Development and validation of a monoclonal ELISA for quantifying vitellogenin in the rainbow trout (*Oncorynchus mykiss*) and its application for studies on environmental oestrogens.

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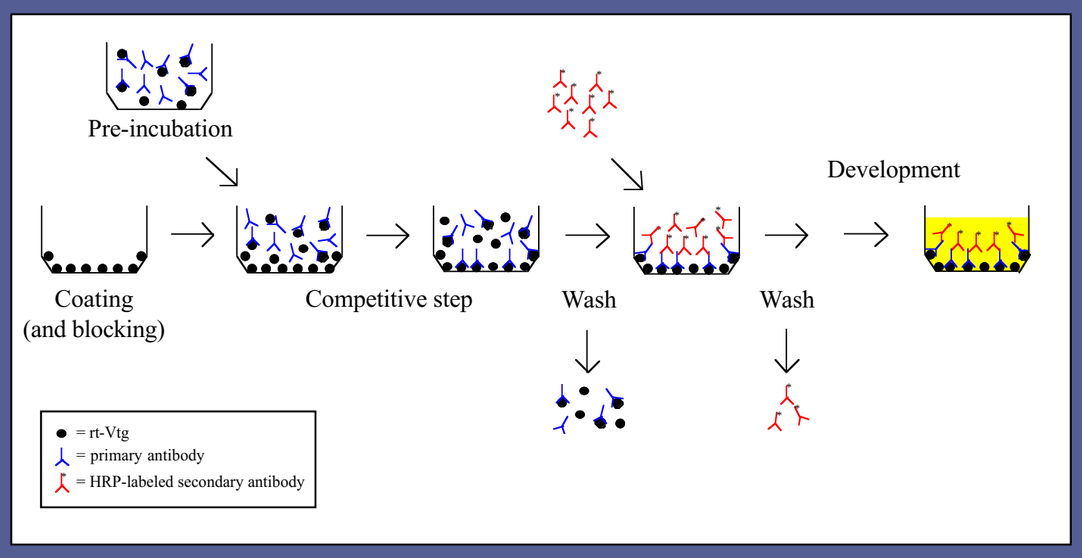
Introduction

Induction of the yolk protein precursor vitellogenin (Vtg) in plasma has proved to be a simple and sensitive biomarker for assessing exposure to environmental estrogens in fish (Tyler et al., 1998; Larsson et al., 1999). The widespread use of Vtg in this regard has lead to the need for standardized assays to quantify Vtg. Monoclonal antibodies, that can be produced from a single clone with a desired specificity and in unlimited amounts, have the potential to help accomplish this. Several governmental organizations, e.g. the OECD and EPA, are now discussing the incorporation of standardized Vtg assays in screening programs for endocrine disruptors.

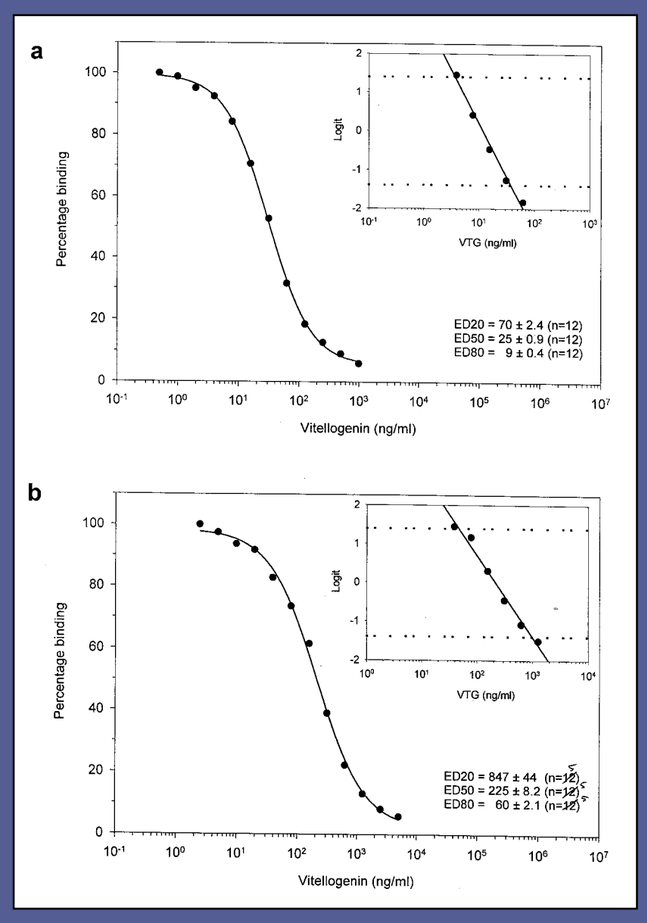
A Vtg ELISA (Enzyme-Linked Immuno-Sorbent Assay) was developed using a monoclonal antibody prepared against Atlantic salmon (*Salmo salar*) vitellogenin (MAb BN-5, Nilsen et al., 1998) and its ability to quantify Vtg in the rainbow trout (*Oncorhynchus mykiss*) compared with a rainbow trout vitellogenin (rt-Vtg) ELISA that employed homologous polyclonal antibodies (PAb). The MAb BN-5 ELISA was validated for use in studies with environmental estrogens by quantifying responses in juvenile female rainbow trout exposed to a series of doses of 4-*tert*-nonylphenol (4-NP) and estradiol-17ß (E2) against the responses measured in the same plasma samples using the homologous PAb rt-Vtg ELISA and radioimmunoassay (RIA, Sumpter, 1985).

Method



Results

Standard curves and working ranges for PAb and MAb-BN5 rt-Vtg ELISA



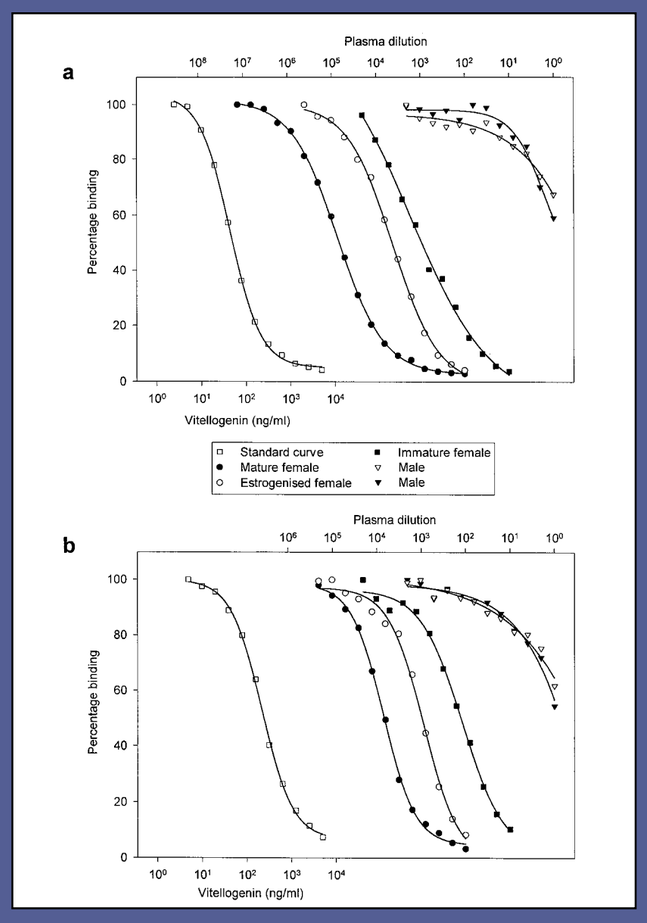
a. PAb ELISA

- Working range: 5ng/ml - 80ng/ml (80% - 20% binding)
- ED_{50}: 25 +/- 0.9ng/ml
- Detection limit: 50ng/ml (ED_{80})
- Inter-assay variation: 12% (ED_{50} , n=12)

a. MAb-BN5 ELISA

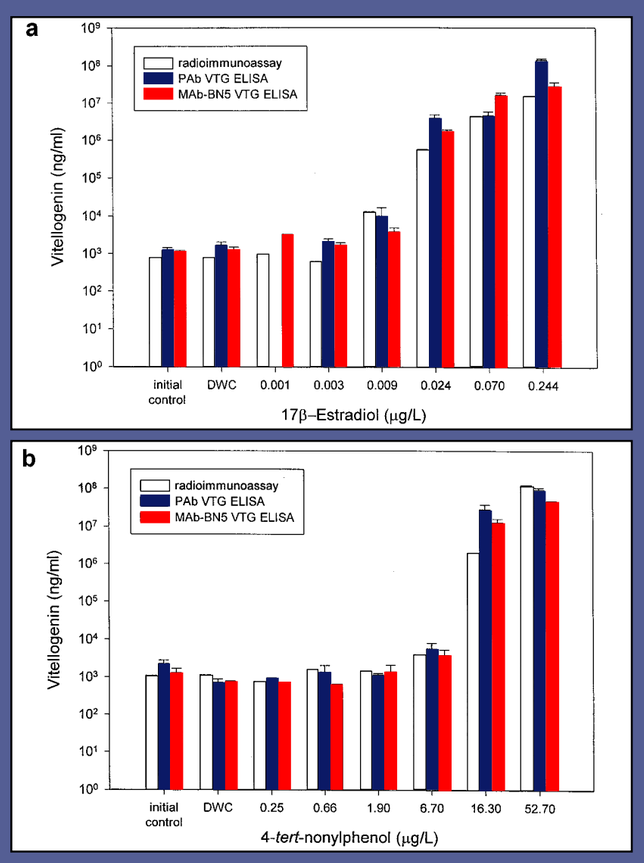
- Working range: 60ng/ml - 850ng/ml (80% - 20% binding)
- ED_{50}: 225 +/- 8ng/ml
- Detection limit: 600ng/ml (ED_{80})
- Inter-assay variation: 8% (ED_{50} , n=5)

PAb (a) and MAb-BN5 (b) standard curves with plasma dilution curves



- Dilutions of plasma from juvenile and maturing female rainbow trout showed good parallelism with the rt-Vtg standard curve over the working range for both assays.
- Plasmas from estrogenized male rainbow trout also diluted parallel with the rt-Vtg standard in both assays.
- In both assays, plasma had an inhibitory effect on antibody binding at low dilutions (plasma effect).
- To avoid plasma effects, plasma samples should be diluted at least 1:10 for both assays.

Vitellogenic responses in juvenile female rainbow trout exposed to 4-NP and E2



- Plasma samples were collected after 14 days of exposure to a series of doses of estradiol-17ß and 4-*tert*-nonylphenol and analysed in the PAb and MAb-BN5 ELISAs and in an established homologous rt-Vtg radioimmunoassay (RIA).
- There were no differences in the vitellogenic responses measured in the PAb and MAb-BN5 Vtg ELISAs or in the RIA.

Further development

Further studies have shown that

- the working range of the MAb-BN5 assay can be expanded to cover a wider area.
- rt-Vtg can be stabilized for inclusion as standard and coating antigen in a standardised kit product.

Conclusions

- The monoclonal MAb-BN5 Vtg ELISA is a robust assay that can be used to quantify Vtg in the rainbow trout for plasma concentrations ranging over 100 000-fold.
- The assay has en excellent utility for studies on environmental estrogens using juvenile female rainbow trout in standardized tests.
- The MAb-BN5 also cross-reacts well with Vtg from other *Salmo* species and may thus have wider potential for developing quantitative Vtg assays for other salmonid fish.

References

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