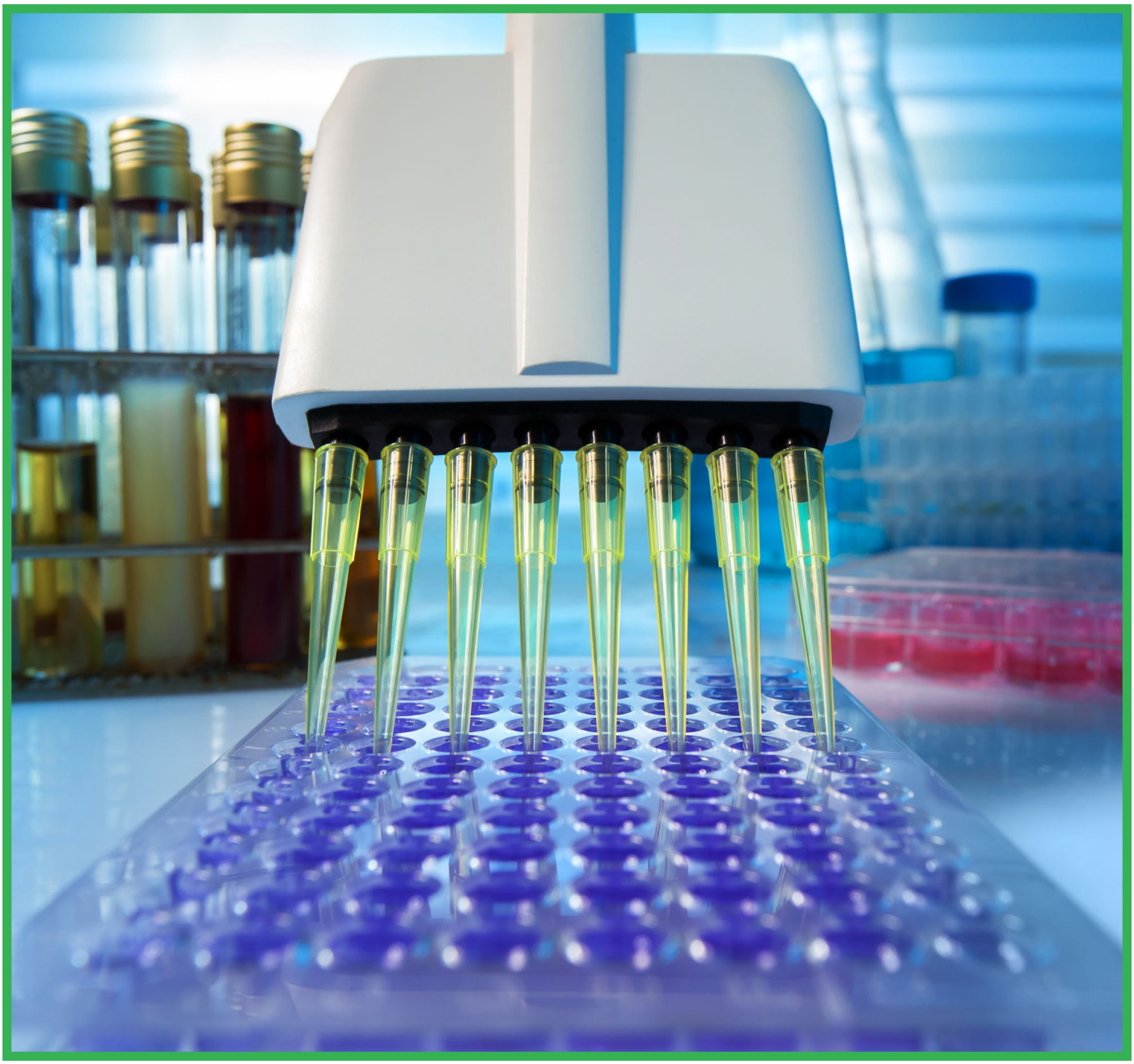
2nd edition

The essential guide to ELISA automation & reading

Explore this comprehensive compendium of ELISA best practises, tips, tricks, and optimized protocols from reading to automation



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Introduction

The enzyme-linked immunosorbent assay (ELISA) is the gold standard for the detection and quantitation of peptides, proteins, antibodies, and hormones in biological samples.

Since its introduction in the early 1970s, ELISA has been applied across nearly every biological discipline and has become one of the most commonly used techniques in clinical testing and life science research. ELISA works by coupling an antibody or antigen to an assay enzyme and relies critically on a highly specific antigen-antibody interaction.

Conventionally, ELISA is performed in multi-well plates (typically a 96- or 384-well) and protocols contain multiple iterations of dispensing reagents, microplate washing, and time-sensitive incubation and shaking, before final detection can be achieved.

Alternatively, innovative workstations automating the entire ELISA workflow from washing, dispensing, shaking, incubating to the final absorbance measurement can help laboratories increasing their productivity and throughput

In this comprehensive eBook, learn about ELISA best practices and troubleshooting tips, and benefit from an extensive compendium of established automation protocols for a wide range of applications.

Contents

Best practices for running your ELISA

In order to achieve accurate and consistent results with your ELISA, you should include the following best practices in your workflow planning.

- **Include blank samples.** Blank samples are buffer or water with no protein included. The use of blank samples enables the subtraction of background absorbance from the rest of the data points and are required for accurate optical density (OD) readings.

- **Include a positive control on each plate.** Positive controls are samples with known concentrations and these help indicate whether the ELISA was correctly executed.

- **Run your samples in replicates.** Running samples and standards in duplicate or triplicate helps determine the average, standard deviation (SD), and coefficient of variation (CV). This practice is required to average out any pipetting error.

Replicates should have a CV of less than 20%. If your CV is higher, check the following:

- Did you use a plate cover during incubation steps? Evaporation of sample could be causing issues

- How did you incubate your samples? Consistent temperatures across the plate are important to ensure reproducible results. Inconsistent and incorrect incubation temperatures and time are typical sources for high background signal

- How did you pipette your samples? Proper pipetting techniques can result in more accurate results. Multichannel pipettes are most commonly used. Electronic pipettes are extremely accurate but require regular maintenance and calibration. You should also only use tips in agreement with the manufacturer´s specification.

- Did you handle your plate and reagents according to good laboratory practice (GLP)? Expired or incorrectly stored reagents, as well as cross-contamination, can lead to incorrect, weak, or no signal.

- How did you wash your samples? Insufficient washing can lead to inconsistent replicates or high background signal. If pipetting manually, invert the plate on absorbent tissue and allow to completely drain to remove any residual fluid. Increasing the number of washing cycles may be useful too. If using an automated plate washer, be sure to recalibrate it so that the tips do not touch the bottom of the wells and be careful when manually dispensing and aspirating into and out of the wells.

- **Include a standard curve on every plate,** if possible. ELISA variations can be introduced by various sources, including the operator, pipetting, incubations, and temperature. Including a standard curve of known concentrations within the microplate can compensate for these variations. If the purified analyte is not available, if its quantification is not possible, or if it is not relevant for the performance of the assay, it's not possible to create a standard curve.

- **Ensure your samples fall within the linear range of your standard curve.** If your samples vary significantly in concentration, test samples at multiple dilutions to ensure that at least one of them falls within the range or the standard curve. The range can be linear or non-linear, depending on the data reduction method used. Be aware that values close to the top or bottom of the standard curve typically display higher error rates.

Sample readings are performed in ELISA readers. Typical ELISA absorbance plate readers such as the Apollo measure the absorbance of samples in a microplate (typically 96 or even 384 wells) by passing light vertically through each well. Thus, they offer a higher throughput than absorbance spectrophotometers, which measure the absorbance of individual samples in cuvettes. Here are some tips when considering buying an ELISA reader:

i. Technical criteria include a:

a. Wavelength range that covers the application you plan to perform; this can either be achieved by filter selections or optical grating technology

b. Dynamic range that is larger than 3 absorbance units

c. Sufficient reading speed to enable the desired throughput, e.g. 10 seconds or below

d. Incubator for consistency in the required temperatures

e. Touchscreen display for easy operation

f. Communication interface (e.g. USB) to either connect a PC or transfer your data

g. Thermal printer that is either integrated or optional

ii. Software considerations include:

a. Availability of different calculation modes enabling analysis and evaluation of your data

b. Easy data import and export options

iii. Other criteria include:

a. Reasonable total cost of ownership (including maintenance costs, accessories, etc.)

b. Availability and quality of technical support and service

Best practices for analyzing your ELISA

ELISA analysis is typically performed using Microsoft™ Excel™ or specialized software programs. Best practices include:

- **Subtract background from all sample readings.** Subtract the absorbance readings of your blank samples from your unknown samples to remove any background signal. If you see higher blank sample signals than usual, this may indicate that there was an error in the assay workflow.

- **Use the recommended data reduction method for your chosen assay,** if available. Try different data reduction methods, e.g. 4- or 5-parameter algorithms, linear, semi-log or log-log. Preferably, use curve-fitting software to generate the standard curve. Use a method that gives the best correlation value. If no software is available, plot the log of concentrations vs the log of O.D. in a linear scale

- **Apply the dilution factor to correct your sample´s absorbance.** Use the standard curve to get the sample concentration in the wells. If your samples were diluted, remember to multiply the concentration in the wells by the dilution factor to obtain the final concentration.

- **Perform statistical tests on your data to evaluate differences.** If technical replicates (repeated measurements of the same sample) were run, determine the variability within your protocol (intra-assay variation), user or equipment. Analyze the data for the average, standard deviation, and CV for the final results. CV >20% indicates a possible error or great inconsistencies.

Manual vs automated ELISA

The ELISA workflow consists of numerous pipetting steps that are time-consuming and tedious and must be performed consistently - from well to well and plate to plate - to guarantee success. In particular, the different washing steps of a typical ELISA protocol should be emphasized. The washing steps are critical as they are essentially crucial for the background signal associated with unbound conjugated antibodies and thus have a direct influence on the signal-to-noise ratio of the assay. Therefore, insufficient washing can result in increased variation and high background, resulting in poor results.

When performed manually, the various ELISA workflow steps can be laborious, time consuming, and require an operator's full attention to ensure assays are consistent and reliable. In addition, the need for multiple instruments for each step can quickly limit sample throughput and decrease a lab's productivity.

As a solution to these challenges, innovative workstations that automate ELISA protocols

can increase the reliability and reproducibility of results, provide researchers with greater walkaway time, and improve workflow efficiency. For low- to medium-throughput labs requiring faster ELISA performance, automated ELISA assay workstations, such as the Crocodile 5-in-one miniWorkstation, and plate washers, such as the Zoom HT Microplate Washer, can be useful additions to ELISA workflows. These instruments can help to optimize well-to-well consistency, maximize productivity and reduce the risk of human error while freeing researchers from repetitive tasks and occupying less lab space than the multiple instruments required for manual processing.

Established automation protocols

Explore this compendium of worked examples that demonstrate how automated workstations can streamline ELISA workflows across a range of applications in veterinary sciences, clinical testing, and food testing.

Veterinary sciences

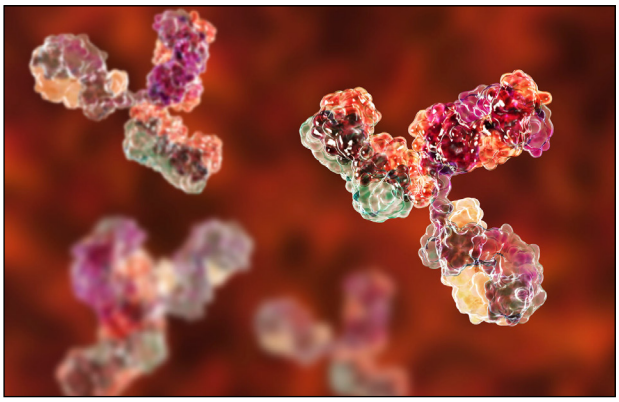
Adaptable bovine pregnancy testing
The IDEXX Milk Pregnancy Test, for use in bovine milk samples, is an enzyme-linked immunoassay for the detection of pregnancy-associated glycoproteins (PAGs). In this application note, learn how the Crocodile miniWorkstation and accompanying Crocodile Control Software enables easy integration of manual and automated steps and can be used to accurately diagnose bovine pregnancy.

High-sensitivity parasite detection
This application note illustrates the high analytical sensitivity of the PrioCHECK® Toxoplasma Ab porcine - an indirect ELISA for the detection of antibodies against the protozoan parasite that causes toxoplasmosis in humans and other species of warm-blooded animal (Toxoplasma gondii) - when automated via the Crocodile miniWorkstation.

The one health concept
In this exclusive interview with The Scientists' Channel, Eric Sellal shares insights into Biosellal´s work developing real-time PCR and ELISA methods for COVID-19 detection. Here he discusses the "one health" concept, which aims to bring human and animal health

research together to achieve a harmonized approach for understanding and control of diseases. Watch the video to learn more about how the integration of the Zoom HT Microplate Washer is instrumental in ensuring quality and throughput in Biosellal's ELISA production line.

Clinical testing



Anti-SARS-CoV-2 antibody detection
Leveraging ELISA for the detection of antibodies against the SARS-CoV-2 virus can provide clinical and research labs with higher throughput, greater sensitivity, and more readily interpreted results than achieved

by alternative tests such as lateral flow. In this application note, learn how the SARS-CoV-2 IgG kit from Virotech Diagnostics can be automated by the Crocodile miniWorkstation to reduce hands-on time, mitigate human error, and ensure assay consistency.

ELISA automation for Alzheimer's research
Cleavage products of the amyloid precursor protein (APP), such as APP ΔC31, play an important role in the pathogenesis of Alzheimer's disease. This application note outlines a convenient and easy-to-use method

for the complete automation of a colorimetric, immunometric immunoassay kit capable of sensitive APP ΔC31 detection in human cell lysate and cerebral spinal fluid samples. With this method, researchers can simply insert a microplate including samples and standards, choose the assay protocol, and walk away, with results generated in just two hours. Explore the protocol step by step, in this video.

Food testing

Reliable detection of aflatoxin in milk
The detection of aflatoxin M1 - a hepatotoxic and carcinogenic mycotoxin – in milk and its derivatives is an important and routine step for many food testing labs. Explore this application note to find out how the MaxSignal® Aflatoxin M1 ELISA Test Kit was automated using the Crocodile 5-in-one ELISA miniWorkstation.

Food allergen testing with a reduced footprint
Bovine serum albumin (BSA) is a common allergen found in milk and beef and can be used to assess foodstuff composition in quality control settings. In this application note, learn how the Crocodile miniWorkstation can be used to determine BSA in food samples, achieving the same functionality while occupying a fraction of the footprint, producing good test-retest reliability and reproducibility, and fully satisfying the required diagnostic sensitivity and specificity.

Optimize your experiment: Find out how to achieve a highly reproducible and accurate ELISA »

Troubleshooting guide: Explore possible solutions to the most common ELISA problems »

Addressing tedious washing and plate-coating protocols

During ELISA, washes are repeated between each step to remove unbound materials and excess liquid is removed to avoid dilution of the solution at each stage. Manually performing washing steps can be tedious, time intensive and, if not performed adequately, lead to high background signal or inconsistent replicates. To overcome this, specialized microplate washers such as the Zoom HT Microplate Washer can be used to achieve high-throughput plate washing, ensure uniformity, and improve assay performance.

With the addition of a dispense module, the Zoom Washer can also be transformed into an efficient washer-dispenser system, supporting ELISA coating applications. The dispense module ensures timely and accurate one or two-channel reagent dispensing with minimal dead volume, which is important for adding antibodies, antigens, and other valuable reagents onto well surfaces.

In this video, learn how the Zoom HT system can perform a triple wash of a 96-well microplate in just 17 seconds, minimize both dead volume during dispensing and residual volume following aspiration, and handle challenging reagents without clogging, making it an ideal solution for both ELISA processing and microplate coating.

Overall, the Crocodile miniWorkstation and Zoom HT Microplate Washer can provide robust, easy-to-use solutions to the challenges posed by manual ELISA assays, at no cost to their analytical sensitivity. By automating ELISA workflows, labs can increase sample throughput, reliability and reproducibility of results, and walkaway time, while reducing the risk of human error and freeing researchers from tedious, repetitive tasks. As demonstrated across the range of applications outlined in this eBook, these systems can therefore be attractive additions to ELISA workflows.

APPLICATION NOTE

AUTOMATION OF THE IDEXX MILK PREGNANCY TEST

Introduction

Accurate and timely detection of pregnancy in dairy cows is an essential component of today's reproductive management programs, as a high reproductive efficiency is a prerequisite for high life-time production from dairy animals. Laboratory methods for pregnancy determination using milk as starting sample have numerous advantages over traditional methods, such as rectal palpation or transrectal ultrasound, as they are safer, equally or less expensive, and do not require trained personnel or special equipment on-farm. Pregnancy-associated glycoproteins (PAGs) constitute a large family of glycoproteins expressed in the outer epithelial cell layer (chorion/trophectoderm) of the placenta of cows and other eutherian species. PAGs can be detected in milk of pregnant cows, thus providing a convenient, specific and sensitive method for pregnancy diagnosis.

The IDEXX Milk Pregnancy Test, for use in bovine milk samples, is an enzyme-linked immunoassay for the detection of PAGs in bovine milk as a marker for pregnancy from ≥ 35 days post-breeding. The test can be used ≥ 60 days post calving.

Material

- Crocodile ELISA MiniWorkstation (Titertek-Berthold)
- Milk Pregnancy Test (Part number 99-41209, IDEXX)
- Adhesive plate covers
- Precision micropipettes or multi-dispensing micropipettes, with suitable disposable tips
- Distilled or deionized water

Methods

All reagents were brought up to room temperature for 30 minutes prior to use. Wash Solution was prepared following the manufacturer's instructions.

Controls and samples were pipetted according to the manufacturer's instructions. 3 samples known to be positive, and 3 samples known to be negative, were used. All controls and samples were run in triplicate.

The plate should be tightly sealed with adhesive cover during incubations to avoid evaporation. Manual steps were included in the program of the Crocodile to allow the user to put or remove the sealing, as needed.

In order to reduce the amount of user intervention and improve automation, the assay was tested covering the plate only in the first incubation (2 h at 37°C) and leaving the plate uncovered in the other incubation steps (20-30 minutes at room temperature); no significant evaporation was observed, and the assay performed as expected.

The Crocodile ELISA miniWorkstation was programmed with the steps summarized in Table 1.

Results

For the assay to be valid, the Positive Control mean minus the Negative Control mean must be greater than or equal to 0.500, and the Negative Control mean must be less than or equal to 0.200. Both criteria were fulfilled running the assay in the Crocodile ELISA miniWorkstation.

Pregnant or open (not pregnant) status is determined by the corrected OD values (S-N: OD of the Sample minus OD of the Negative Control) for each sample: if the S-N value is less than 0.100, the animal is considered not pregnant (open); if it is equal to or greater than 0.250, the animal is considered pregnant; and if is less than 0.250 but greater than or equal to 0.100, the animal should be re-checked to confirm pregnancy status. All negative samples were determined as negative, and all positive samples were determined as positive. There were no false positives or false negatives. Results are summarized in Figure 1:

ID	OD	S-N	Result
Negative Control	0,136	0,000	
Positive Control	1,992	1,856	
Negative Sample #1	0,170	0,034	NEGATIVE
Positive Sample #1	2,129	1,993	POSITIVE
Positive Sample #2	1,233	1,097	POSITIVE
Positive Sample #3	3,717	3,581	POSITIVE
Negative Sample #2	0,099	-0,037	NEGATIVE
Negative Sample #3	0,106	-0,030	NEGATIVE

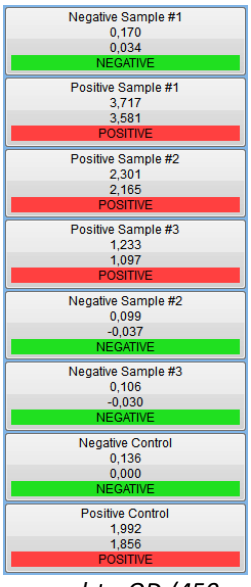


***Figure 1**. Left: Table displaying the results for controls and samples; OD values correspond to OD (450 nm) - OD (620 nm). Data shown are averages of triplicate measurements. Right panel: screenshot of data reduction software (MikroWin).*

Summary:

The assay fulfilled both validation conditions and all positive and negative samples were correctly determined. The assay procedure is simple and involves only the addition of controls and samples, while the instrument is processing all necessary dispense, wash, incubation and reading steps. The addition of manual steps in the Crocodile Control Software allows the user to remove the cover when required, thus providing an easy and convenient integration of manual and automated steps. In consequence, the Crocodile ELISA miniWorkstation, in combination with the MikroWin data reduction software, provides a convenient and easy-to-use method to automate the IDEXX Milk Pregnancy Test.

Acknowledgements:

Samples kindly provided by Franziska Breitenwieser (Milchprüfring Baden-Württemberg)

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Table 1.1. Summary of steps programmed in the Crocodile Control Software

#	Step name	Description and parameters
1	**Incubator ON**	**Incubation** Incubator On, Temperature: 37°C
2	**Incubator heat up**	**Manual** "Insert plate when the incubator reaches 37°C and press Continue", Duration: 00:10:00, Mode: User Continue, Position: Insert Position
3	**Sample Incubation**	**Shaking** For 02:00:00, at Incubator, with 1 mm Amplitude at 5 Hz
4	**Incubator OFF**	**Incubation** Incubator Off
5	**Remove adhesive cover**	**Manual** "Please remove cover", Duration: 00:02:00, Mode: user continue, Position: Insert position, Alarm Notification: sound of choice, 0 %
6	**Wash Solution priming**	**Washing** Method: Prime Washer, Wash Solution Inlet: 1, Cycles: 3, Volume: 800 µL
7	**Wash**	**Washing** Method: Standard, Wash Solution Inlet: 1, Cycles: 4, Volume: 300 µL, Delay: 1 s, Wait: 200 ms, Dispenser Depth: 1300 (Plate Offset: -50), Aspiration Depth: 2910* (Plate Offset: 20), Sweep: 4 mm @ 2 mm/s
8	**Aspiration**	**Washing** Method: Aspirate Only, Cycles: 1, Delay: 1s, Wait: 500 ms, Dispenser Depth: 1300 (Plate Offset: -50), Aspiration Depth: 2920* (Plate Offset: 20), Sweep: 4 mm @ 2 mm/s
9	**Detector priming**	**Dispensing** Volume: 850 µL, Inlet: 1, Method: Priming
10	**Detector distribution**	**Dispensing** Volume: 100 µL, Inlet: 1, Method: Standard
11	**Mix**	**Shaking** For 00:00:10 at Shaker Position with 1 mm Amplitude at 5 Hz
12	**Detector incubation**	**Manual** Message: "Incubating at Room Temperature", Duration: 00:30:00, Mode: Auto Continue, Position: Insert Position
13	**Wash**	**Washing** Method: Standard, Wash Solution Inlet: 1, Cycles: 4, Volume: 300 µL, Delay: 1 s, Wait: 200 ms, Dispenser Depth: 1300 (Plate Offset: -50), Aspiration Depth: 2910* (Plate Offset: 20), Sweep: 4 mm @ 2 mm/s
14	**Aspiration**	**Washing** Method: Aspirate Only, Cycles: 1, Delay: 1s, Wait: 500 ms, Dispenser Depth: 1300 (Plate Offset: -50), Aspiration Depth: 2920* (Plate Offset: 20), Sweep: 4 mm @ 2 mm/s

* Aspiration depth may have to be optimized for individual Crocodile instruments

>> **Continues in next page**

Table 1.2. Summary of steps programmed in the Crocodile Control Software.

#	Step name	Description and parameters
15	**Conjugate priming**	**Dispensing** Volume: 850 µL, Inlet: 2, Method: Priming
16	**Conjugate distribution**	**Dispensing** Volume: 100 µL, Inlet: 2, Method: Standard
17	**Mix**	**Shaking** For 00:00:10 at Shaker Position with 1 mm Amplitude at 5 Hz
18	**Conjugate incubation**	**Manual** Message: "Incubating at Room Temperature", Duration: 00:30:00, Mode: Auto Continue, Position: Insert Position
19	**Wash**	**Washing** Method: Standard, Wash Solution Inlet: 1, Cycles: 4, Volume: 300 µL, Delay: 1 s, Wait: 200 ms, Dispenser Depth: 1300 (Plate Offset: -50), Aspiration Depth: 2910* (Plate Offset: 20), Sweep: 4 mm @ 2 mm/s
20	**Aspiration**	**Washing** Method: Aspirate Only, Cycles: 1, Delay: 1s, Wait: 500 ms, Dispenser Depth: 1300, Aspiration Depth: 2920*, Sweep: 4 mm @ 2 mm/s
21	**Substrate priming**	**Dispensing** Volume: 850 µL, Inlet: 3, Method: Priming
22	**Substrate distribution**	**Dispensing** Volume: 100 µL, Inlet: 3, Method: Standard
23	**Mix**	**Shaking** For 00:00:10 at Shaker Position with 1 mm Amplitude at 5 Hz
24	**Substrate incubation**	**Manual** Message: "Incubating at Room Temperature", Duration: 00:20:00, Mode: Auto Continue, Position: Insert Position
25	**Stop solution priming**	**Dispensing** Volume: 850 µL, Inlet: 4, Method: Priming
26	**Stop solution distribution**	**Dispensing** Volume: 100 µL, Inlet: 4, Method: Standard
27	**Mix well**	**Shaking** For 00:00:10 at Shaker Position with 1 mm Amplitude at 5 Hz
28	**Measure**	**Reading** Reference Measurement, Filter 1: 450 nm, Filter 2: 620 nm

* Aspiration depth may have to be optimized for individual Crocodile instruments

APPLICATION NOTE

ANALYTICAL SENSITIVITY OF SAMPLES TESTED WITH THE CROCODILE MINIWORKSTATION IN COMPARISON TO HAND PROCESSING USING PRIOCHECK® TOXOPLASMA AB PORCINE ELISA FROM PRIONICS AG

Introduction:

An ELISA protocol contains typical routine steps such as the addition of different reagents, incubations, microplate washing steps and OD-measurements. Laboratory benches are often cluttered by large instruments or multiple instruments required for assay procedure. Lack of space negatively affects productivity. The new Crocodile miniWorkstation combines the functionality of five individual instruments in a footprint the size of a standard stand-alone ELISA reader. This note will demonstrate the diagnostic sensitivity and specificity of the system using the ELISA test PrioCHECK® Toxoplasma Ab porcine (Prionics AG).

Toxoplasmosis is caused by the protozoan parasite Toxoplasma gondii, which belongs to the family of Sarcocystiidae. Toxoplasma infections are widespread in humans and many other species of warm-blooded animals. Occurrence is worldwide, however, the prevalence in human and animal populations varies greatly among countries.

Materials:

Instrumentation:	Crocodile miniWorkstation Single channel pipette (20-200 µl)
Instrumentation for the manual test:	Tecan HydroFlexTM Tecan SunriseTM
Reagents:	PrioCHECK® Toxoplasma Ab porcine. Product N.: 7610230; Lot TX100401M; exp Date April 30th 2011 Demineralized water
Consumables:	Solution reservoirs Pipette tips

Method:

Test procedure
Analytical sensitivity is addressed by diluting positive samples and evaluating the dilution at which the samples can still be detected as positive. To determine the analytical sensitivity, two positive samples were diluted using serial dilutions from undiluted to 1:64. Both serial dilutions were run in triplicates using the Crocodile miniWorkstation, in parallel, duplicates were tested manually; a Tecan reader was used to measure the OD values from the manually processed samples.

Assay principle

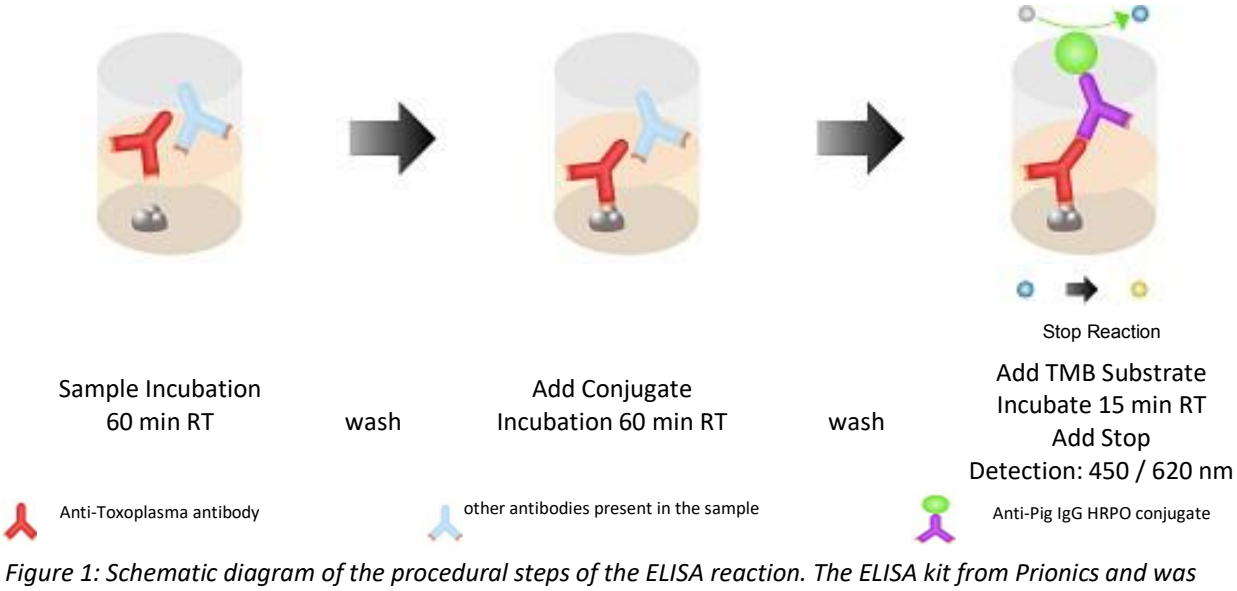

Figure 1: Schematic diagram of the procedural steps of the ELISA reaction. The ELISA kit from Prionics and was performed as described in the kit instructions. The absorbance of each well was measured at 450 nm with a reference measurement at 620 nm.

The PrioCHECK® Toxoplasma Ab porcine is an indirect ELISA for the detection of antibodies against Toxoplasma gondii. The test follows a short four step ELISA protocol. Test samples are incubated in plates coated with Toxoplasma antigen at room temperature. Plates are then washed and an enzyme labelled anti-pig antibody is added. The signal is measured and if colour develops the sample is positive for anti-Toxoplasma antibodies.

Reagent and sample dilution were performed as described in the test procedure document.
The assay program for the **Crocodile** is listed on the last page.

Results:

Validation criteria:

The mean OD450 of the Positive Controls must be >1,2
The mean percentage of positivity of the weak Positive Controls must be > 35%
The mean OD450 of the Negative Controls must be < 0,15

sample ID	Crocodile	manual
PC	2,32	2,16
PC	2,3	1,99
wPC	1,01	0,92
wPC	1,01	0,91
NC	0,09	0,08
NC	0,1	0,08

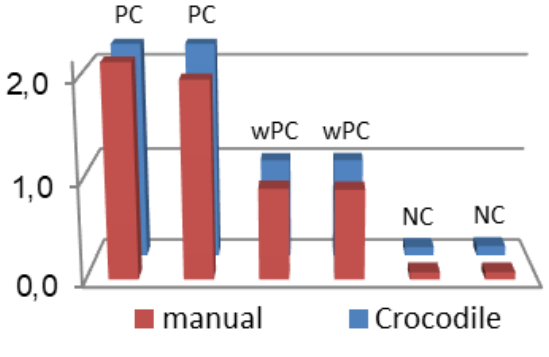

Figure 2: Positive (PC), Negative (NC) and weak Positive (wPC) Controls were determined in duplicates. OD is $OD_{450-620}$. The picture shows Positive (PC), Negative (NC) and weak positive (wPC) Controls in relation to the measured $OD_{450-620}$ values.

$OD_{450-620}$	Crocodile		manual	
Dilution Factor	**Vial 1**	**Vial 2**	**Vial 1**	**Vial 2**
1:1	2,11	2,08	2,06	1,97
1:2	1,30	1,25	1,23	1,17
1:4	0,68	0,65	0,66	0,63
1:8	0,39	0,38	0,38	0,36
1:16	0,22	0,22	0,22	0,21
1:32	0,15	0,15	0,14	0,14
1:64	0,12	0,10	0,11	0,10
NC	0,09	0,10	0,08	0,07

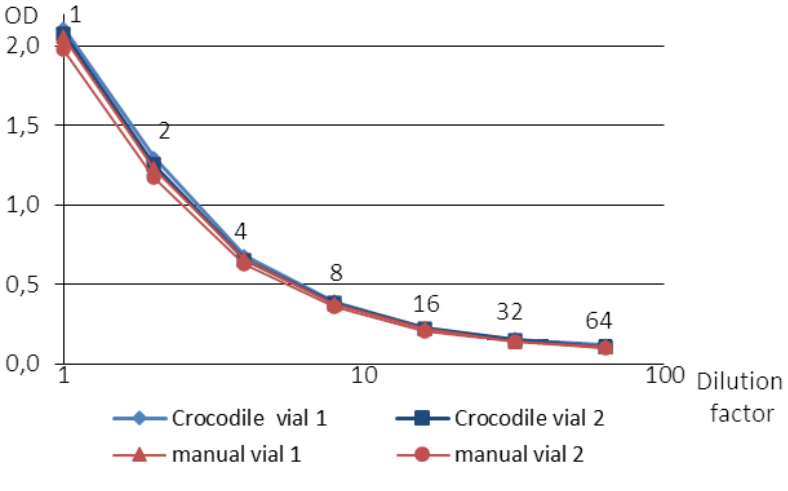

Figure 3: The table and the graph show the average results of $OD_{450-620}$ values of two different dilution series measured in triplicates (Crocodile) and duplicates (manual).

Summary:

The mean $OD_{450-620}$ values of samples analyzed with the **Crocodile** are comparable to the $OD_{450-620}$ values of samples processed manually.
Using the PrioCHECK® Toxoplasma Ab porcine a mean OD_{450} of < 0,15 is defined as negative. The Crocodile miniWorkstation was able to detect a dilution of 1:32 with a mean $OD_{450-620}$ value of 0,15, whereas for the manually processed samples the mean $OD_{450-620}$ value in this dilution was 0,14.

Conclusions:

Using the Crocodile miniWorkstation for the assay procedure is extremely simple and involves only the addition of the samples.
This application note demonstrates that the analytical sensitivity of the PrioCHECK® Toxoplasma Ab porcine using the Crocodile miniWorkstation is equivalent to analytical sensitivity achieved by manual processing of the test.

Acknowledgement:

We wish to thank Prionics AG for the supply of reagents and Pascal Schacher, Mario Pürro and Daniel Zwald for their technical support.

www.prionics.com

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Assay Program:

#	Step Name	Description
1	**Incubate 1**	**Incubation** Incubator On Temperature: 22.3 °C Duration: 01:00:00
2	**Prime Wash 1**	**Washing** Method: Prime Dispenser Wash Solution Inlet: 1 Cycles: 7 Volume: 1000ul Dispenser Depth: 1300 Aspiration Depth: 1300 Count: 96
3	**Wash 1**	**Washing** Method: Soak Wash Wash Solution Inlet: 1 Wash Fluid Cycles: 4 Volume: 300ul Dispenser Depth: 1500 Aspiration Depth: 3000 Sweep: 5mm @ 1mm/s Count: 96
4	**Prime Conjugate 2**	**Dispensing** Volume 800ul Inlet 2 Label "Conjugate " Method: Priming Count: 1
5	**Conjugate 2**	**Dispensing** Volume 100ul Inlet 2 Label "Conjugate " Method: Standard Count: 96
6	**Incubate 2**	**Incubation** Incubator On Temperature: 22.3 °C Duration: 01:00:00
7	**Wash 2**	**Washing** Method: Soak Wash Wash Solution Inlet: 1 Wash Fluid Cycles: 4 Volume: 300ul Dispenser Depth: 1500 Aspiration Depth: 3000 Sweep: 5mm @ 1mm/s Count: 96
8	**Manual 1**	**check for remaining liquid** Duration: 00:02:00 Mode: Auto Continue Position: Insert Position
9	**Prime TMB 3**	**Dispensing** Volume 800ul Inlet 3 Label "TMB " Method: Priming Count: 1
10	**TMB 3**	**Dispensing** Volume 100ul Inlet 3 Label "TMB " Method: Standard Count: 96
11	**Incubate 3**	**Incubation** Incubator On Temperature: 22.3 °C Duration: 00:15:00

#	Step Name	Description
12	**Prime Stop 4**	**Dispensing** Volume 800ul Inlet 4 Label "Stop " Method: Priming Count: 1
13	**Stop 4**	**Dispensing** Volume 100ul Inlet 4 Label "Stop " Method: Standard Count: 96
14	**Shake 1**	**Shaking** for 00:01:00 at Shaker Position with 1mm Amplitude at 20Hz
15	**Measure 1**	**Reading** Reference Measurement Filter 1: 450 nm (Pos:2) Filter 2: 620 nm (Pos:4) Count: 96

APPLICATION NOTE

AUTOMATION OF THE VIROTECH SARS-CoV-2 IgG ELISA KIT

Abstract

The SARS-CoV-2 virus is the causative agent of COVID-19, a disease that has led to a global pandemic of unprecedented proportions. The detection of antibodies against SARS-CoV-2 in the blood of individuals and the associated infections is very valuable for both, research and diagnostics. In the following, we describe the automation of the Virotech SARS-CoV-2 IgG ELISA with the Crocodile 5-in-one ELISA miniWorkstation, which offers a convenient solution for the detection of antibodies against SARS-CoV-2.

Introduction

COVID-19 (coronavirus disease 2019) is an infectious disease caused by SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2). This new virus was first detected in December 2019 and has since spread globally. The resulting pandemic has caused severe global socioeconomic disruption, including the largest global recession since the Great Depression of the 1930s [1]. While in many cases the disease results in mild symptoms, several possible complications can lead to death. The estimated global death-to-case ratio of COVID-19 is 2.8% [2]. At the time of publication of this Application Note, there are neither vaccines nor specific antiviral treatments available for COVID-19, and all aspects of the disease are therefore subject to intensive research.

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Detecting antibodies against SARS-CoV-2 in the blood of individuals (meaning that the individual has been exposed to the virus) is very valuable, not only as a diagnostic tool, but also for research and epidemiological studies. The methods most frequently used to detect such antibodies are rapid tests, based on lateral flow, and ELISA (enzyme-linked immunosorbent assay). Rapid tests are quick (10-20 minutes) and can be performed at the point of care (POC). ELISA tests, on the other hand, have to be performed in a laboratory and need more time (typically 1-3 hours), but are easier to interpret, have higher throughput, and in some cases can be used quantitatively, providing more information about the immunity status of the subject.

ELISA assays have many advantages, but the protocols are repetitive and time-consuming. This makes automation highly desirable. The Crocodile 5-in-one ELISA miniWorkstation offers a complete automation solution for low- to medium-throughput laboratories.

Virotech Diagnostics offers 3 different kits for the qualitative detection of antibodies against SARS-CoV-2, respectively for IgG, IgM and IgA. The tests are highly specific and reliable. This Application Note reviews the automation of the Virotech SARS-CoV-2 IgG ELISA with the Crocodile ELISA miniWorkstation and provides optimized protocols.

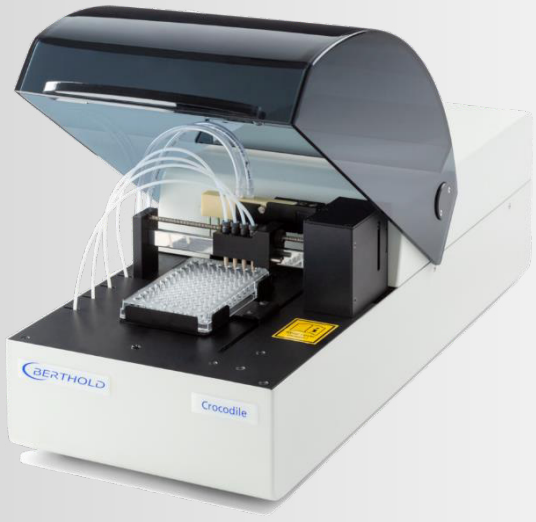
SINGLE PLATE ELISA

WALKAWAY AUTOMATION

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

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

- All-in-One ELISA automation
- Ultra-compact footprint saving precious bench space
- User-friendly open system software for maximum assay flexibility
- Plug & play setup



Materials

- Crocodile 5-in-one ELISA miniWorkstation LB 925 (Berthold Technologies).
- Virotech SARS-CoV-2 IgG ELISA kit (Order number EC123G00).
- Precision micropipettes or multi-dispensing micropipettes, with suitable disposable tips.
- Various plastic and glass containers for the preparation of dilutions.
- 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.

The Crocodile ELISA miniWorkstation was programmed with the steps summarized in **Table 1**.

Blank, controls (Positive, Negative and Calibrator) and samples were pipetted according to the manufacturer's instructions. A total of 44 patient samples were tested.

Results were calculated and interpreted according to the manufacturer's instructions. Briefly:

1. OD value of the Blank was subtracted from the values of all controls and samples
2. The Cut-off value was calculated.
3. OD units of controls and samples were converted to Virotech Units (VU).
4. Samples were classified as follows:
   - VU < 9.0: Negative
   - VU 9.0-11.0: Doubtful (must be repeated)
   - VU > 11.0: Positive

#	Step name	Description and parameters
1	**Sample Incubation**	**Incubation** Incubator ON, Temperature: 37° C, Duration: 00:30:00
2	**Wash Solution priming**	**Washing** Method: Prime Washer, Wash Solution Inlet: 1, Cycles: 6, Volume: 1000 µL
3	**Wash**	**Washing** Method: Soak Wash, Wash Solution Inlet: 1, Cycles: 4, Volume: 300 µL, Delay: 1 s, Wait: 200 ms, Dispenser Depth: 1593 (Plate Offset: -27), Aspiration Depth: 2930* (Plate Offset: 36), Sweep: 4 mm @ 2 mm/s
4	**Conjugate priming**	**Dispensing** Volume: 1000 µL, Inlet: 1, Method: Priming
5	**Conjugate addition**	**Dispensing** Volume: 100 µL, Inlet: 1, Method: Standard
6	**Conjugate incubation**	**Incubation** Incubator ON, Temperature: 37° C, Duration: 00:30:00
7	**Wash**	**Washing** Method: Soak Wash, Wash Solution Inlet: 1, Cycles: 4, Volume: 300 µL, Delay: 1 s, Wait: 200 ms, Dispenser Depth: 1593 (Plate Offset: -27), Aspiration Depth: 2930* (Plate Offset: 36), Sweep: 4 mm @ 2 mm/s
8	**Substrate priming**	**Dispensing** Volume: 1000 µL, Inlet: 3, Method: Priming
9	**Substrate addition**	**Dispensing** Volume: 100 µL, Inlet: 3, Method: Standard
10	**Substrate incubation**	**Incubation** Incubator ON, Temperature: 37° C, Duration: 00:30:00
11	**Turning incubator Off**	**Incubation** Incubator Off
12	**Stop solution priming**	**Dispensing** Volume: 1000 µL, Inlet: 4, Method: Priming
13	**Stop solution addition**	**Dispensing** Volume: 50 µL, Inlet: 4, Method: Standard
14	**Mixing**	**Shaking** For 00:00:10 at Incubator with 2 mm Amplitude at 5 Hz
15	**Measurement**	**Reading** Reference Measurement, Filter 1: 450 nm, Filter 2: 620 nm
	**Depth settings have to be optimized for each individual Crocodile unit*	

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

Results

All validation criteria for the Blank, Positive, Negative and Calibrator controls were met. In parallel, the assay was processed manually (using a multichannel pipette and manual washer) with the same samples.

The results were analyzed with the optional MikroWin software, providing convenient color-coded classification of the samples (see Figure 1). Of the 44 samples tested, 35 were classified as negative (marked green) and 9 as positive (marked red); no sample was classified as doubtful. No differences were found between the assay analyzed on the Crocodile and the manually processed control.

Figure 1. Results obtained for the controls and patient samples tested, calculated and classified using the MikroWin software. Each well position contains the following information (top to bottom):

1. Sample ID
2. Calculated VU
3. Classification

Summary

The Crocodile 5-in-one ELISA miniWorkstation can significantly facilitate the performance of the Virotech SARS-CoV-2 IgG ELISA assay: 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. This greatly reduces hands-on time and allows the staff of the laboratory to concentrate on other tasks. The obtained data met the validation criteria of the kit, and no differences were found between the assay analyzed on the Crocodile and the manually processed control. Finally, the optional MikroWin software provided a convenient way to interpret the results.

Acknowledgements

Experiments were performed in the laboratories of ZAKlab GmbH in Balingen, Germany.

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APPLICATION NOTE

AUTOMATION OF THE ENZO APP ΔC31 ELISA KIT

Introduction

Unlike the β-amyloid and tau fibril formation pathways leading to amyloid plaques and tangles, APP ΔC31 reveals an alternative, unique pro-apoptotic mechanism leading to Alzheimer's disease. APP ΔC31 is the stable amyloid precursor protein fragment created from a caspase cleavage event of the APP695 molecule at Asp664 leaving a smaller 31-residue intracellular fragment. Both the APP ΔC31 and 31-residue fragments are pro-apoptotic and are present in 4-fold greater levels in Alzheimer's disease patients. Given that the smaller 31-residue fragment has a short half-life and is difficult to measure, the use of this APP ΔC31 ELISA provides for the first time a sensitive research tool to measure the levels of the APP caspase cleavage from tissue, biological fluids, and cells.

The APP ΔC31 ELISA kit is a complete, colorimetric, immunometric immunoassay kit for the quantitative determination of human APP ΔC31 in cell lysate, serum, plasma, and cerebral spinal fluid samples with results in just 2 hours.

Materials

- Crocodile miniWorkstation (Berthold Technologies)
- MikroWin module, quantitative and qualitative data reduction package (Titertek-Berthold)
- APP ΔC31 ELISA kit (#ADI-900-227, Enzo)
- ddH_20, pipette and tips, sample tubs, Vortex mixer

Methods

All reagents were brought up to room temperature for 30 minutes prior to use. According to manufacturer instructions wash buffer and 8 standards were prepared.

We changed the first steps in the assay procedure to have it more convenient for automation purposes: At the beginning 50µl of samples and standards in duplicate were transferred to the assay plate. For standard 0 (S0) 50µl of assay buffer was pipetted.

Automation with the Crocodile miniWorkstation was performed as shown on table 1 starting in dispensing antibody.

Adjust aspiration depth in your assay setup as to avoid cross-contamination through direct contact between aspiration needles and well surfaces.

The plate was read at 450 nm absorbance. After blanking the reader against the average blank OD, a standard curve was calculated by using MikroWin and fitted with four parameter algorithms.

Results

Standard	Concentration (pM)	OD average (Minus Blank OD)
Blank	-	0
S0	0	-0.002
S1	1500	2.135
S2	750	1.242
S3	375	0.602
S4	187.5	0.293
S5	93.75	0.139
S6	46.88	0.091
S7	23.44	0.036
S8	11.72	0.020

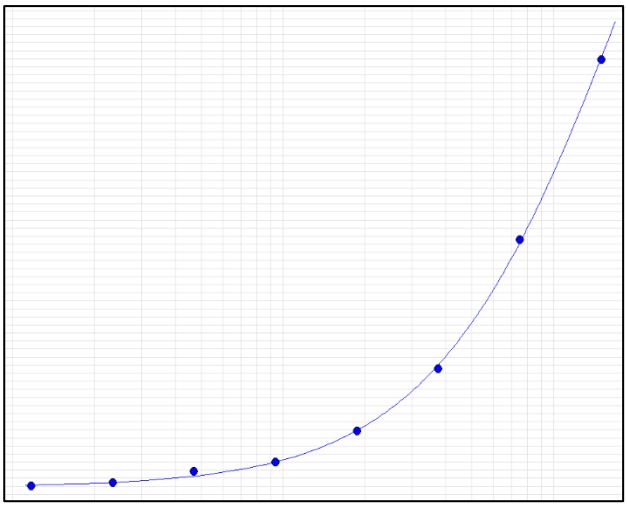

Figure 1. Standard curve fitted with four parameter algorithm. Y-axis linear, X-axis logarithmic.

Conclusion

The standard curve showed excellent fitting, so Crocodile provides a convenient and easy-to-use method for the automation of the Enzo APP ΔC31 ELISA kit. The assay procedure is extremely simple and involves only the addition of standards and controls while the instrument is processing all necessary dispense, wash, incubation and reading steps.

Changing the first steps in the assay procedure (pipetting antibody and standards/samples) doesn't affect the assay results at all.

Acknowledgement

Special thanks to Miriam Cortes-Caminero and Erica Brooks from Enzo for their support.

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Table 1: Assay program with Crocodile Control Software

#	Step Name	Description
1	**Prime Antibody**	**Dispensing** Volume 1000 µl Inlet 1 Label “Antibody” Method: Priming Well Count: 1
2	**Dispense Antibody**	**Dispensing** Volume 50ul Inlet 1 Label “Antibody” Method: Standard Well Count: 20
3	**Incubate 1**	**Shaking** for 01:00:00 at Incubator with 2mm Amplitude at 10 Hz
4	**Wash 1**	**Washing** Method: Standard Wash Solution Inlet: 1 Wash buffer Cycles: 3 Volume: 300 µl Delay: 2s Wait: 500ms Dispenser Depth: 1300 (Plate Offset 50) Aspiration Depth 2725 (Plate Offset 20) Sweep 5mm @1mm/s Well Count 96
5	**Prime Conjugate**	**Dispensing** Volume 1000 µl Inlet 2 Label “Conjugate” Method: Priming Well Count: 1
6	**Dispense Conjugate**	**Dispensing** Volume 100ul Inlet 2 Label “Conjugate” Method: Standard Well Count: 20
7	**Incubate 2**	**Shaking** for 00:30:00 at Incubator with 2mm Amplitude at 10 Hz
8	**Wash 2**	**Washing** Method: Standard Wash Solution Inlet: 1 Wash buffer Cycles: 3 Volume: 300 µl Delay: 2s Wait: 500ms Dispenser Depth: 1300 (Plate Offset 50) Aspiration Depth 2725 (Plate Offset 20) Sweep 5mm @1mm/s Well Count 96
9	**Prime Substrate**	**Dispensing** Volume 1000 µl Inlet 3 Label “Substrate” Method: Priming Well Count: 1
10	**Dispense Substrate**	**Dispensing** Volume 100ul Inlet 3 Label “Substrate” Method: Standard Well Count: 20
11	**Incubate 3**	**Shaking** for 00:30:00 at Incubator with 2mm Amplitude at 10 Hz
12	**Prime Stop**	**Dispensing** Volume 1000 µl Inlet 4 Label “Stop” Method: Priming Well Count: 1
13	**Dispense Stop**	**Dispensing** Volume 100ul Inlet 4 Label “Stop” Method: Standard Well Count: 20
14	**Measure**	**Reading** Single Wavelength Filter 1: 450nm (Pos:2) Well Count: 96

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.

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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).

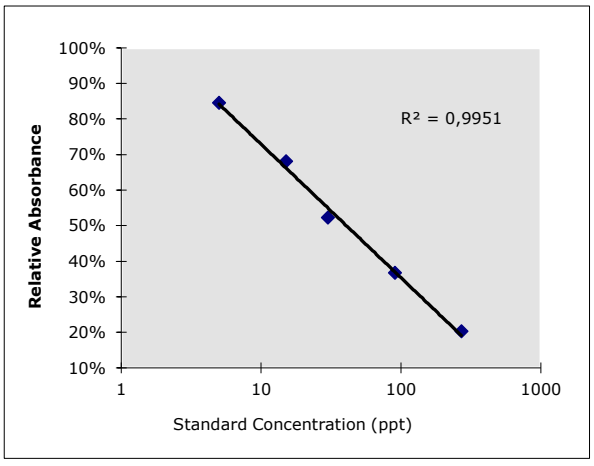

Figure 1. *Representative aflatoxin M1 standard curve obtained with the Crocodile.*

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

Experiments were performed in the laboratories of Generon S.p.A. by Luca Pacchioni.

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APPLICATION NOTE

DIAGNOSTIC SENSITIVITY AND SPECIFICITY OF SAMPLES TESTED WITH THE CROCODILE MINIWORKSTATION AND BOVINE SERUM ALBUMIN ELISA KIT FROM SEDIUM R&D

Introduction:

An ELISA protocol contains typical routine steps like the addition of different reagents or incubations, microplate washing and OD-measurement. The usage of physically large instruments or the requirement for multiple instruments to perform assay functions leads to a crowded and cluttered work area that decreases productivity. The new Crocodile miniWorkstation provides the same functionality as five individual instruments in a footprint only slightly larger than a standard stand-alone reader. This note will show the diagnostic sensitivity and specificity of the system using the ELISA test Bovine Serum Albumin ELISA kit (Sedium R&D).

Beef is a significant component of food for a considerable part of the word population. The prevalence of allergies to BSA in no doubt is less than that to other food allergens. However, allergologists do recommend the determination of BSA as a foodstuff allergen. Not negligible is also the fact that the presence or absence of BSA in foodstuff can be exploited as the parameter utilisable for assessment/evaluation of the specified foodstuff composition as well as of observance of the produce specified for the foodstuff production.

Description:
Bovine Serum Album ELISA kit is a competitive immunoassay for the determination of the bovine serum album (BSA) in the food samples. The kit results are always negative for food matrices which naturally do not contain BSA.

Materials:

Instrumentation:	Crocodile miniWorkstation
	Single channel pipette (20-200 µl)
Reagents:	Bovine Serum Albumin ELISA Kit Cat.No.: FA 00308/48; Lot 004;
	exp Date: August 31st, 2011
	Demineralized water
Consumables:	Solution reservoirs
	Pipette tips

Method:

Definitions and test procedure:

- Reliability (Test-retest reliability) is the variation in measurements taken by a single person or instrument on the same item and under the same conditions. For this purpose, four standard and two control samples were tested in three independent runs as duplicates to determine the CV%.

- Reproducibility is defined as the ability to independently reproduce results e. g. by testing in different plates on different days. For this purpose, 21 samples were tested in two independent runs to determine the correlation coefficient “r” and the coefficient of determination “r²”.

- Diagnostic sensitivity is defined as the ability to correctly identify BSA contaminated samples whereas diagnostic specificity is defined as the ability to correctly identify non-contaminated samples. To determine diagnostic sensitivity and specificity 21 samples were analysed using the Crocodile miniWorkstation. 9 of the used samples were spiked with BSA and 12 samples were confirmed negative samples. The limit of qualification for this test kit was described at 3,76 ppm.

- Reagent and Sample dilution was performed as described in the test procedure document. Controls and samples were determined in duplicates.

Assay principle:

In the wells of a microtiter plate, walls of which are coated with sheep anti-BSA antibody, the sample to be analysed, while combined with BSA conjugate, is homogenized with horse – radish peroxidase. During the step of incubation, BSA will be bound to the wells walls. BSA present in the sample and BSA bound in the conjugate will compete mutually for access to the binding sites present in limited number in the antibody against BSA. After a subsequent incubation step, the wells are washed and the peroxidase bound to the wells walls is detected by means of a chromogenic substrate (TMB), added to the system. Intensity of thus development coloration is inversely proportional to the BSA concentration in calibrators and analysed samples.
Reagent and Sample dilutions were performed as described in the test procedure document.

Results:

Reliability:
The standard deviation (SD) is the way of describing how dispersed a set of values is from the mean. The coefficient of variation (CV) is defined as the ratio of standard deviation to the mean. The CV is a standardization of the SD that allows comparison of variability of an assay. The inter-assay variance of the kit is described with 13,6% CV.

Sample	OD	OD	CV%
STD0	2,095	1,973	3,0
STD1	1,36	1,249	4,3
STD2	0,854	0,813	2,5
STD3	0,578	0,466	10,7
STD4	0,261	0,245	3,2

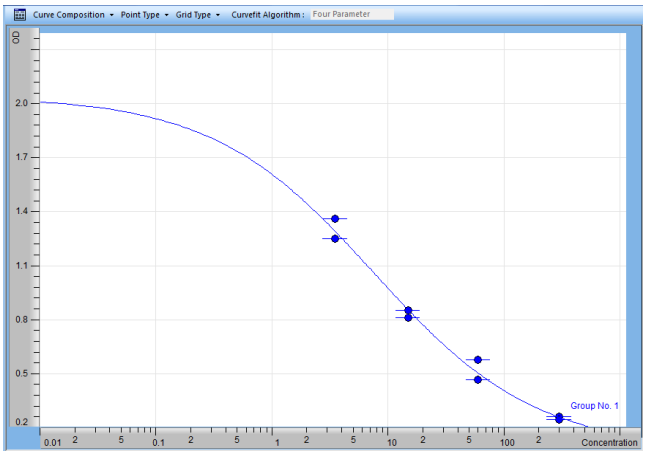
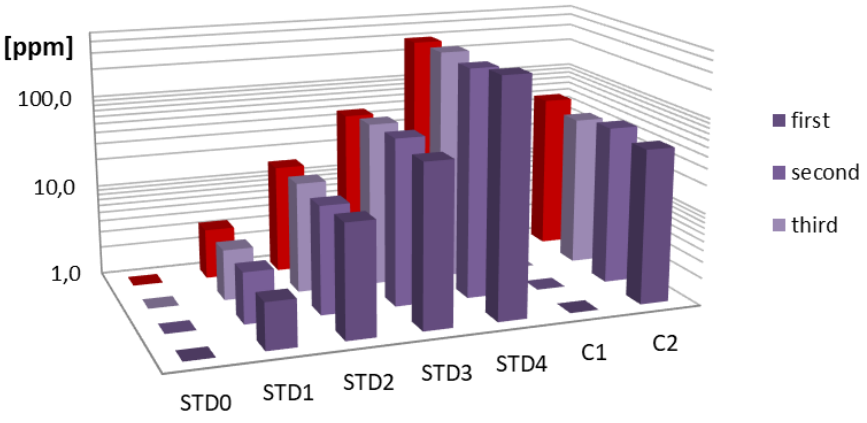


Figure 1: Standards were determined in duplicates. OD is OD450. The table shows the results for one run and the CV% for each sample. The diagram shows the related standard curve with Four Parameter Curvefit Algorithm using MikroWin 2010.



OD	first	second	third	Calibrator	CV%
STD0	0,0	0,0	0,0	0,0	0,0
STD1	3,2	3,6	3,5	3,5	6,6
STD2	16,4	14,7	15,7	15,0	5,5
STD3	56,1	63,2	59,4	50,0	6,0
STD4	353,4	296,2	317,0	300,0	9,0
C1	0,0	0,0	0,0	0,0	0,0
C2	46,5	51,9	43,1	50,0	9,4

Figure 2: Table and graph of the mean values of the calculated concentration from standard and control samples of 3 independent runs.

Validation criteria for reproducibility:

The linear correlation coefficient "r" measures the strength and the direction of a linear relationship between two measurements. A correlation between two independent tests greater than 0,8 is generally described as strong whereas a correlation less than 0,5 is generally described as weak.

The coefficient of determination "r²" denotes the strength of the linear association between two tests. This coefficient is a measure of how well a regression line represents the percentage of the data that is closest to the line of the best fit.

A perfect correlation between two measurements would be indicated with an r=1 and r^2=1.

Validation criteria for diagnostic sensitivity and specificity:

Diagnostic sensitivity is defined as the ability to correctly identify contaminated samples, whereas diagnostic specificity is defined as the ability to correctly identify non-contaminated samples.

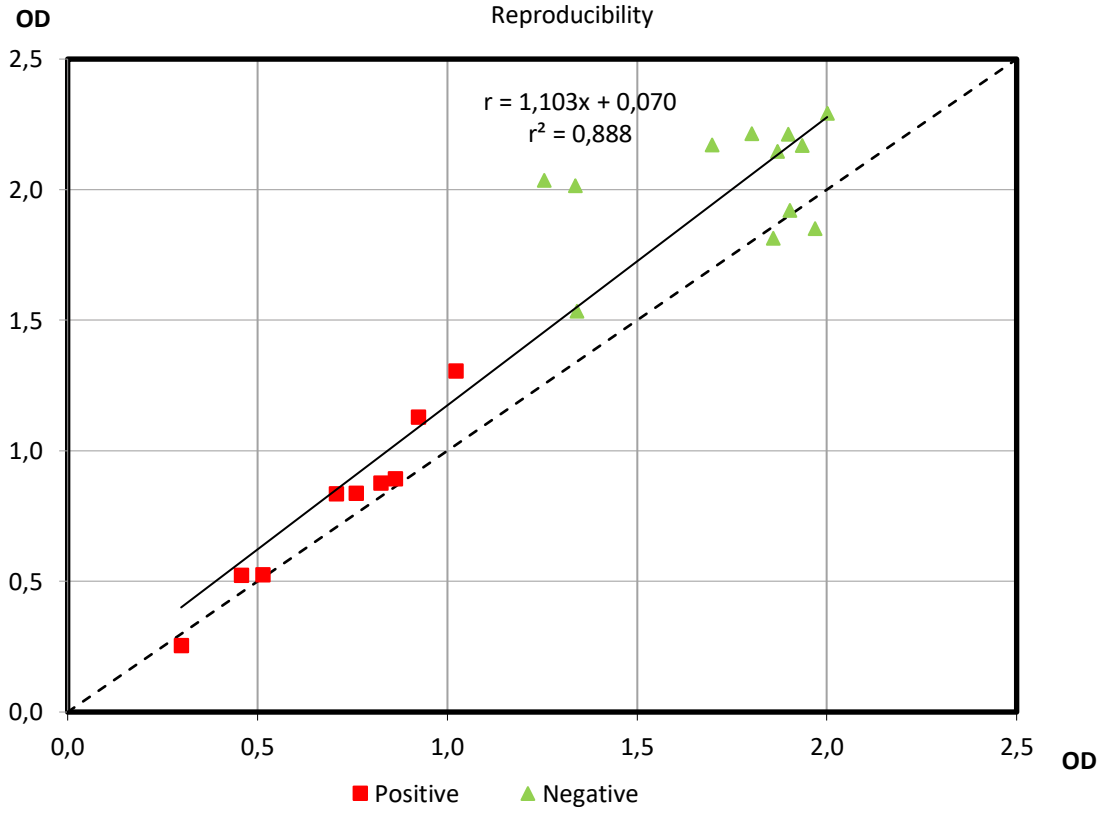


Figure 3: The graph shows the relation between the OD_{450} values of two independent tests containing results of 9 positive and 12 negative samples (samples with less than 3,76 ppm BSA concentration). The linear correlation coefficient "r" (r= 1,103x + 0,07) measures the strength and the direction of a linear relationship between both measurements. The coefficient of determination "r²" (r² = 0,888) denotes the strength of the linear association between both tests.

Summary:

To test the reliability of the assay processed by the Crocodile miniWorkstation, four standard and two control samples were tested in three independent runs in duplicates. The concentration was determined by the mean value of each data pair. The CV% of the resulting concentration for each sample of the three runs was calculated and compared with the nominal concentration. The inter-assay variance of the kit is described with 13,6% CV

To test the reproducibility of an assay, 21 samples were tested in two independent runs with the BSA ELISA kit from Sedium R&D. The linear correlation coefficient "r" was determined with r= 1,103x + 0,07 and the coefficient of determination "r^2" with r^2 = 0,888.

To test the diagnostic sensitivity and specificity of an assay processed by the Crocodile miniWorkstation, 9 positive (more than 3,76 ppm BSA) and 12 negative samples were tested in two independent runs with the BSA ELISA kit from Sedium R&D.

Conclusions:

Using the Crocodile for the assay procedure is extremely simple and involves only the addition of the samples.
The Crocodile miniWorkstation is excellent suitable for the performance of the BSA ELISA test. This Application note demonstrates that reliability, reproducibility and diagnostic sensitivity and specificity of the kit was fully achieved using the Crocodile miniWorkstation.

The test-retest reliability, determined with three independent measurements showed good CV%s in the range between 5,5 and 9,4. The determined concentrations have been very close to the estimated values.

The Crocodile miniWorkstation achieved a good reproducibility in OD450 measurements. This is demonstrated by the resulting correlation coefficient on "r" with r = 1,103x + 0,07 and the coefficient of determination "r^2"with r^2 = 0,888. A correlation between two independent tests greater than r = 0,8 is generally described as strong.

All samples with more than 3,76 ppm BSA were identified correctly as positive, showing the diagnostic sensitivity by using the combination Crocodile miniWorkstation and ELISA BSA kit from Sedium R&D.

Samples with less than 3,76 ppm BSA were identified correctly as negative, showing the diagnostic specificity by using the combination Crocodile miniWorkstation and ELISA BSA kit from Sedium R&D.

Acknowledgement:

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www.sedium-rd.cz/

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Optimize your ELISA experiments

There are several ways to optimize ELISA experiments to ensure that the assay will perform with high reproducibility and accuracy.

During optimization, parameters such as temperature and humidity must be kept constant to ensure standardized results. Below, we provide tips on how to optimize ELISA with regards to various ELISA assay components and well-to-well consistency.

Optimizing ELISA assay components

Typically, optimization of ELISA assay components (antibodies, buffers, samples) is performed by testing different solutions to identify the ideal working concentration for each component. For the capture antibody, dilutions in coating buffer ranging from 5 to 15 µg/ml for unpurified antibodies and 1 to 12 µg/ml for affinity-purified antibodies are prepared. Affinity-purified antibodies are recommended for optimal signal-to-noise ratio. Samples should be added in a high and a low concentration, reflecting the expected working range. For the detection antibody, a similar dilution setup can be performed in standard diluent, ranging from 1 to 10 µg/ml for unpurified antibodies and 0.5 to 5 µg/ml for affinity-purified antibodies.

There is a wide range of commercial blocking buffers to choose from, some of which contain BSA. The manufacturers of these buffers usually provide extensive optimization protocols. If the blocking buffer is not preformulated, try different concentrations of the protein, such as BSA.

Some types of samples can cause matrix effects, for instance, serum or plasma. These can be reduced by diluting the sample. Standard diluents should reflect the matrix of the sample as closely as possible and linearity of the dilutions be checked to ensure a good dynamic range.

Ensuring well-to-well consistency

ELISA is typically performed using multiwell plates. If you decide to pipette your ELISA manually, make sure that all your pipettors are well calibrated. To avoid inconsistent performance, it is good practice to visually inspect that the liquid level in the pipette tip and plate wells is at an identical level while pipetting your assay. For this, automated ELISA workstations such as Crocodile miniWorkstation can help to optimize well-to-well consistency and provide faster performance than achieved manually.

ELISA troubleshooting

When performing ELISA experiments, several problems can occur that may influence the results. The following table describes some of the most common problems and possible solutions.

Weak or no signal

Possible causes	Solution
Incorrect reagent storage	Double check if you have followed the recommendations of your kit manufacturer
Antibody stored at 4°C for several weeks or subjected to repeated freeze/thaw cycles	Use a fresh aliquot of antibody
Expired reagents	Do not use reagents that are past the expiration date
Incorrect dilutions prepared	Check if your calculations are correct. Use a higher concentration of detection reagent.
Incorrect assay setup	Repeat assay and ensure reagents have been prepared according to the protocol and are added in the correct order
Incorrect plate reader settings	Make sure your microplate reader settings reflect the recommended wavelength/filters
Not enough antibody used	Increase the concentration of your primary and/or secondary antibody
Wells damaged with pipette or washing tips	Be careful when dispensing and aspirating into and out of wells when pipetting manually. Recalibrate your automated plate washers so that the tips do not touch the bottom of the wells if required.

High background signal

Possible causes	Solution
Incorrect incubation temperature	Follow your kit manufacturer's recommendation or optimize the incubation temperature
Incorrect incubation times	Follow your kit manufacturer's recommendation or optimize the incubation time
Insufficient washing	If pipetting manually, invert plate on absorbent tissue and allow to completely drain to remove any residual fluid. Increasing the number of washing cycles may also be useful.
Substrate exposed to light	Perform substrate incubation in the dark
Reactions not stopped	Ensure you use stop solution as recommended in your protocol to avoid overdevelopment

Inconsistent replicates

Possible causes	Solution
Insufficient washing	If pipetting manually, invert plate on absorbent tissue and allow to completely drain to remove any residual fluid. Increasing the number of washing cycles may also be useful.
No plate-binding of capture antibody	Prepare both coating and blocking steps as recommended in the protocol. Make sure you're using an ELISA plate.
Incorrect plate sealing	Use plate sealers during incubation to avoid well-to-well contamination, don't reuse plate sealers.

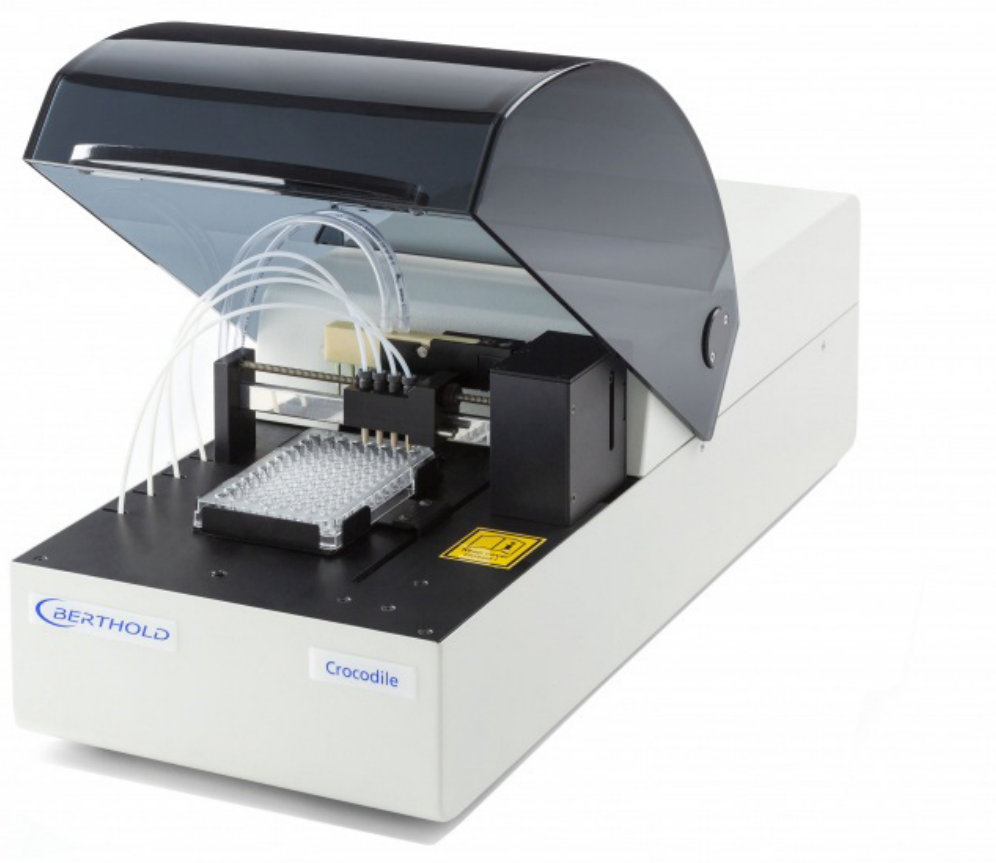
Featured products

Crocodile 5-in-one miniWorkstation

★★★★★

"Aside from the obvious benefits of hands-free operation, we find that day-to-day reproducibility is better with the Crocodile than with manual assays. In particular, we find it very useful for assays that require critical timing and/or temperature control. We also regularly just use the plate reader functionality to take advantage of the interface with Microwin for data interpolation."

Andrew Woodhead, Invitron



★★★★★

"We chose this equipment because of its robustness, ease of use and configuration (reader + washer). We are quite satisfied because all procedures, enzyme assays, specific product quantification, protein quantification and antimicrobial/antiadhesion assays can be carried out on microtiter plates and quantified using the Crocodile reader. Excellent product and good value for money."

Augusto Etchegaray, Pontifical Catholic University of Campinas

Zoom HT Microplate Washer

★★★★★

"Produces good-quality results and is quite easy to use. It represents decent value for money. After-sales care is excellent."

Paul Mitchell, Tridelta Development Ltd.

★★★★★

"It does a great job at washing microplates, pushing the background level to the very minimum."

Alessandro Ustione, Washington University in St Louis - School of Medicine