APPLICATION NOTE

AUTOMATION OF THE BIOO MAXSIGNAL® AFLATOXIN M1 ELISA TEST KIT

Abstract

Ensuring food safety requires reliable and convenient methods to detect the presence of harmful substances in food. Aflatoxin M1 is a hepatotoxic and carcinogenic mycotoxin that can be potentially present in milk and its derivatives; therefore, assays for its detection are routinely performed in many food testing laboratories. The automation of the MaxSignal® Aflatoxin M1 ELISA Test Kit using the Crocodile 5-in-one ELISA miniWorkstation reduces labour and provides excellent results.

Introduction

Milk is a good source of many nutrients but can be also a source of intake of harmful substances such as mycotoxins. Aflatoxin M1 is the main secondary metabolite found in milk secreted by animals and nursing mothers who have consumed food contaminated with aflatoxin B1 (1). Even though it is less toxic than its parent compound, aflatoxin M1 has hepatotoxic and carcinogenic effects (2, 3, 4); furthermore, it is thermostable and is not readily destroyed or removed by chemical and physical treatments typically used in food processing (5); therefore, its presence in raw milk implies its presence also in the derivatives (yogurt, cream, butter and cheese).

Many of the available assays to detect aflatoxin M1 are ELISA assays; the repetitive protocols and the number of samples to be processed make automation highly desirable, and the Crocodile 5-in-one ELISA miniWorkstation is a good automation solution for low- to medium-throughput laboratories.

The MaxSignal® Aflatoxin M1 ELISA Test Kit is a high sensitivity competitive enzyme immunoassay for the quantitative analysis of aflatoxin M1 in milk, characterized by extreme precision and robustness. This work assesses the automation of the MaxSignal® Aflatoxin M1 ELISA Test Kit with the Crocodile ELISA miniWorkstation and provides an optimized protocol for it.

Luca Pacchioni

Generon S.p.A. - Via San Geminiano, 4 - 41030 San Prospero (MO) – Italy

Francesc Felipe Legaz

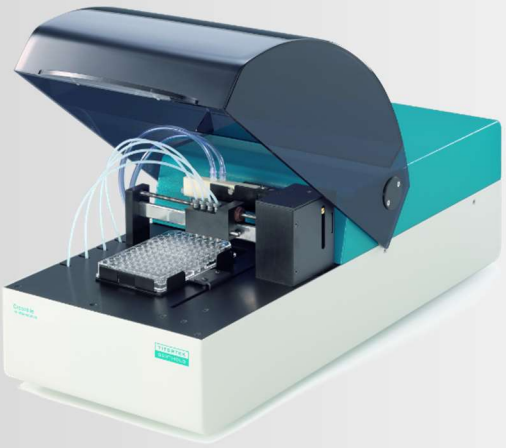
Berthold Technologies GmbH & Co. KG – Calmbacher str. 22, 75323 Bad Wildbad, Germany

The Berthold Technologies Crocodile 5-in-one ELISA miniWorkstation

The Crocodile 5-in-one ELISA miniWorkstation is a compact liquid handling system integrating dispenser, shaker, incubator, washer and reader using the bench space of an ELISA reader.

The use of the Crocodile reduces assay time by eliminating the need to move plates between dispenser, shaker, incubator, washer and reader.

The Crocodile is a bench-top instrument that has been designed specifically with ease of use in mind. It is easy to operate, extremely reliable and requires only minimal routine maintenance. You can automate any ELISA, or other assays involving dispensing, shaking, incubation, washing or absorbance reading, thanks to the flexibility of its software. And, in addition, all steps performed by the instrument are perfectly documented.



Materials

- Crocodile ELISA MiniWorkstation LB 925 (Berthold Technologies).
- MaxSignal® Aflatoxin M1 ELISA Test Kit (Order number 1060-05, Bioo Scientific).
- Precision micropipettes or multi-dispensing micropipettes, with suitable disposable tips.
- Distilled or deionized water.

Methods

All reagents were brought up to room temperature for 1 h prior to use. Wash Solution was prepared following the instructions given in the user manual of the kit.

Standards and samples were pipetted according to the manufacturer's instructions. 6 samples with a known aflatoxin M1 concentration (from 7,5 ppt to 70 parts per trillion, ppt) were prepared by adding the appropriate amount of 0,5 µg/mL aflatoxin M1 (Trilogy Analytical Laboratory, order number TSL-143) to reconstituted powder milk; in addition, one sample with no Aflatoxin M1 was used as negative control (Whole milk powder ERM® certified Reference Material, aflatoxin M1, zero level from Sigma-Aldrich, order number ERMBD282-30G). All standards and samples were run in duplicate. Two independent experiments were performed.

The Crocodile ELISA miniWorkstation was programmed with the steps summarized in **Table 1**. Incubation times were adjusted to take into account the time used by the instrument in the priming steps.

Aflatoxin M1 concentrations were calculated as indicated in the kit insert with the help of the MaxSignal® ELISA Analysis Program in Excel, which is provided by the manufacturer.

#	Step name	Description and parameters
1	**Sample Incubation**	**Incubation** Incubator Off, Duration: 00:43:40
2	**Wash Solution priming**	**Washing** Method: Prime Washer, Wash Solution Inlet: 1, Cycles: 6, Volume: 1000 µL
3	**Wash**	**Washing** Method: Standard, Wash Solution Inlet: 1, Cycles: 3, Volume: 250 µL, Delay: 1 s, Wait: 500 ms, Dispenser Depth: 1300 (Plate Offset: -45), Aspiration Depth: 3075* (Plate Offset: 41), Sweep: 5 mm @ 4 mm/s
4	**Conjugate priming**	**Dispensing** Volume: 1200 µL, Inlet: 1, Method: Priming
5	**Conjugate addition**	**Dispensing** Volume: 100 µL, Inlet: 1, Method: Standard
6	**Mix**	**Shaking** For 00:00:10 at Incubator with 1 mm Amplitude at 5 Hz
7	**Conjugate incubation**	**Incubation** Incubator Off, Duration: 00:15:00
8	**Wash**	**Washing** Method: Standard, Wash Solution Inlet: 1, Cycles: 3, Volume: 250 µL, Delay: 1 s, Wait: 500 ms, Dispenser Depth: 1300 (Plate Offset: -45), Aspiration Depth: 3075* (Plate Offset: 41), Sweep: 5 mm @ 4 mm/s
9	**TMB priming**	**Dispensing** Volume: 1200 µL, Inlet: 3, Method: Priming
10	**TMB addition**	**Dispensing** Volume: 100 µL, Inlet: 3, Method: Standard
11	**Mix**	**Shaking** For 00:00:10 at Incubator with 1 mm Amplitude at 5 Hz
12	**TMB incubation**	**Incubation** Incubator Off, Duration: 00:14:00
13	**Stop solution priming**	**Dispensing** Volume: 1200 µL, Inlet: 4, Method: Priming
14	**Stop solution addition**	**Dispensing** Volume: 100 µL, Inlet: 4, Method: Standard
15	**Mix**	**Shaking** For 00:00:10 at Incubator with 1 mm Amplitude at 5 Hz
16	**Measure**	**Reading** Single Wavelength, Filter 1: 450 nm
** Depth settings have to be optimized for individual instruments*		

Table 1. Summary of steps programmed in the Crocodile Control Software.

Results

The standard curves obtained with the Crocodile ELISA miniWorkstation showed excellent fitting (Figure 1) and were very similar to the curves obtained when performing the assay manually (data not shown).

The measured concentrations were closer to the expected concentrations (with a maximum difference of a 10%, see Table 2) than when the assay was performed manually (maximum difference of a 18%, data not shown).

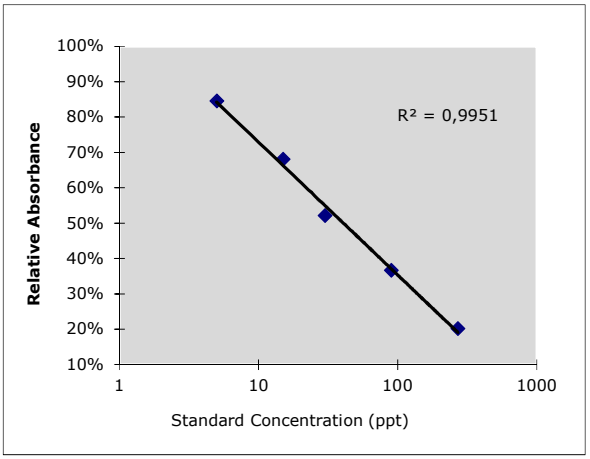


Figure 1. *Representative aflatoxin M1 standard curve obtained with the Crocodile.*

Sample number	Expected concentration (ppt)	Measured concentration (ppt)
1	0,0	0,0 ± 0,0
2	7,5	7,0 ± 0,3
3	10,0	11,3 ± 0,5
4	11,0	11,8 ± 1,8
5	15,0	14,3 ± 0,8
6	30,0	31,5 ± 1,7
7	70,0	71,6 ± 2,5

Table 2*. Expected and measured aflatoxin M1 concentrations. Measured concentrations are averages of 2 experiments and are represented as average ± standard error of the mean.*

Summary

The assay procedure is simple and involves only the addition of controls and samples, while the instrument performs the various dispensing, washing, incubation and reading steps automatically. The standard curve obtained in the Crocodile showed excellent fitting, and the calculated concentrations were in all cases very close to the expected concentration. In consequence, the Crocodile ELISA miniWorkstation provides a convenient and easy-to-use method to automate the Bioo MaxSignal® Aflatoxin M1 ELISA Test.

Acknowledgements

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References

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Berthold Technologies GmbH & Co. KG
Calmbacher Straße 22
75323 Bad Wildbad
GERMANY
Phone: +49 7081 177 0
Email: bio@berthold.com

www.berthold.com