# DEVELOPMENT OF QUANTITATIVE VITELLOGENIN-ELISA ASSAYS FOR FISH TEST SPECIES USED IN ENDOCRINE DISRUPTOR SCREENING

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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., *Crit. Rev. Toxicol*. 28:319-361, 1998; Larsson et al., *Aquatic Toxicol.* 45: 91-97, 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 guidelines for standardized Vtg assays in screening programs for endocrine disruptors, using the standard OECD test species rainbow trout (*Oncorhynchus mykiss*), carp (*Cyprinus carpio*), fathead minnow (*Pimephales promelas*), zebrafish (*Danio rerio*), and medaka (*Oryzias latipes*).

We have developed a line of specific monoclonal (MAb) and polyclonal (PAb) antibodies reacting with Vtg from these five species, and present here the development of sensitive, simple, and robust ELISA assays for Vtg in these species.

## Methods

**Fig. 1. MAb-PAb-sandwich assay**
(Carp Vtg-ELISA, zebrafish Vtg-ELISA and Medaka Vtg-ELISA)

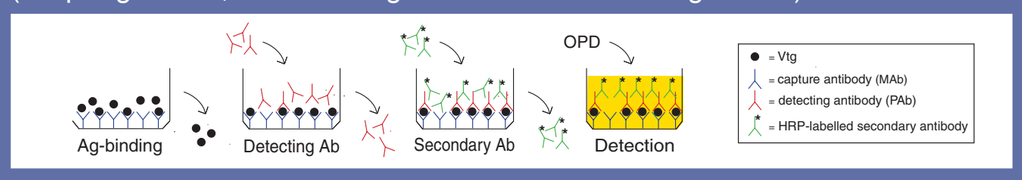
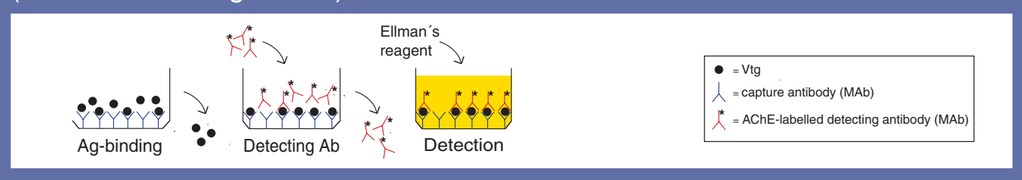



**Fig. 2. MAb-MAb-sandwich assay**
(Rainbow trout Vtg-ELISA)




## Carp/fathead minnow Vtg-ELISA

This assay is a MAb-PAb sandwich assay (see Fig. 1) utilizing carp-specific antibodies. Using both log-log and semi-log transformations, two standard curves are obtained expanding the working range and sensitivity of the assay. Fathead minnow (FHM) Vtg may be quantitated in the assay using carp Vtg as standard with a slightly lower precision of the results (Fig. 3B).

**Fig. 3A. Carp-Vtg standard curve and intra- / inter-assay variation**

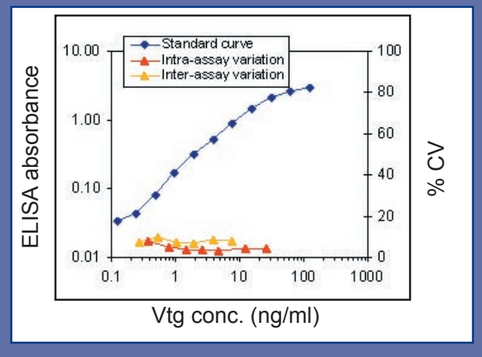
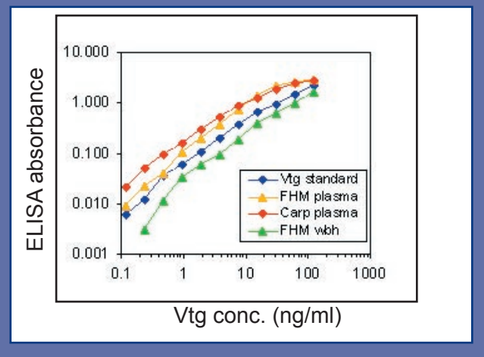



**Fig. 3B. FHM plasma and wbh dilute near parallel with carp-Vtg standard**




| **Table 1: Assay characteristics** | **Log-log** | **Semi-log** |
|---|---|---|
| Working range | 0.24-7.8 ng/ml | 7.8-62.5 ng/ml |
| Intra-assay variation (n=12) | 2.7-11.6 %CV | 2.1-9.1 %CV |
| Inter-assay variation (n=10) | 8.4-9.4 %CV | 7.9 %CV |

## Rainbow trout Vtg-ELISA

This ELISA is a MAb-MAb sandwich assay (see Fig. 2) utilizing two different salmonid-Vtg specific MAbs. The detecting antibody is labelled with with AChE, an enzyme with a high turnover that gives an increased sensitivity in ELISA assays. Purified Vtg and plasma samples from E2-treated fish give parallel dilution curves, demonstrating that Vtg in plasma may be accurately quantitated with this assay.

**Fig. 4A. Rainbow trout-Vtg standard curve and intra-assay variation**

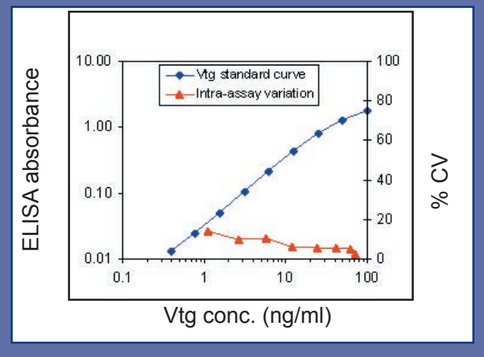
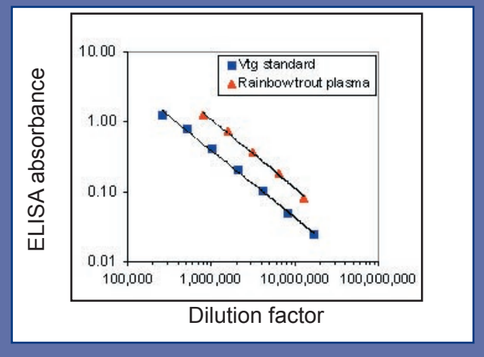



**Fig. 4B. Parallell dilution of rainbow trout-Vtg and plasma**




| **Table 2: Assay characteristics** | |
|---|---|
| Working range | 1.5 ng/ml - 50 ng/ml |
| Intra-assay variation (n=12) | 13.8 - 5.3 %CV (within working range) |

## Zebrafish Vtg-ELISA

This assay utilizes zebrafish-specific antibodies in a MAb-PAb sandwich assay (see Fig. 1).
The assay is well suited for quantitation of Vtg in zebrafish whole body homogenate, as purified Vtg and homogenate from E2-treated fish yield parallell dilution curves (Fig. 5B).

**Fig. 5A. Zebrafish-Vtg standard curve and intra-assay variation**

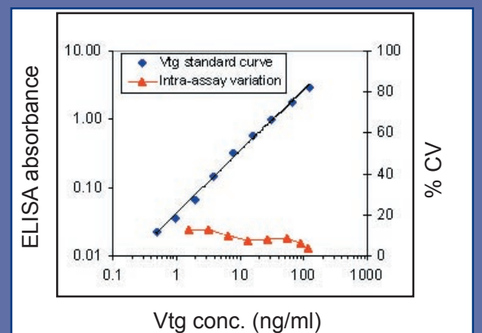
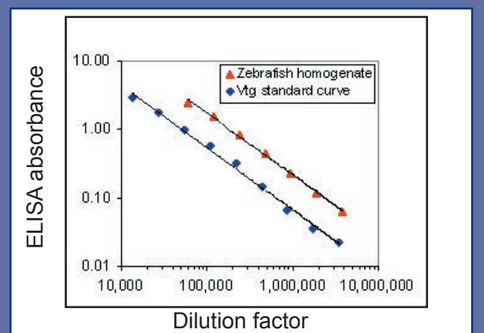



**Fig. 5B. Parallell dilution of zebrafish-Vtg and whole body homogenate**




| **Table 3: Assay characteristics** | |
|---|---|
| Working range | 0.49 ng/ml - 125 ng/ml (250-fold) |
| Intra-assay variation (n=12) | 12.9-3.9 %CV |

## Medaka vitellin/Vtg-ELISA


This assay under development utilizes a MAb specific for vitellin from medaka in a MAb-PAb sandwich assay (Fig. 1).
The assay is well suited for quantitation of Vtg in medaka plasma samples, as purified medaka vitelline and plasma from E2-treated fish yield parallell dilution curves (Fig. 6B).

**Fig. 6A. Medaka vitellin standard curve**

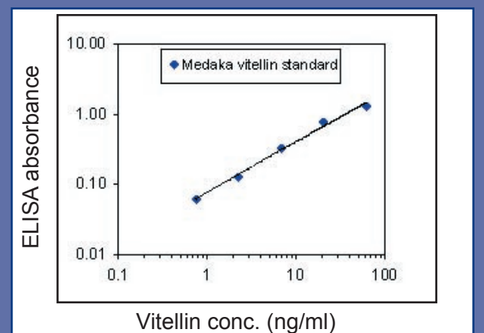
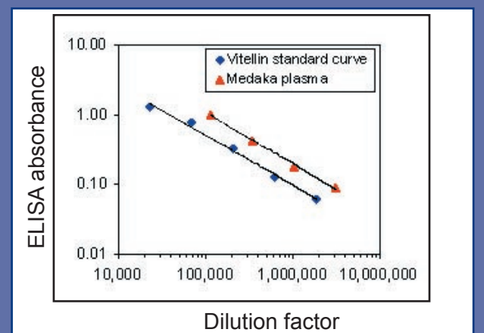



**Fig. 6B. Parallell dilution of medaka-vitellin and plasma**




| **Table 4: Assay characteristics** | |
|---|---|
| Working range | 0.8 ng/ml - 62 ng/ml |

## Conclusions

- Sensitive and robust Vtg assays have been developed for the standard OECD test species rainbow trout, carp and zebrafish
- The carp-Vtg ELISA may be used for quantitation of Vtg in fathead minnow
- A medaka Vtg ELISA is currently under development
- Stable and robust ELISA kits will be available for all these species during 2001