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Method Article

Updating the ELISA standard curve fitting process to reduce uncertainty in estimated microcystin concentrations

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A B S T R A C T

This study is aimed at exploring the optimal ELISA standard curve fitting process for reducing measurement uncertainty. Using an ELISA kit for measuring cyanobacterial toxin (microcystin), we show that uncertainty associated with the estimated microcystin concentrations can be reduced by defining the standard curve as a four-parameter logistic function on the natural log concentration scale, instead of the current approach of defining the curve on the concentration scale. The model comparison method is outlined in this paper, allowing it to be transferable to test different statistical models for other ELISA test kits.

A R T I C L E I N F O

Keywords: Cyanobacteria, Drinking water, Harmful algal bloom, Nonlinear regression

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Subject area	Mathematics
More specific subject area	Applied Statistics in Life Science
Method name	ELISA Standard Curve Fitting Method
Name and reference of original method	U.S. EPA [15] & Abraxis product (520011) documentation http://www.abraxiskits.com/moreinfo/PN520011USER1.pdf (more on online supporting materials)
Resource availability	The statistical software R: https://www.r-project.org/, Github: https://github.com/songsqian/ELISA

Method details

The Enzyme-Linked Immunosorbent Assay (ELISA) is a biochemical technique for detecting the presence of a substance (usually, an antigen or protein) in a water sample [5]. The basic principle of ELISA is the use of an enzyme-linked antibody attached to a solid surface to attract the antigen of interest. Once the antigen in the water sample and the antibody are bound, a color change can be detected and used to quantify the concentration of the substance. ELISA is widely used in immunology and other medical settings. The use of ELISA for monitoring cyanobacterial toxins (microcystins or MC) was discussed by Chu et al. [1]. ELISA can be used to detect all known microcystin congeners and to quantify total microcystin concentration [3]. With the advent of commercial microtiter plate kit for microcystins [13], ELISA has quickly become a commonly used method for quantifying cyanobacterial toxins associated with harmful algal blooms (HABs). As such, this paper will focus on the use of ELISA for measuring MC, but the methods presented are applicable for other ELISA test kits.

Because MC are known to cause damage to the nervous systems and liver [4] at high concentrations, the World Health Organization proposed a provisional limit of 1 μg/L in drinking water [16]. Additionally, the US Environmental Protection Agency recommends the upper limit of the 10-day mean MC concentration be 0.3 μg/L for pre-school age children and 1.6 μg/L for the rest of the population [14]. The effects of MC concentrations on the public were felt by the city of Toledo, Ohio, USA, between August 2nd and 4th, 2014 when MC concentration from one tap water sample was shown to be much higher than 1 μg/L, prompting the city to issue a "Do Not Drink," advisory, affecting nearly half a million residents. The MC concentration of this sample was measured by Toledo Collins Park Water Treatment Plant using an ELISA test kit. Although thresholds for acceptable exposure to MC are precisely defined in the advisory, ELISA-measured MC concentrations are unfortunately highly variable [10]. In recognition of the high variability, many quality control procedures related to lab operations were developed (e.g., Ohio EPA [6]). The statistical side of ELISA (the mathematical form of the standard curve, curve fitting method, and concentration estimation method) is not affected by these quality control measures intended to reduce operational uncertainty; Qian et al. [10] showed the estimation uncertainty due to statistical reasons is considerable. As such, this is where we aim to apply new methods to reduce the statistical model uncertainty associated with ELISA test kits. This paper presents an experimental method for comparing alternative mathematical forms of the standard curve, with an emphasis on evaluating the estimation uncertainty.

Experimental design for comparing alternative models

A general approach for comparing alternative models is to compare models' predictions to the same testing data with known values. We can fit alternative models to the same training dataset and apply them to a testing dataset. The model with the highest predictive accuracy is the preferred model. To compare the predictive uncertainty of alternative standard curve models, we used an ELISA kit from Abraxis, Inc. (kit #PN520011OH, lot #16F0230), which comes with five non-zero concentration solutions (0.15, 0.4, 1, 2, and 5 μg/L) and a quality control solution (0.75 μg/L). We diluted these solutions by the following factors: 1, 1.5, 2, 2.5, 3, 3.5, and 4, each with two replicates, resulting in 84 non-zero concentration solutions. We used six replicates of zero concentration solutions and the remaining six of the 96 wells of the ELISA kit were filled with a dilution sequence of a water sample