BS ISO 23893-3:2013

BSI Standards Publication

Water quality — Biochemical and physiological measurements on fish

Part 3: Determination of vitellogenin

National foreword

This British Standard is the UK implementation of ISO 23893-3:2013.

The UK participation in its preparation was entrusted to Technical Committee EH/3/5, Biological Methods.

A list of organizations represented on this committee can be obtained on request to its secretary.

This publication does not purport to include all the necessary provisions of a contract. Users are responsible for its correct application.

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Compliance with a British Standard cannot confer immunity from legal obligations.

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Part 3: Determination of vitellogenin

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ISO copyright office
Case postale 56 • CH-1211 Geneva 20
Tel. + 41 22 749 01 11
Fax + 41 22 749 09 47
E-mail copyright@iso.org
Web www.iso.org

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 23893-3 was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 5, *Biological methods*.

ISO 23893 consists of the following parts, under the general title *Water quality — Biochemical and physiological measurements on fish*:

— *Part 1: Sampling of fish, handling and preservation of samples*

— *Part 2: Determination of ethoxyresorufin*-O-*deethylase (EROD)* [Technical Specification]

— *Part 3: Determination of vitellogenin*

Introduction

Vitellogenin (Vtg) is a large phospholipoglycoprotein produced as the yolk protein precursor in the liver of oviparous vertebrates, such as fish. Vtg is secreted from hepatocytes through the secretory pathway, enters the circulation and is taken up by the growing oocyte. Plasma Vtg concentrations are normally an indication of the maturational status of the female fish, for reviews see References [2] [18]. More than a decade ago, several studies demonstrated that male fish caught in rivers and streams had high concentrations of plasma Vtg (e.g. References [14][23]), caused by chemicals acting like oestrogens present in the environment. Since then, numerous studies have shown the fish Vtg to be a highly responsive biomarker for oestrogenic compounds in both *in vitro* hepatocyte cell cultures, *in vivo* aquaria studies, and field studies, for reviews see References [1][2][10][13][16][20][26]. Hence, Vtg in fish has become an accepted biomarker of xenooestrogenic and antioestrogenic exposure of chemicals, effluents, and discharges, and has been proposed in chemical testing, as well as environmental monitoring programmes, e.g. Reference [13].

However, recent genetic and immunological analyses have demonstrated a general multiplicity of Vtg forms in fish, References [9][10]. The concentrations of circulating Vtg proteins (or Vtg gene transcripts) during oogenesis and their degree of induction by oestrogens appear to vary among species and among different types of Vtg within a species. The kinetics of induction of distinct types of Vtg by oestrogens in fish appears to depend on environmental factors (e.g. water temperature and photoperiod), life history stage, sex, and the concentration and type of oestrogenic compound. Based on these findings, it is important that the Vtg targets in a bioassay for oestrogens in a specific species be demonstrated to be an oestrogen-responsive form, and that the assay be validated with the species in question before embarking on a monitoring programme, Reference [10].

The scientific literature contains a multitude of publications on procedures for determining Vtg in fish samples, using immunoassays. Whereas radioimmunoassays (RIA) were a predominant method in the 1980s and early 1990s, e.g. References [4][29], enzyme-linked immunosorbent assays (ELISAs) are the dominating principle today. Both the sandwich and competitive ELISA principles provide sensitive results without the use of radioactive isotopes, and have been successfully applied to determine Vtg levels in several fish species, e.g. References [3][6][8][12][15][17][19][21][22][24][25][27][28][31][32].

This part of ISO 23893 presents a generalized protocol for both the sandwich and competitive ELISA for use in quantification of Vtg in fish blood plasma samples. The application of standardized methods is strongly advised within monitoring programmes.

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WARNING — Persons using this document should be familiar with normal laboratory practice. This document does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user to establish appropriate safety and health practices and to ensure compliance with any national regulatory conditions.

IMPORTANT — It is absolutely essential that tests conducted in accordance with this document be carried out by suitably qualified staff.

1 Scope

This part of ISO 23893 specifies a method for measuring vitellogenin (Vtg) concentrations in a fish plasma sample employing an enzyme-linked immunosorbent assay (ELISA) method.

It applies to fish that are sampled in the environment (fresh, estuarine or salt water) and to fish exposed to substances or effluents in a laboratory. The method is quantitative when using Vtg antibodies and a Vtg standard well characterized with the species of choice.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 23893-1, *Water quality — Biochemical and physiological measurements on fish — Part 1: Sampling of fish, handling and preservation of samples*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1
limit of detection
LOD
lowest content that can be measured with reasonable statistical certainty

EXAMPLE The LOD is often expressed as the reagent blank value plus three times its standard deviation.

Note 1 to entry: The method LOD is determined by taking the sample dilution factor into the calculation.

3.2
limit of quantification
LOQ
content equal to or greater than the lowest concentration point in the calibration curve

EXAMPLE The LOQ is often expressed as the reagent blank value plus 10 times its standard deviation (Reference [11]).

Note 1 to entry: LOQ is also determined by taking the sample dilution factor into the calculation.