Application note 2020/09:
Automation of the Agrodiag PorCoV test (1/2)

INTRODUCTION

The Agrodiag PorCoV kit is a multiplex ELISA-like test for the simultaneous serodetection and differentiation of the three porcine enteric coronaviruses currently circulating in North America swine herds, *i.e.* porcine epidemic diarrhea virus (PEDV), transmissible gastroenteritis virus (TGEV) and porcine deltacoronavirus (PDCoV) in a single reaction, saving cost and time. Enteric disease caused by high virulent TGEV and PEDV strains is characterized by vomiting, severe diarrhea, and high mortality (up to 100%) in piglets less than 2 weeks of age. Although PDCoV is less pathogenic and infectious than TGEV and PEDV, they are clinically and histopathologically indistinguishable. Laboratory testing is critical for routine diagnosis, detecting infection and evaluating immunity.

Here we present the automation of the Agrodiag PorCoV test kit on the Crocodile ELISA miniWorkstation, aiming to reduce to a minimum the user intervention through the duration of the assay. The design of the dot-microarray has been adjusted so results interpretation does not require any sophisticated reader and can be performed with bare eyes. Validation of the assay has been performed using 40 serum samples of known porcine coronavirus status collected from pigs experimentally inoculated with PEDV, PDCoV, TGEV Miller, and TGEV Purdue or culture medium (negative control), which infection was previously demonstrated by PCR (viral shedding) and ELISA (seroconversion).

MATERIAL

- Agrodiag PorCoV kit*
- Crocodile ELISA MiniWorkstation (Berthold Technologies GmbH)
- Samples known positive to PEDV (n=7), TGEV Purdue (n=6), TGEV Miller (n=6), PDCoV (n=4), and samples known negative (n=17)*
- Precision micropipettes with suitable disposable tips
- Distilled or deionized water

* More information on chip fabrication steps and serum sample collection is available in Agrodiag PorCoV: a multiplex immunoassay for the differential diagnosis of porcine enteric coronaviruses, Malbec et al., *Journal of Immunological Methods*, 2020.

METHODS

All reagents were brought up to room temperature 30 minutes before use. Wash Solutions, Conjugate Solutions, and samples dilution were prepared according to the manufacturer's instructions. Note that some samples were mixed to simulate positivities to multiple strains. All serum samples were tested in duplicates.

After the sample dilution step and dispense in the 96 wells microplate, all incubation and washing steps were automated with the Crocodile miniWorkstation (Figure 1). The plate was left unsealed through the whole process to limit user intervention, and the assay performed as expected.

The Crocodile ELISA miniWorkstation programmed steps are summarized in Supplementary Table 1.

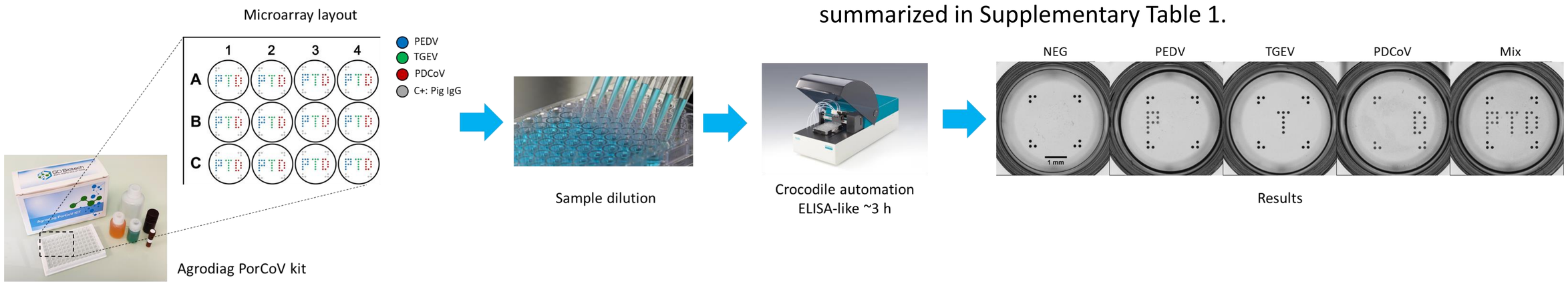

Figure 1. Description of the assay: samples are dispensed in the wells printed with microarrays, before automation steps on the Crocodile miniWorkstation leading to different visual outcomes (Negative, Positive to PEDV, TGEV, PDCoV, or positive to all strains).

RESULTS

In this version of the test, results are interpreted visually by the operator. Samples are considered positive to a strain when the corresponding letter is clearly visible at the surface on the well. All positive samples were found positive and all negative samples were found negative. There were no false positives or negatives, and no cross reactivity was observed. Incubation plan and results are displayed in Figure 2.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	Neg #56	Neg #90	PEDV #41	TGEV P #62	PDCoV #79	Blank	Neg #56	Neg #90	PEDV #41	TGEV P #62	PDCoV #79
B	Neg #13	Neg #58	Neg #91	TGEV P #44	TGEV P #63	Mix 2a	Neg #13	Neg #58	Neg #91	TGEV P #44	TGEV P #63	Mix 2a
C	Neg #14	Neg #60	PEDV #36	TGEV P #45	TGEV P #64	Mix 2b	Neg #14	Neg #60	PEDV #36	TGEV P #45	TGEV P #64	Mix 2b
D	Neg #15	Neg #85	PEDV #37	TGEV P #51	TGEV P #65	Mix 3	Neg #15	Neg #85	PEDV #37	TGEV P #51	TGEV P #65	Mix 3
E	Neg #16	Neg #86	PEDV #38	TGEV P #52	TGEV P #66	Mix 3	Neg #16	Neg #86	PEDV #38	TGEV P #52	TGEV P #66	Mix 3
F	Neg #17	Neg #87	PEDV #39	TGEV P #53	PDCoV #75	Mix 3	Neg #17	Neg #87	PEDV #39	TGEV P #53	PDCoV #75	Mix 3
G	Neg #18	Neg #88	PEDV #40	TGEV P #54	PDCoV #76	Mix 3	Neg #18	Neg #88	PEDV #40	TGEV P #54	PDCoV #76	Mix 3
H	Neg #55	Neg #89	PEDV #41	TGEV M #61	PDCoV #78	Mix 3	Neg #55	Neg #89	PEDV #41	TGEV M #61	PDCoV #78	Mix 3

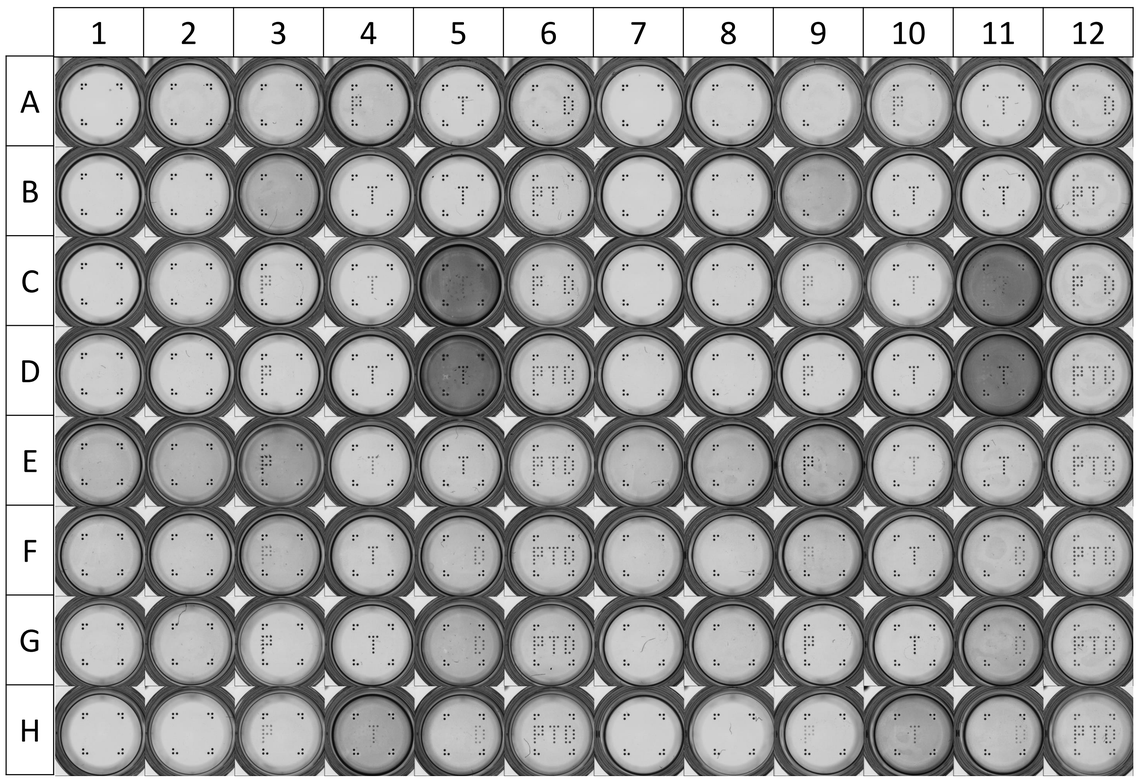

Figure 2. Plan of incubation with positive samples in red (left) and associated results (right). Samples and blank are incubated in duplicate. Mix 2a = PEDV #40 + TGEV P #54 ; Mix 2b = TGEV P #54 + PDCoV # 79 ; Mix 3 = PEDV #40 + TGEV P #54 + PDCoV # 79.

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CONCLUSION

The assay fulfilled validation conditions and all positive and negative samples could be correctly determined. Washing steps give homogeneous background while automation allows for the operator to deal with other occupations. The microarray design in shape of letters makes the results easy to interpret with no need for a reader.

ACKNOWLEDGEMENTS

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SUPPLEMENTARY

Table 1. Summary of the 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 01:00:00 at Incubator with 1 mm Amplitude at 10 Hz
4	Wash Solution priming	Washing Method: Prime Dispenser, Wash Solution Inlet: 1, Cycles 6, Volume: 1000 µL, Delay 1s, Wait: 100 ms, Dispenser Depth: 1300 (Plate Offset: 0), Aspiration Depth: 1300 (Plate Offset: 0)
5	Wash	Washing Method: Standard, Wash Solution Inlet: 1, Cycles 3, Volume: 200 µL, Delay 1s, Wait: 100 ms, Dispenser Depth: 2910 (Plate Offset: -40), Aspiration Depth: 2910 (Plate Offset: -40)*, Sweep: 4mm@ 4mm/s
6	Conjugate priming	Dispensing Volume: 2000 µL, Inlet: 1, Method: Priming
7	Conjugate distribution	Dispensing Volume: 100 µL, Inlet: 1, Method: Standard
8	Conjugate incubation	Shaking For 01:00:00 at Incubator with 1 mm Amplitude at 10 Hz
9	Wash	Washing Method: Standard, Wash Solution Inlet: 1, Cycles 3, Volume: 200 µL, Delay 1s, Wait: 100 ms, Dispenser Depth: 2910 (Plate Offset: -40), Aspiration Depth: 2910 (Plate Offset: -40)*, Sweep: 4mm@ 4mm/s
10	Substrate priming	Dispensing Volume: 2000 µL, Inlet: 2, Method: Priming
11	Substrate distribution	Dispensing Volume: 100 µL, Inlet: 2, Method: Standard
12	Substrate incubation	Manual Duration: 00:15:00, Mode: Auto Continue, Position: Insert Position
13	Water wash priming	Washing Method: Prime Dispenser, Wash Solution Inlet: 2, Cycles 6, Volume: 1000 µL, Delay 1s, Wait: 100 ms, Dispenser Depth: 1300 (Plate Offset: 0), Aspiration Depth: 1300 (Plate Offset: 0)
14	Wash	Washing Method: Standard, Wash Solution Inlet: 2, Cycles 3, Volume: 200 µL, Delay 1s, Wait: 100 ms, Dispenser Depth: 2910 (Plate Offset: -40), Aspiration Depth: 2910 (Plate Offset: -40)*, Sweep: 4mm@ 4mm/s
15	Dry	Incubation Incubator: On, Temperature: 37°C, Duration: 00:30:00
16	Incubator OFF	Incubation Incubator Off

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

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