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 6.5% [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.

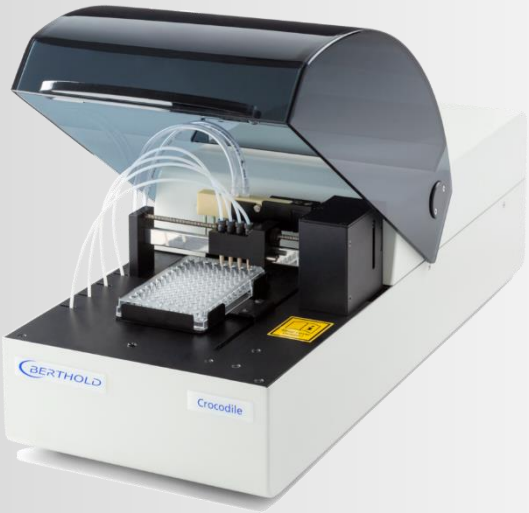
SINGLE PLATE ELISA

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

Sample 1 1,897 neg	Sample 5 3,549 neg	Sample 13 2,986 neg	Sample 21 1,390 neg	Sample 29 22,122 POS	Sample 37 1,352 neg
Sample 2 0,864 neg	Sample 6 3,812 neg	Sample 14 1,221 neg	Sample 22 2,535 neg	Sample 30 2,329 neg	Sample 38 2,385 neg
Sample 3 4,620 neg	Sample 7 0,901 neg	Sample 15 0,469 neg	Sample 23 2,103 neg	Sample 31 3,005 neg	Sample 39 34,610 POS
Sample 4 17,446 POS	Sample 8 11,643 POS	Sample 16 2,216 neg	Sample 24 2,385 neg	Sample 32 17,784 POS	Sample 40 2,779 neg
Positive Control 14,648 POS	Sample 9 21,972 POS	Sample 17 0,808 neg	Sample 25 4,977 neg	Sample 33 1,953 neg	Sample 41 0,714 neg
Negative Control 0,225 neg	Sample 10 4,113 neg	Sample 18 21,953 POS	Sample 26 18,648 POS	Sample 34 3,192 neg	Sample 42 4,657 neg
Calibrator 6,667 neg	Sample 11 1,371 neg	Sample 19 1,502 neg	Sample 27 1,746 neg	Sample 35 4,188 neg	Sample 43 4,469 neg
Blank 0,000 neg	Sample 12 8,169 neg	Sample 20 1,164 neg	Sample 28 2,310 neg	Sample 36 1,465 neg	Sample 44 28,676 POS

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; 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; in addition, the MikroWin optional software provided a convenient way to interpret the results. In consequence, the Crocodile 5-in-one ELISA miniWorkstation is suitable to easily automate the Virotech SARS-CoV-2 IgG ELISA.

Acknowledgements

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References

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2. COVID-19 Dashboard by the Center for Systems Science and Engineering (CSSE) at Johns Hopkins University (JHU), retrieved 25 May 2020: https://gisanddata.maps.arcgis.com/apps/opsdashboard/index.html#/bda7594740fd40299423467b48e9ecf6

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