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Ultra-Sensitive Salmonid Vitellogenin ELISA: A new tool allowing accurate determination of vitellogenin in male and juvenile fish

Recently we have introduced a new collection system allowing a non-invasive, repeatable, and effective mucus sampling for vitellogenin determination in fish. This new sampling method opens the possibility to study vitellogenin concentrations over time or over treatment periods even in small juvenile fish. Juvenile fish are useful in the effect assessment of wastewater effluents and therefore we specifically developed a very sensitive vitellogenin ELISA for Salmonids. This sandwich ELISA requires two immune incubations, first with the capture antibody for 2 hours and second with the detection antibody for 1 hour, both at room temperature. At the end, the color reaction is measured at 450 nm and a 4-parameter curve fit should be used for automatic data reduction and calculation. The linear standard range is between 0.012 and 1.00 ng/ml (undiluted samples) covering an OD range between approximately 0.05 and 2.8 at 450 nm. The lower limit of detection (LLD) was calculated to be 0.002 ng/ml. The intra- and inter-assay CVs were between 3.7-5.2% and 6.4-9.5%, respectively. This ultra-sensitive vitellogenin ELISA can be used to measure vitellogenin in Atlantic salmon (*Salmo salar*), Brown trout (*S. trutta*), Chum salmon (*Oncorhynchus keta*); Pink salmon/humpback salmon (*O. gorbuscha*), Rainbow trout (*O. mykiss*), Brook trout (*Salvelinus fontinalis*), "Common whitefish, European whitefish" (*Coregonus lavaretus*), Maraena whitefish (*C. maraena*). With this new assay, changes in vitellogenin level can be accurately determined even in male and juvenile salmonid fish.

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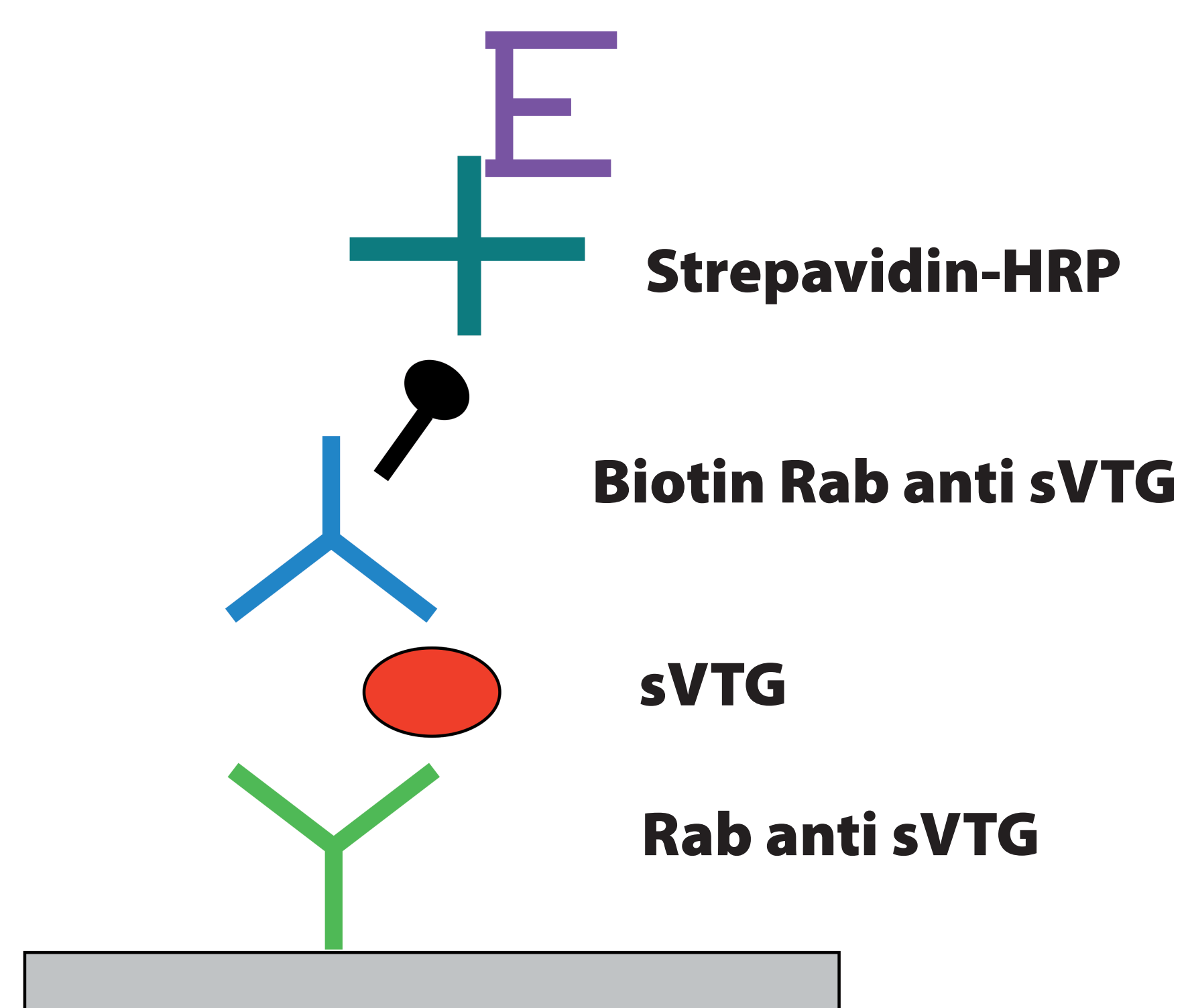
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INTRODUCTION AND OBJECTIVES

Recently it was shown, that vitellogenin can be accurately measured in the epidermal mucus of fish (Allner et.al. 2016). Vitellogenin measurement in mucus offers a broad range of advantages - one of them is based on a non-invasive sampling technique, offering the opportunity in taking multiple samples from individual fish. Based on a non-physiological vitellogenin increase juvenile and male fish are used as indicator organism for estrogenic endocrine disruptors. In these fish it is of special interest to understand the vitellogenin increase from baseline values during sexual maturation and due to estrogenic active substances. For this purpose we developed an ultra sensitive Salmonid vitellogenin ELISA test to measure vitellogenin in response to environmentally relevant concentrations of estrogen compounds.

MATERIALS AND METHODS

Test principle



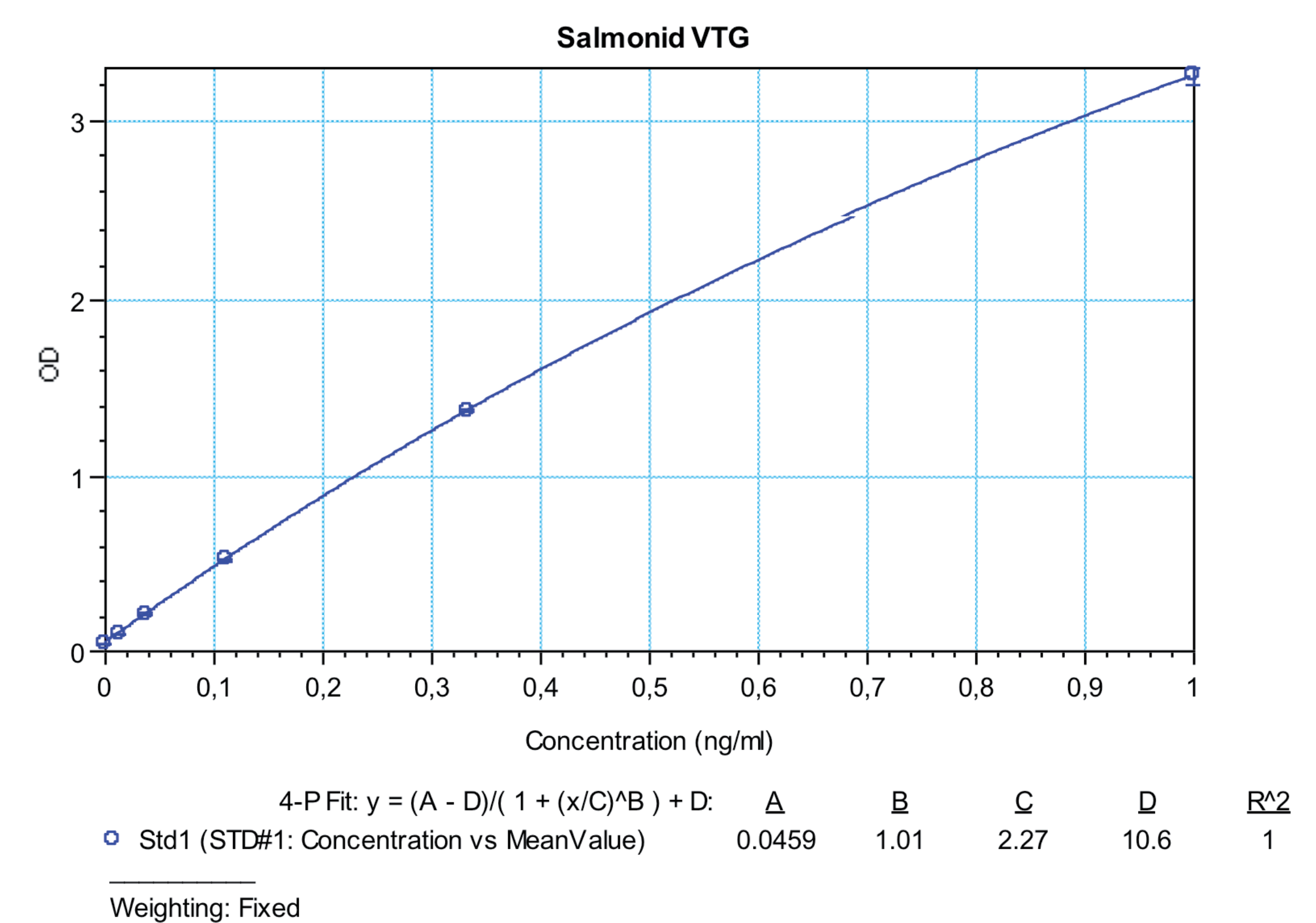
Samples:

The Ultra sensitive Salmonid Vitellogenin ELISA can be used in epidermal mucus samples (TECO Mucus Collection Set), in blood and tissue homogenates from male or juvenile fish.

ASSAY PROCEDURE

Add 50 µL Matrix solution into each well (multichannel pipette)
Add 50 µL of each standard, prepared controls and (pre-diluted) samples into wells
Incubate plate for overnight 120 min ± 10 min on a shaker (500rpm) at RT (20-28°C)
Wash plate 5 times using Wash Buffer
Add 100 µL Biotinylated AB in each well (multichannel pipette)
Incubate plate for 60 min ± 5 min on a shaker (500rpm) at RT (20-28°C)
Wash plate 5 times using Wash Buffer
Add 100 µL SA-HRP-Conjugate in each well (multichannel pipette)
Incubate plate for 30±5 Minuten on a shaker (500rpm) at RT (20-28°C)
Wash plate 5 times using Wash Buffer
Add 100 µL TMB Substrate Solution in each well (multichannel pipette)
Incubate the plate for 15-30 minutes, in the dark, on a shaker (500rpm) at RT (20-28°C)
Add 100 µL Stop Solution in each well (multichannel pipette)
Measure the color reaction within 10 minutes at 450nm (reference filter between 560 - 650 nm) A 4-parameter curve fit should be used for automatic data reduction

RESULTS



TEST CHARACTERISTICS

Standard range 0.012 ng/ml - 1.0 ng/ml
Sensitivity LLOQ: 0.012 ng/ml
 LLD: 0.002 ng/ml

Intra-assay CV	Mean (ng/ml)	SD (ng/ml)	CV (%)
Sample 1	0.101	0.005	5.2
Sample 2	0.652	0.024	3.7
Sample 3	0.044	0.002	3.9
Mean			4.3

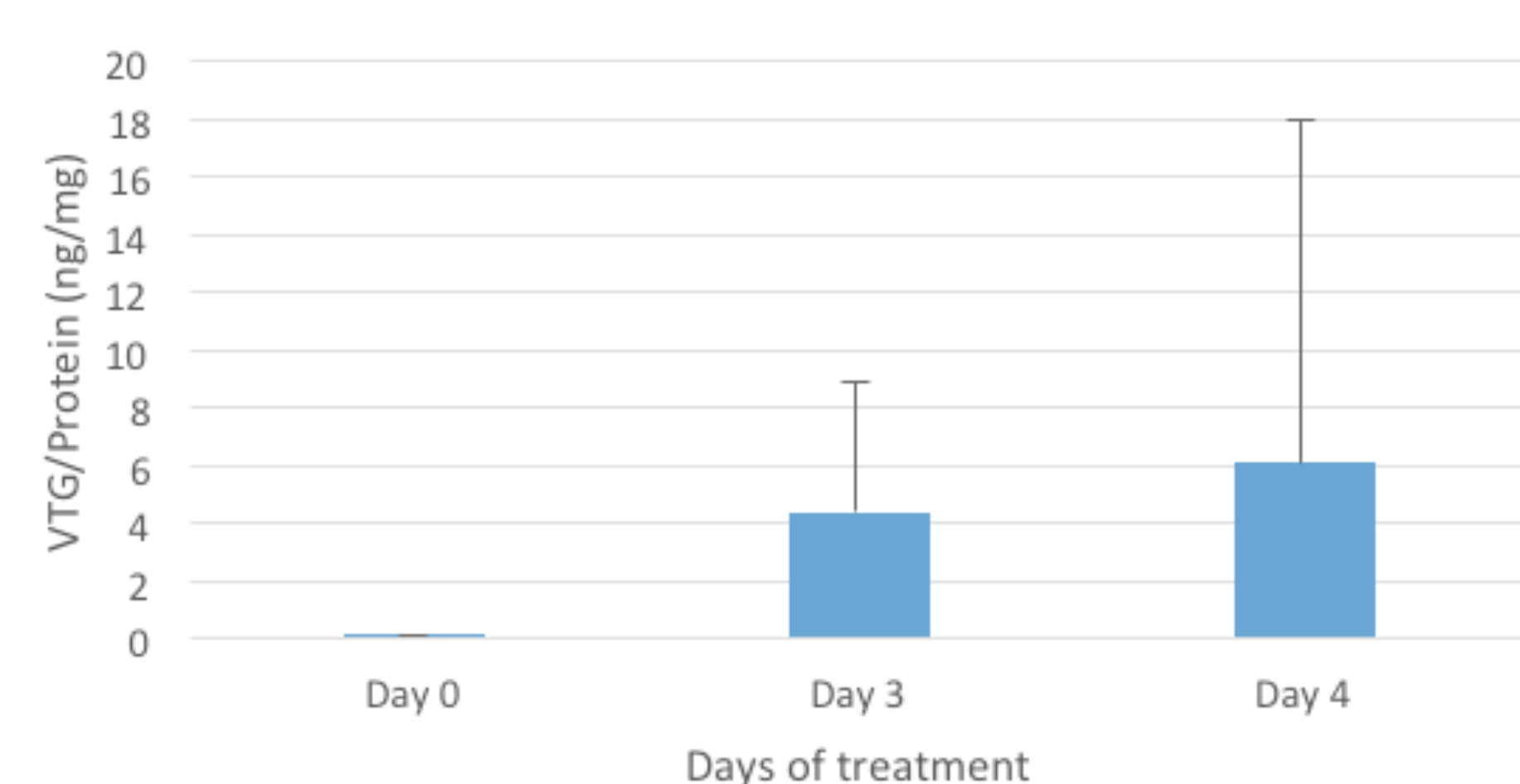
Inter-assay CV

Sample 1	0.129	0.012	9.5
Sample 2	0.783	0.050	6.4
Mean			8.0

Crossreactivities to different fish species

Atlantic salmon (Salmon salar)
 Brown trout (Salmo trutta)
 Chum salmon (Oncorhynchus keta)
 Pink salmon/humpback salmon (Oncorhynchus gorbuscha)
 Rainbow trout (Oncorhynchus mykiss)
 Brook trout (Salvelinus fontinalis)
 "Common whitefish, European whitefish" (Coregonus lavaretus)
 Maraena whitefish (Coregonus maraena)

E2 treatment in Brown trout (500 ng/L)



CONCLUSION

The ultra sensitive salmonid vitellogenin ELISA shows despite relatively short immune incubation times of total 180 min. a very high sensitivity. The linear standard range between 0.012 ng/ml and 1 ng/ml with mean intra-assay and inter-assay CVs of 4.4% and 8.0% respectively allow an accurate measurement of vitellogenin above 0.012 ng/ml in different salmonid species. In non-treated juvenile rainbow trouts (data not shown) no concentration above the detection limit of 0.002 ng/ml was detected. However within 3 days after exposure to E2 a measureable change in vitellogenin concentrations was observed and thus the proof of vitellogenin in salmonid mucus in trace amounts reflects a reliable biomarker for the exposure to exogenous estrogens.