of the dilution parallelism, the initial diluted sample was analysed in triplicate. Dilution of the plasma with high concentration of cTnI was made in the following steps: 4:1, 3:2, 2:3, 1:4, 1:9, 1:19 and 1:99. All diluted samples were analysed in duplicates.

Intra-assay precision

The intra-assay precision was calculated using the obtained values in the dilution parallelism. To further investigate the intra-assay precision, another plasma sample from the horse with previously established high cTnI concentration, was diluted with equine lithium heparin plasma with known low cTnI concentration (< 2 ng/L), in the following steps: 4:1, 3:2, 2:3, 1:4 and 1:9. The samples were run in duplicates.

Samples from healthy horses

19 horses, four fillies and 15 geldings, with a mean age of 11.5 years (range 6 – 19 years) were included in the study. The horses consisted of 17 Warmbloods (seven German Warmbloods, five Polish Warmbloods, three Dutch Warmbloods, one Irish Warmblood and one Swedish Warmblood), one English Thoroughbred and one American Paint horse. Three of the horses were privately owned whereas the rest of the horses belonged to a riding school located in Uppsala, Sweden. All horses underwent a standard clinical examination, including a thorough auscultation of the heart as well as a one-minute ECG recording, in order to check for signs of arrhythmia. The ECG recording was obtained using an Alive Cor Vet for iPhone 4S. None of the horses showed any significant findings on clinical examination or on the ECG recordings. Based on the examination, none of the horses showed signs of cardiovascular disease, or other disease.

Blood sample collection

Venous blood samples were collected in 4 ml lithium heparin tubes[6] from one of the jugular veins, using Vacutainer technique[7]. All samples were collected at rest with the horse standing calmly in the box stall. After collection, the samples were stored on ice, and transported to the Swedish University of Agricultural Sciences where the samples were centrifuged at 3000 rpm during 10 minutes using the Hettich EBA 20 centrifuge. All samples were centrifuged and stored at – 80 ° C within four hours after collection. The samples were thawed at room temperature 30 minutes prior to analysis with the Meritas POC analyzer. In the dilution parallelism, the samples were kept on ice at 4 ° C after being diluted, and then thawed until they reached room temperature before they were assayed. In the intra-assay precision study, the samples were kept on ice after being diluted, and then thawed until they reached room temperature before they were assayed.

[5] Samples were sent to Akademiska Sjukhuset in Uppsala, Sweden, where determination of cTnI concentration was made using Abbott Architect cTnI assay.

[6] BD vacutainer Li-Hep, Belliver Industrial Estate, Plymouth, UK.

[7] BD Vacutainer precision glide 0.9 x 38 mm, Belliver Industrial Estate, Plymouth, UK.

Statistical analysis

All values are expressed as mean ± standard deviation (SD). The intra-assay precision, defined as the variation between measured cTnI concentrations of plasma samples from the same horse or samples in the same dilution step, run in two or three consecutive runs, was expressed as coefficient of variation in percentage (CV%[8]). The expected value in the dilution parallelism was calculated by using the mean concentration of the undiluted sample with known high cTnI concentration. The linearity of the dilution parallelism was determined via t-based, ordinary Least Squares regression analysis with a 95 % confidence interval, using Analyse-it Method Validation software Version 3.70.1 and Microsoft Excel 2013.

[8] The CV% was calculated by dividing SD by the mean of two or three aliquots multiplied by 100

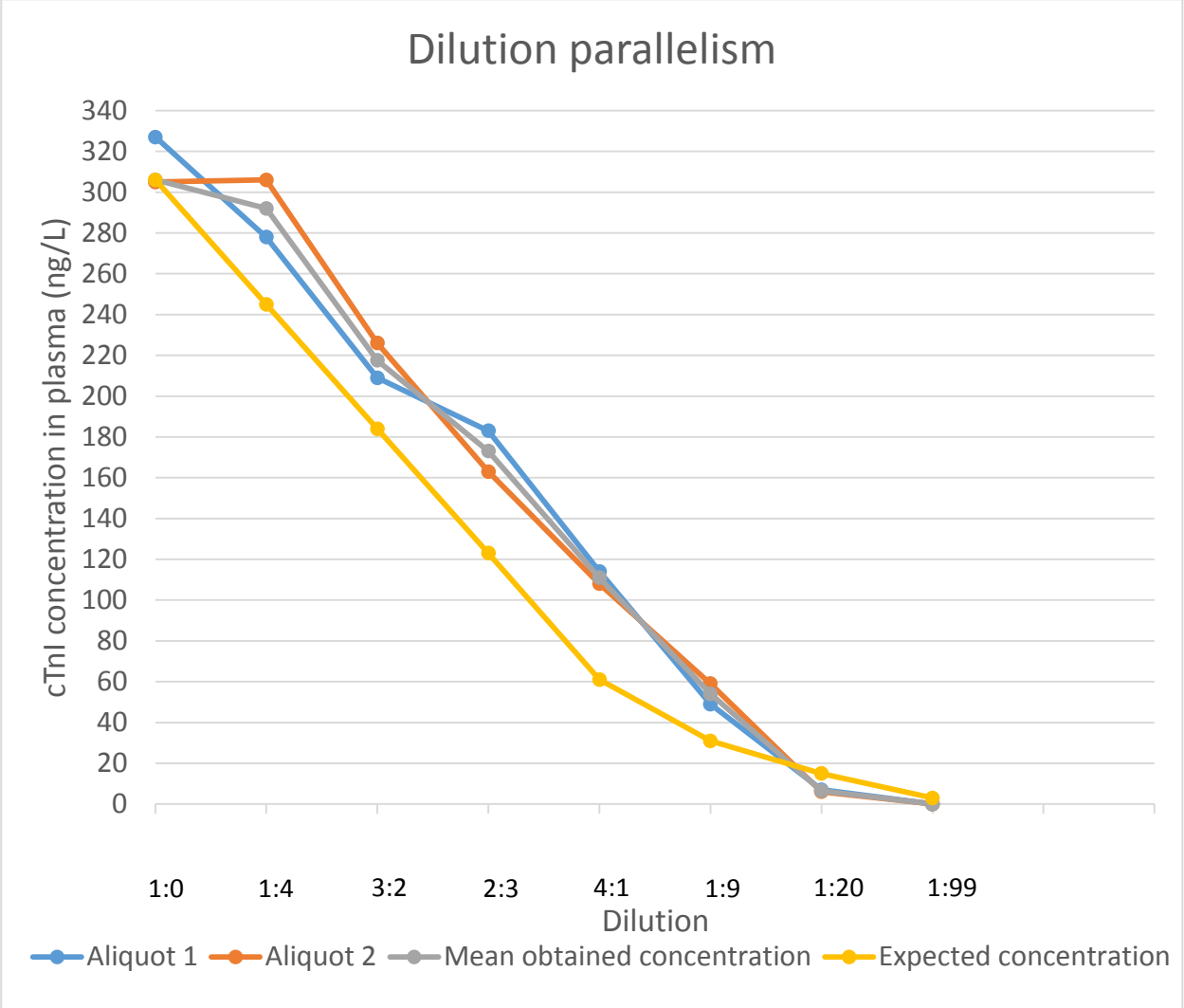
RESULTS

Evaluation the Meritas Troponin I Test

Dilution parallelism

A triplicate of the plasma sample with previously established high concentration of cTnI was run where the measured concentration was 327 ng/L in the first run, 305 ng/L in the second run and 288 ng/L in the third run[9]. The average concentration of the undiluted sample was 306 ng/L.

Figure 1.Dilution parallelism with results of measurement 1 (Aliquot 1), measurement 2 (Aliquot 2), mean concentration and expected concentration.



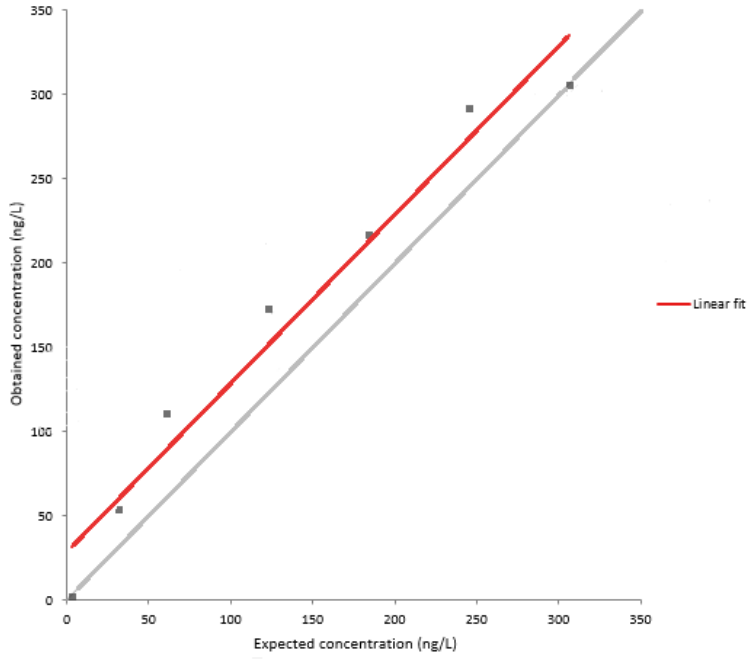
[9] Data from the third run is not included in table 1.

Table 1. Measured concentrations in the dilution parallelism

Dilution	Measured concentration (ng/L) 1	Measured concentration (ng/L) 2	Mean concentration (ng/L)	Expected value (ng/L)	Measured/ expected value (ng/L)	Standard deviation	CV %
1:0	327	305	306	306	1.0	19.5	6
4:1	278	306	292	245	1.2	19.8	6.8
3:2	209	226	217	184	1.2	12.0	5.5
2:3	183	163	173	123	1.4	14.1	8.1
1:4	114	108	111	61	1.8	4.2	3.8
1:9	49	59	54	31	1.8	7.1	13.1
1:19	7	6	6.5	15	0.4	0.7	10.9
1:99	< 1	3	2	3	0.3	1.4	70.7

In the regression analysis, using a 95 % confidence interval, the curve of the obtained concentrations demonstrated a linear curve parallel with the curve of the expected concentration where the slope of the lines ranged from 0.77 to 1.23 (SD 0.09). The intercept was 28.6 ng/L and when using a 95 % confidence interval, ranged from -10 to 67.8 ng/L (SD 15.13) (figure 2). The correlation coefficient was 0.981.

Figure 2. Plot of the obtained verses the expected concentrations for cTnI.



Intra-assay precision study

Measured concentrations below 12 ng/L were not included in the intra-assay precision study since 12 ng/L is the limit of detection of the Meritas Troponin I test, and therefore CV% is expected to vary.

In the dilution parallelism the CV% ranged from 3.8 – 13.1 (mean 7.2 ± SD 2.9). After exclusion of the highest and lowest values, the CV% ranged from 5.5 – 8.1 (mean 6.6 ± SD 0.98). In addition to the obtained CV% in the dilution parallelism, intra-assay precision was further investigated by diluting another plasma sample, collected from the same horse used in the dilution parallelism study. The CV% ranged from < 0.1 – 11.0 (mean 6.7 ± SD 3.8). When highest and lowest values were excluded, CV% ranged from 4.9 – 10.2 (mean 7.1 ± SD 2.4). (Table 2).

The mean CV%, when combining the results mentioned above, was 6.8 (range < 0.1 – 13.1) ± SD 3.3. When the lowest and the highest values were excluded, CV% ranged from 3.8 – 11.0, and mean CV% was 6.9 ± SD 2.2.

Table 2. Measured and mean concentrations, standard deviation and CV% obtained from a diluted plasma sample from a horse with previously established high cTnI concentration.

Dilution	Measured concentration (ng/L) 1	Measured concentration (ng/L) 2	Mean concentration (ng/L)	Standard deviation	CV %
1:0	1377	1277	1327	70.7	5.3
4:1	444	519	482	53.0	11.0
3:2	556	498	527	41.0	7.8
2:3	396	369	283	19.1	4.9
1:4	274	237	256	26.1	10.2
1:9	85	84	84.5	0.7	< 0.1

Plasma cTnI concentration in healthy horses

Three horses (horse 4, 7, 19) were excluded from the study as the test was not able to quantify the concentration of cTnI in the samples. All other horses had cTnI concentrations below or near the detection limit of the test. In five of the horses, CV% was > 37.2, and in the remaining horses the CV% was < 20.2. (Table 3). Horse 10 was the only horse that had plasma cTnI concentration above the detection limit of the test, and there the CV% was 12.9.

Table 3. Equine cTnI concentration in plasma in healthy horses.

Horse	Measured concentration 1 (ng/L)	Measured concentration 2 (ng/L)	Standard deviation [10]	Mean[11] (ng/L)	CV %
Horse 1	1	< 1	0	1	0
Horse 2	2	7	3.5	4.5	78.6
Horse 3	1	< 1	0	1	0
Horse 5	10	10	0	10	0
Horse 6	< 1	< 1	0	1	0
Horse 8	< 1	< 1	0	1	0
Horse 9	< 1	< 1	0	1	0
Horse 10	15	18	2.1	16.5	12.9
Horse 11	1	7	4.2	4	106.1
Horse 12	6	8	1.4	7	20.2
Horse 13	12	7	3.5	9.5	37.2
Horse 14	< 1	8	4.9	4.5	110
Horse 15	3	4	0.7	3.5	20.2
Horse 16	< 1	6	3.5	3.5	100
Horse 17	< 1	< 1	0	1	0
Horse 18	< 1	< 1	0	1	0

[10] In the calculations all values < 1 were calculated as 1.
[11] In the calculations all values < 1 were calculated as 1.

DISCUSSION

The present study indicates that the Meritas POC analyzer may have the capability to measure equine cTnI and, accordingly, could the analyzer be a candidate for further validations. In the intra-assay precision study, the test had a mean CV% of 6.7 (range < 0.1 – 13.1) for samples with cTnI concentration > 12 ng/L. However, in the blood samples with cTnI concentrations < 12 ng/L, CV% varied extensively, which is an expected result due to the fact that CV% can vary a great deal in samples with concentrations below the limit of detection of an assay/test. Therefore, cTnI concentrations in equine plasma < 12 ng/L should be interpreted with care, due to the fact that CV% can vary. To further evaluate the Meritas POC analyzer a more comprehensive validation should be performed to investigate if the test may be used clinically in equine medicine.

All obtained cTnI-concentrations in the dilution parallelism were higher than the expected, calculated concentrations, except for the 1:20 and 1:99 dilutions. This could indicate that measured equine plasma cTnI concentrations may be false high in samples with a cTnI concentration exceeding the limit of detection. Nonetheless, the concentrations could have been higher due to matrix-effects, which can be seen when plasmas from different individuals are mixed.

Only one of the epitopes, epitope 91, targeted by the Meritas Troponin I test differed between human cTnI and equine cTnI. The human epitope 91 is an alanine, whereas the equine corresponding epitope is a glutamic acid. Alanine is non-polar and hydrophilic whereas glutamic acid is negatively charged which may affect the three dimensional structure of the protein, thus alter the binding capacity of the antibodies used in the Meritas Troponin I test. An altered binding capacity may affect the result of the analysis in equine plasma samples since the Meritas Troponin I test is produced for human use, thus optimized and standardized for human plasma. To investigate the measurement accuracy; a sample with a known concentration of cTnI could be measured and then the obtained value could be compared with the known value e.g. by doing a spike recovery.

In the dilution parallelism and the intra-assay precision study, a majority of the first analysed samples (64%, 9/14) had higher concentrations than the following samples in the duplicate/triplicate series. In horses, no study investigating the stability of equine plasma stored at room temperature has been made (Kraus et al. 2012). Human and canine plasma cTnI samples are known to be unstable in room temperature. In room temperature, the cTnI concentration decreases approximately 10% after 24 h, 50% after 48 h and 70% after 72 h, in canine plasma, and 20% after 72 hours in human plasma (Venge, Lindahl & Wallentin 2001, Oyama & Solter 2004). As the cTnI has a short half-life in plasma in the body, it is likely that equine cTnI also is unstable at room temperature. Therefore, there is a possibility that the measured concentrations in the first assayed samples were higher, than in the following samples, due to the fact that cTnI in equine plasma is unstable at room temperature. However, due to the possible instability of equine plasma cTnI at room temperature, the samples in the present study were kept on ice and/or at 4 ° C. In addition, the maximal time between the analyses in each duplicate/triplicate was 20 minutes.

In the present study, the mean CV% in the intra-assay precision study was 6.7%. In human cardiology, the recommended CV of cTnI assays is up to 10% at the decision limit, where the decision limit is defined as a value that exceeds the 99th percentile of a reference control group (Antman et al. 2000). The cut off value for human cTnI is defined as "maximal concentration of cardiac troponin exceeding the 99th percentile of values (with optimal precision defined by total CV 10%) for a reference control group on at least one occasion during the first 24 h after the clinical event…".[12] In horses, assay-specific 99th percentile upper reference limits can be determined (Slack et al. 2012). However, there is no defined cut off value for plasma cTnI concentrations in horses and therefore a recommended CV% cannot be defined. Therefore it is difficult to determine if the CV% is acceptable or not.

The healthy horses in the present study all had mean cTnI concentrations below or close to the limit of detection. Previous studies in clinically healthy horses have shown that they generally have low cTnI concentrations (below or close to the detection limit of the assay) with reported concentrations of 0 – 350 ng/L, depending on the assay used (Phillips et al. 2003, Begg, Hoffmann & Begg 2006, Nostell et al. 2008, Kraus et al. 2010, Slack et al. 2012). Thus, the result obtained in this study is in agreement with results from previous studies.

There are several examples of scenarios where access to a bedside test for measurement of cTnI is desirable. An example of a scenario where a quick cTnI result is desirable would be in the case of colic. In colic horses admitted for surgery an elevated cTnI value is associated severity of disease, poorer prognosis, and the horse is less likely to survive. 9/25 horses with strangulating obstruction and 9/19 with inflammatory disease, had cTnI values up to 9310 ng/L. (Nath et al. 2012a). In a study by Radcliffe et al. (2012) non-surviving colic horses had several fold higher mean preoperative cTnI value than the surviving horses. Thus, a higher concentration of plasma cTnI was associated with poorer prognosis, indicating that analysis of cTnI concentration in horses with colic may be of prognostic value. The cTnI concentration can be part of the clinical examination when considering admitting a colic horse for laparotomy. However, a decision whether a horse should undergo laparotomy or not should not be based on a single blood variable, but rather as one of several clinical variables used for decision making. Another clinical setting where cTnI may have a clinical value is in myopathies. Increased cTnI concentrations are an indication of myocytes damage and repeated measurements will give the veterinarian information about the course of the myocardial damage. If the cTnI values remain elevated the horse has an ongoing myocytolysis and exercise should be avoided (Jesty et al. 2009). By doing follow ups on the cTnI value, together with clinical examination and other diagnostic methods the veterinarian can decide when the owners can start exercising the horse again.

[12] Laboratory Medicine guidelines for biomarkers of acute coronary syndromes and heart failure issued by the National Academy of Clinical Biochemistry in 2007.

Study limitations

Handling of the samples could have influenced the results in the study. The time between the duplicate/triplicate runs could have influenced the result, where a longer time would result in a lower second measured concentration. Thus, affecting both the result of the dilution parallelism and the intra-assay precision study.

In the present study, the highest cTnI concentration measured was 1377 ng/L, whereas the measuring range of the Meritas Troponin I test is 12 – 30,000 ng/L. Therefore, it would have been interesting to use a plasma sample with a higher concentration in the dilution parallelism, but also in the intra-assay precision study. Another factor that could have influenced the results is the dilution of the samples. In order to perform an accurate dilution, calibrated pipettes were used.

None of the samples were run immediately after collection in the study due to practical reasons. This differs from clinical practice where the samples are mostly run after collection, without being frozen.

This study should be considered as an initial evaluation of the Meritas Troponin I test. In order to investigate the clinical potential of the Meritas Troponin I test in equine medicine, a comprehensive validation is required.

CONCLUSIONS

In the present study an initial evaluation of the Meritas Troponin I test was made, where the dilution parallelism demonstrated a linearity indicating different concentrations of equine cTnI in lithium-heparin plasma may be measured using Meritas Troponin I test. The mean CV% in the intra-assay precision study was 6.7% in samples with cTnI concentrations > 15 ng/L. The test tended to give higher concentrations than expected, which means that there is a risk of getting false high results. The intra-assay precision study and the dilution parallelism indicated that measured cTnI concentrations in equine plasma below 15 ng/L should be interpreted with care. All healthy horses had low concentrations of cTnI in plasma, a result consistent with previous studies in horses.

In conclusion, having a bedside test is beneficial for both the patient, the animal owner and the veterinarian. In clinical practice, measurement of cTnI is useful when the patient has a suspected myocardial damage. The Meritas Troponin I test demonstrated, in the present study, an acceptable curve in the dilution parallelism, a CV% below 10%, and plasma cTnI concentrations in healthy horses close to or below the limit of detection. This makes the test a possible candidate for a future validation in order to properly investigate its possible clinical value in equine medicine.

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