Sequencing of the southern white rhinoceros (*Ceratotherium simum simum*) cardiac troponin I gene and analytical validation of a point-of-care cardiac troponin I immunoassay

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Background:

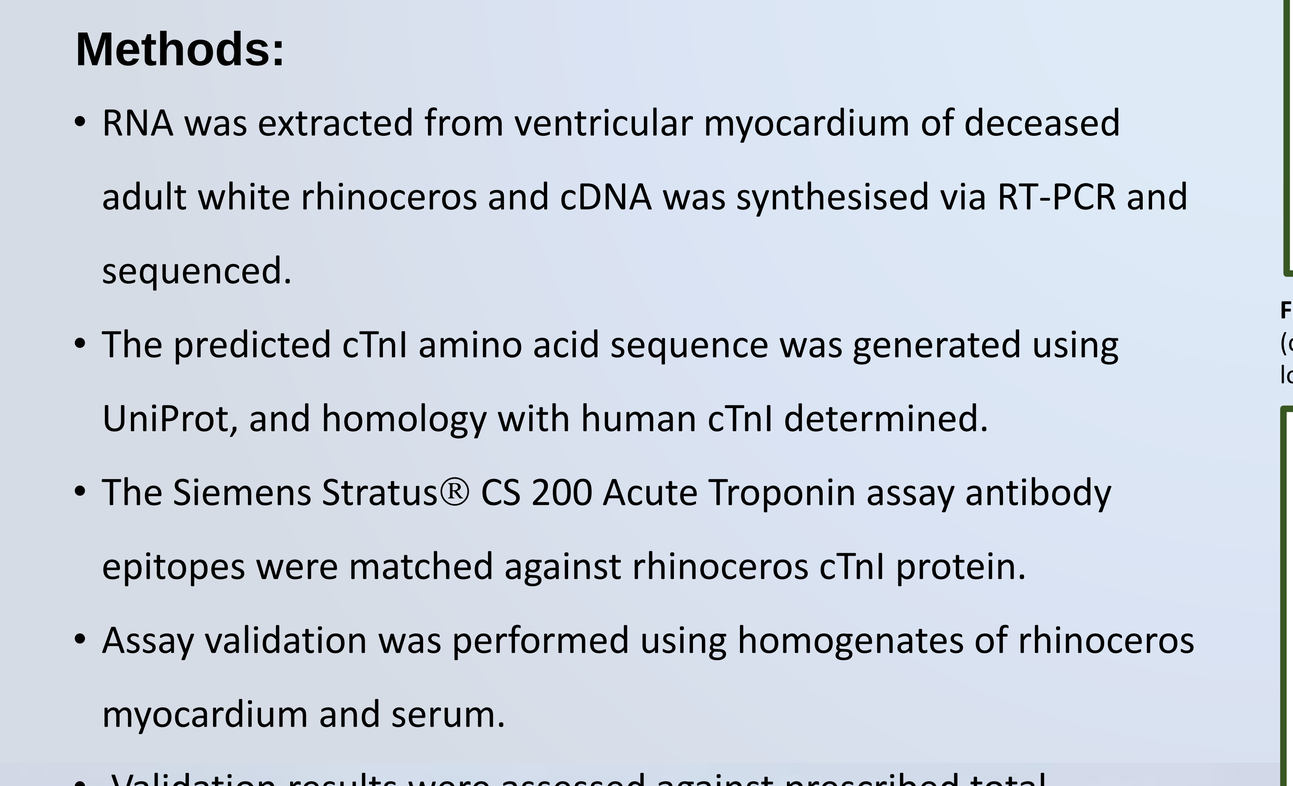
- Conservation strategies for the near threatened white rhinoceros (*Ceratotherium simum simum*) often involve chemical immobilisation and translocation.
- Dehydration, acid-base disturbances, hypoxaemia, negative energy balance, stress-induced immunomodulation and skeletal muscle injury are common adverse changes in translocated rhinoceros.
- Investigation into potential concurrent myocardial injury in transported rhinoceros is limited due to a lack of validated immunoassays.

Objectives:

- Firstly, to determine the mRNA transcript sequence of white rhinoceros' cardiac troponin I (cTnI) and evaluate sequence homology.
- Secondly, to validate a point-of-care cTnI immunoassay with antibody epitopes that match the predicted rhinoceros cTnI protein sequence.

Methods:

- RNA was extracted from ventricular myocardium of deceased adult white rhinoceros and cDNA was synthesised via RT-PCR and sequenced.
- The predicted cTnI amino acid sequence was generated using UniProt, and homology with human cTnI determined.
- The Siemens Stratus® CS 200 Acute Troponin assay antibody epitopes were matched against rhinoceros cTnI protein.
- Assay validation was performed using homogenates of rhinoceros myocardium and serum.
- Validation results were assessed against prescribed total allowable error (TE_a) of 70%.



Results:

- Nucleotide sequence identity of the rhinoceros cTnI gene with human and equine cTnI genes was high (97% and 96%, respectively).
- Predicted amino acid sequence matched assay antibody epitope-binding sites.
- The assay was linear within a range of 0.05-38.39 ng/mL.
- Imprecision ranged from 1.9%-8.0%.
- The proportional systematic error was -1.87% and was < TE_a.
- Limit of the blank was below the detection limit of the assay (<0.03 ng/mL) and the limit of detection was 0.04 ng/mL.

cttcttgccccttctctgcctcctgcgactcctggccagaagctcagcATGGCGGACCAGAGCGGCAATGCGGC
GCCCGCCCCCATCCGACGCCGCTCCTCGGCCAACTACCGCGCCTACGCCACCGAGCCGCACGC
CAAGAAAAAGTCTAAGATCTCCGCCTCGAGAAAACTGCAGCTGAAGACCCTGATGCTGCAGA
TTGCGAAGCAGGAGCTGGAGCGGGAGGCGGAGGAGCGCCGCGGAGAGAAGGGGCGCGCC
CTGAGCACGCGGTGCCAGCCTCTGGAGTTGGCCGGGCTGGGCTTCGAGGAGCTGCAGGATT
TGTGCCGACAGCTCCATGCCCGCGTGGACAAGGTGGATGAGGAGAGATACGACGTGGAGGC
GAAAGTCACCAAGAACATCACGGAGATCGCAGATCTGACCCAGAAGATCTTTGACCTTCGGG
GCAAGTTTAAGCGGCCCACCCTGCGGAGGGTGCGGATCTCTGCGGATGCTATGATGCAGGCG
CTGCTGGGGGCCAGGGCTAAGGCGACCTTAGACCTGCGGGCCCACCTCAAGCAGGTGAAGA
AGGAGGATACAGAGAAGGAAAACCGGGAGGTGGGAGACTGGCGCAAGAACATCGACGCGC
TAAGCGGAATGGAGGGCCGCAAGAAAAAGTTTGAGGGCTGAgctggcctgcccaccgctctggccct
gaggatggccctgaggaaa

Figure 1: Nucleotide sequence of southern white rhinoceros (*Ceratotherium simum simum*) cardiac troponin I (cTnI) mRNA. The coding regions are indicated in uppercase letters; the untranslated regions are indicated in lowercase letters.

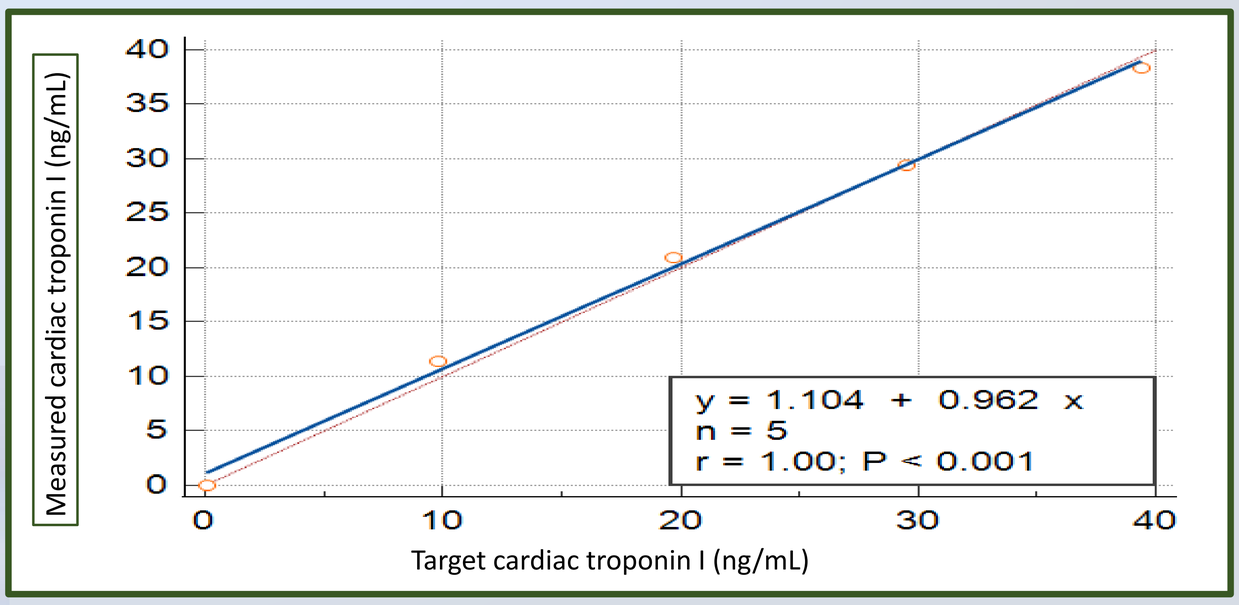


Figure 2: Reportable range of cardiac troponin I assay of the Siemens Stratus® CS 200 Acute Care™ Analyser

Conclusion:

The Stratus CS 200 is suitable for measurement of cTnI in white rhinoceros and can be used to investigate potential myocardial injury in this species.

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