

— Homologous ELISA procedure for the determination of penaeid shrimp vitellogenin .



Vitellogenin (VTG) was isolated from the haemolymph of *Penaeus vannamei* by a three step procedure including ultracentrifugation, gel filtration and ion exchange chromatography. VTG was used to raise polyclonal antibodies that were purified by ion exchange chromatography. A two step competitive assay was developed in which VTG could be quantitated by its capacity to inhibit the binding of antibody to the VTG previously adsorbed onto a solid phase. Sensitivity from equilibrium and from non-equilibrium assays was 41 and 2.3 ng/ml respectively. Estimates of within-assay and between-assay variabilities of standard curves were 4.2 and 9.6% respectively. The inhibition curves for dilutions of haemolymph from vitellogenic females, egg yolk extracts and purified vitellin (VTL) were parallel to the standard VTG curve, haemolymph from immature females and males showed no cross-reactivity. The antibodies directed against VTG recognize but partially VTL as shown by displacement curves. Recovery tests were near 100%.

Thus the procedure was considered to be suitable for the measurement of haemolymphatic VTG. The VTG enzyme-linked immunosorbent assay (ELISA) developed in this study was validated by detecting physiological VTG changes in female shrimps after being fed squid extracts.

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