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# Development and application of a monoclonal antibody-based sandwich ELISA for vitellogenin in carp (*Cyprinus carpio*).

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## Introduction

In teleost, vitellogenin (Vtg) is synthesised in the liver in response to estrogens and transported by the blood to the growing oocytes where it is incorporated by micropincytosis (Selman and Wallace, 1982). Generally, Vtg is not induced in normal male fish but male fish are capable of synthesis Vtg in reponse to exogenous estrogen and xenoestrogens. Thus Vtg induction in male fish triggered by expose to estrogen is a powerful and well established biomarker for detecting endocrine disrupting chemicals (Tvler et al., 1999).

In the last decade, enzyme linked immunosorbent assay (ELISA) for measuring Vtg concentration in a variety of fish species have been developed with purpose of detecting xenoestrogenic effects. But most of these ELISAs have been developed using polyclonal antibodies (pAbs) against vtg . pAbs could have failed to detect small quantities of non specific antibodies that react to other proteins than Vtg.

Carp have been selected for the study as they are widely distributed freshwater fish, and they are tolerant species that can persist in highly polluted environments. The development of an ELISA is very important, capable of the precise quantification of small amounts of vtg in carp, as a biomarker for endocrine disrupting chemicals.

In this study, we purified Vtg and developed monoclonal antibody (mAb) and pAb for carp Vtg (cVtg). Using these mAb and pAb, a sandwich ELISA was developed and its validation was assessed.

## Material and Methods

Vtg was purified from E2-treated male carp serum by FPLC systems which were equipped with Mono Q anion exchange column and Superdex gel filtration column, and then mAb and pAb against cVtg were producted.

A sandwich ELISA was produced by the method of Nishi et al. (2002) with modification. The wells were coated with 100$\mu\ell$ of mAb (diluted 1:2000 in coating buffer) and incubated at 4℃ overnight, and then 100$\mu\ell$ of standard (purified-vtg) or samples were added. After 100$\mu\ell$ of pAb was added at 1 : 5000 dilution and then 100 $\mu\ell$ of goat anti-rabbit IgG biotin conjugategoat (Sigma; diluted 1:100,000) was added. The wells were added 100$\mu\ell$ of a streptavidin-peroxidase conjugated solution and then filled with 100$\mu\ell$ of a freshly prepared substrate solution. The reaction was stopped after 15min by the addition of 50$\mu\ell$ 2M $H_2SO_4$ and absorbances were determined at 450nm with a microtiter plate reader.

## Result and Discussion

cVtg purified by the rapid two-step of FPLC was characterized and its properties were determined by SDS-PAGE and western blot analysis. Two cVtgs of 160kDa and 180kDa were identified.

A sandwich ELISA for cVtg was developed by using the mAb and pAb. In selecting the pair that gave high sensitivity, mAb was used for microtiter plate coating and pAb was used for capturing antigen.

The assay range of the standard curve was between 2-250ng/ml, and intra- and inter-assay variations determined from plasma samples were within 11%. Recovery rate of carp vtg added to plasma was 87-101%.

## References

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