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Validation of *Arxula* Yeast Estrogen Screen assay for detection of estrogenic activity in water samples: Results of an international interlaboratory study

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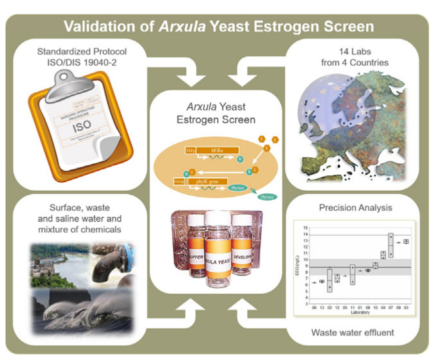
HIGHLIGHTS

- Validation of *Arxula* Yeast Estrogen Screen (A-YES®) for water and waste water
- International interlaboratory trial with 14 participants and 9 samples
- Repeatability and reproducibility of A-YES® are comparable to chemical methods.
- A-YES® is a suitable tool for monitoring water quality and waste water treatment.

GRAPHICAL ABSTRACT



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ABSTRACT

Endocrine-active substances can adversely impact the aquatic ecosystems. A special emphasis is laid, among others, on the effects of estrogens and estrogen mimicking compounds. Effect-based screening methods like *in vitro* bioassays are suitable tools to detect and quantify endocrine activities of known and unknown mixtures. This study describes the validation of the *Arxula*-Yeast Estrogen Screen (A-YES®) assay, an effect-based method for the detection of the estrogenic potential of water and waste water. This reporter gene assay, provided in ready to use format, is based on the activation of the human estrogen receptor alpha. The user-friendly A-YES® enables inexperienced operators to rapidly become competent with the assay.

Fourteen laboratories from four countries with different training levels analyzed 17β-estradiol equivalent concentrations (EEQ) in spiked and unspiked waste water effluent and surface water samples, in waste water influent and spiked salt water samples and in a mixture of three bisphenols. The limit of detection (LOD) for untreated samples was 1.8 ng/L 17β-estradiol (E2). Relative repeatability and reproducibility standard deviation for samples with EEQ above the LOD (mean EEQ values between 6.3 and 20.4 ng/L) ranged from 7.5 to 21.4% and 16.6 to 28.0%, respectively. Precision results are comparable to other frequently used analytical methods for estrogens. The A-YES® has been demonstrated to be an accurate, precise and robust bioassay. The results have been included in the ISO draft standard.

The assay was shown to be applicable for testing of typical waste water influent, effluent and saline water. Other studies have shown that the assay can be used with enriched samples, which lower the LOD to the pg/L range.

The validation of the A-YES® and the development of a corresponding international standard constitute a step further towards harmonized and reliable bioassays for the effect-based analysis of estrogens and estrogen-like compounds in water samples.

1. Introduction

Endocrine-disrupting compounds (EDC) pose a global threat to human health and the environment (Bergman et al., 2012). Concerns about the effects of natural estrogens and estrogen mimics to the environment stem from their widespread use in personal care products (Karpuzoglu et al., 2013), industrial chemicals (Rochester et al., 2015), livestock breeding and agriculture (Gall et al., 2011) and human pharmaceutical products (Maitre 2013). Several examples include the use of estrogens in contraceptives, hormonal replacement therapy or for treating menopausal and post-menopausal symptoms. Depending on their use patterns, EDCs enter aquatic ecosystems from different human and animal sources (Adeel et al. 2017). The elimination of estrogenic micro-pollutants in conventional waste water treatment plants is incomplete so far and effluents are one of the major sources of estrogens and estrogenic transformation products into watercourses (Filby et al., 2007; Racz and Goel, 2010; Gehrmann et al., 2016). Because of the hormone-like action of estrogens and estrogen acting compounds and their ubiquity in environment, a number of efforts have been made to investigate their impacts on the ecosystems. To dates, there are numerous laboratory and field studies showing the adverse effects of natural estrogens and estrogenic EDCs in marine organisms that result in altered reproductive output in gastropods and altered sexual maturation in wild roach (Oehlmann et al., 2006; Benstead et al., 2011; Jobling et al., 2002; Jobling and Tyler, 2003; Lange A. et al., 2008).

To address the problem of EDCs and micro-pollutants in general, the European Union Water Framework Directive (WFD) was adopted in 2000 (European Commission 2000). The WFD aims to improve overall surface water quality by (i) achieving a good ecological and chemical status for surface waters and (ii) a good chemical and quantitative status for ground waters in Europe. The Article 8 of the WFD calls for the necessity of monitoring programs to establish a coherent and comprehensive overview of the water status within each river basin. Typically targeted chemical analysis strategies are applied for monitoring the chemical status. These methods are focused on the type and concentration of chemicals in a sample, and can precisely identify single known substances within environmental samples. However, they require *a priori* knowledge about the substances to be monitored. For technical and economic reasons, it is not possible to analyze, detect and quantify all substances that are present in the aquatic environment (Wernersson et al., 2015). Chemical monitoring is, therefore, performed instead only on regulated and harmful substances.

Unlike other analytical techniques, effect-based bioassays present a different approach to monitor water quality. Using bioassays offers a possible assessment of the cumulative effect of all compounds present in a sample (Silva et al., 2002). Thus, the effect of both known and unknown substances is captured (Leusch et al., 2014; Kunz et al., 2015).

Bioassays can complement a chemical analysis in various ways. They can provide additional information, or can be used as screening tools that initiate a chemical analysis in case of positive results, or can be used if routine chemical methods do not reach the required detection limits. For example, the environmentally relevant concentrations for the estrogens 17β-estradiol (E2) and 17α-ethinylestradiol (EE2) are in the low ng/L or pg/L range (Kunz et al., 2015). Here, routine analytical methods are often not sensitive enough, allowing bioassays to be used to quantify the estrogenic activity of a water sample in terms of a 17β-estradiol equivalent concentration (EEQ) (Kunz et al., 2015).

Realizing the problem of chemical mixtures in water quality monitoring, the CMEP (Chemical Monitoring and Emerging Pollutant) group, which acts in the context of Common Implementation Strategy (CIS) of the WFD, started activities on effect-based bioassays and published a technical report on aquatic effect-based monitoring tools (Wernersson et al., 2015). Beyond this report, several other recommendations for integration of bioassays in water quality monitoring are also available (Brack et al., 2016; Brack et al., 2017; Altenburger et al., 2015; Hecker and Hollert, 2011; Hamers et al., 2013; Di Paolo et al., 2016, Tousova et al. 2017).

A prerequisite for the implementation of effect-based tools in water quality monitoring is the availability of reliable and standardized test methods. On an international level, the ISO (International Organization for Standardization) is the relevant body for the development of standards. The scope of the ISO Technical Committee 147 is standardization in the field of water quality with emphasis on environmental issues. In this context, the standardization of assays for the determination of the estrogenic potential of water and waste water were included in the working program of ISO TC 147 in 2013, with *Arxula* Yeast Estrogen Screen (A-YES®) assay among them (ISO/DIS 19040-2) (ISO Standards Catalogue).

The present study describes the validation of the A-YES® within the framework of an interlaboratory trial in the context of the