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 Effect of prolonged captivity on the hemolymph profile of *Tachypleus gigas* using the various anticoagulant formulations

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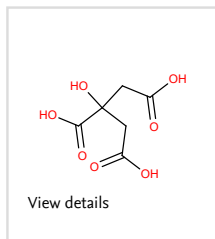
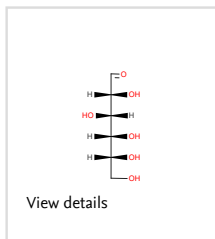
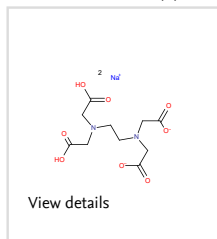
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**Abstract**

Horseshoe crab (HSC) amebocyte cells degranulate to form a gel clot when in contact with endotoxins. This phenomenon is the basis of both the horseshoe crab immune system and the detection of endotoxin in biologicals. Horseshoe crab captive rearing has been suggested as a promising method for continuous amebocyte cell harvesting. Hence, we aimed to investigate the amebocyte cells quality in *Tachypleus gigas* pre and post bleeding under captivity. Wild and captive horseshoe crabs (5 months captivity) were fed with Meretrix meretrix (highly preferred diet by *T. gigas*) and bled in 6 anticoagulant formulations (A, B, C, D, E, and F). No profound difference in cell density was observed between captive and wild groups with the mean value of  $0.883 \times 10^7$  cells/mL and  $0.917 \times 10^7$  cells/mL, respectively. Cell viability of the captive group was significantly lower than the wild group ( $F = 808.075$ ,  $p < 0.001$ ). Anticoagulant formulation greatly affected cell viability and cell morphology in captive and wild groups ( $p < 0.001$ ). Amebocyte cells collected from the wild *T. gigas* using optimum anticoagulant (formula C) showed  $0.6 \times 10^7$  cells/mL cell density and 86.9% cell viability. Simultaneously, morphology analysis revealed the percentage of contracted, granular flattened, and degranulated flattened cells were 14.62%, 71.39%, and 14%, respectively. The anticoagulant formulations showed varying capabilities in maintaining cell viability due to their buffering and chelating capacity. We postulate that selection of the best anticoagulant recipe is crucial to inaccurate blood profiling of horseshoe crabs. Prolonged captivity and single feed impact the quality of amebocyte cells in the crabs. © 2021 The Authors

**Author keywords**
 Amebocyte cells; Anticoagulant; Captive rearing; Living fossil; *Tachypleus gigas*
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[Back to results](#) | 1 of 1

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