STUDY REPORT

Test Performance of the Shandong Diano Bio-tech Co., Ltd Detection Kit for 2019-novel Coronavirus Antigen (Fluorescence Immunochromatography Method) with Clinical Samples and comparison with alternative testing methods

v1

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Study Report v.1

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Test Manufacturer	Diano Bio-tech Co.

INHOUDSOPGAVE

1. Study Title

Test Performance of the Shandong Diano Bio-tech Co., Ltd Detection Kit for 2019-novel Coronavirus Antigen (Fluorescence Immunochromatography Method) with Clinical Samples and comparison with alternative testing methods.

2. Sumary

Introduction: Availability and implementation of SARS-CoV-2 antigen rapid testing is crucial for the management of the current COVID-19 pandemic. There is a variety of clinical testing methods of which Shandong Diano Bio-tech Co., Ltd Detection Kit is a recent addition. It is imperative to collect and report its analytical and clinical performance. The Diano Bio-tech assay has obtained the CE-IVD market approval which implies adequate analytical and clinical performance. CE-IVD status requires post-market surveillance of its performance characteristics on an ongoing basis. This report therefore describes the results from a small clinical study to assess and compare its performance with several alternative Sars-CoV-2 detection systems. Note that this study uses the Diano Bio-tech Co., Ltd Detection Kit in conjunction with non-recommended sample collection buffer VTM. Paired PCR and Diano Bio-tech results are tabulated and used for interpretation.

Sudy Oblectives: The primary objective of the study is to assess the limit of detection of the Diano Bio-tech Co., Ltd Detection Kit for use in conjunction with de-identified and previously collected clinical samples after one cycle of freeze storage. A subset of the experiments has already been interpreted and been reported (Appendix A: *'Report on the evaluation of the Diano Bio-tech Detection kit for 2019-novel Coronavirus antigen in mild symptomatic population'*.). A secondary objective of the study is to evaluate the potential impact of a deliberate deviation from the recommended sample buffer as it may contain *Interfering Substance(s)*. The results in this report observed that the sensitivity was lower than expected when using the test with the historic sample dilutant (VTM) used during biobanking which is a deviation from the instructions for use (IFU) of the Diano Bio-tech Sars-CoV-2 assay (Appendix B)).

Study Type: Limit of detection assessment, using retrospective positive Covid-19 clinical samples and investigation of *Interfering Substance(s)* in the sample buffer.

Study population: This study used previously collected and biobanked, de-identified surplus clinical samples from Sars-CoV-2 positive individuals visiting two different routine testing locations in the Netherlands (Cities of Rotterdam (sample S-001, S-002 and S-003) and

Comicro Hoorn (S-004 and S-005)). The surplus samples have been collected and biobanked in VTM buffer (Mediaproducts B.V., Groningen, The Netherlands) until use for this study. Note that the dilutant used (VTM) is a deviation from the Instructions For Use from Diano Bio-tech Co., Ltd (Appendix B).

3. STUDY DESIGN

This study was performed without a formal Study Protocol. The Diano Bio-tech Sars-CoV-2 assay was supplied to two independent laboratories. One lab was Microvida, part of the Amphia Hospital in Breda, The Netherlands and performed the PCR and Diano Bio-tech Sars-CoV-2 assay for samples S-001, S-002 and S-003. The second lab was the Brightlabs Venlo , The Netherlands, and performed the PCR and Diano Bio-tech Sars-CoV-2 assay for samples S-004, and S-005. Sample dilutions are as indicated in the result section.

4. ACCEPTANCE CRITERIA

This study shall demonstrate parallel decreasing signals for the PCR and the Diano Bio-tech Sars-CoV-2 assay upon serially diluting the clinical samples.
This study shall document a possible superiority of the IFU recommended dilution buffer over the non-IFU buffer (VTM) when used for serial dilutions.

5. DEVIATIONS

Due to the retrospective approach (for selecting the biobanked samples), there are no deviations.

6. METHOD

The antigen test was clinically evaluated in a population consisting of mainly mild symptomatic cases attending the municipal health service drive-through test location in Breda and Etten Leur, the Netherlands (GGD West Brabant). Participants were informed of the evaluation on site. A participant information letter was handed out. Verbal informed consent was requested for a second swab from the throat and nasal cavity up to the nasal bridge (TNS) for the antigen test. The TNS for the RT-PCR (Zeesan SARS-CoV-2 Test Kit for Real-time PCR) was taken first, after which the TNS for antigen test was taken. In the test location Breda, the TNS for the antigen test were immediately stored in dry sterile test tubes and transported within 20 minutes to the local laboratory. In the local laboratory swabs were inserted into the extraction buffer and vigorously plunged up and down for 15 seconds. The swab was removed while pressing the swab to the side of the tube. Swabs were set at a temperature between 4 and 8 °C to the main laboratory (Microvida location Amphia Breda) and analyzed according to manufacturer's instructions on a Diano Automatic immunofluorescence Analyzer AFS3000B. Samples were

analyzed the same day or at the latest within 24 hours after storage at 5°C. In the test location Etten Leur, the TNS for the antigen test were immediately stored in dry sterile test tubes and stored at a temperature between 4 and 8 °C (2 sampling days) or at a temperature of -20 °C (3 days) before transportation to the central laboratory under the same temperature conditions. In the central laboratory, after a thaw step of 15 minutes, the same procedure was applied as described above. Technicians worked in standard personal protection equipment. The swabs for RT-PCR were sent to Microvida location Bravis Roosendaal for regular PCR testing (cobas® SARS-CoV2 test on cobas® 6800 or cobas® 8800 or an in-house PCR assay as described by Kluytmans-van den Bergh et al.). The antigen test was analytically evaluated by diluting SARS-CoV-2 stock provided by Erasmus MC in 10-fold series (10-1 to 10-8) viral transport medium (Mediaproducts B.V., Groningen, The Netherlands) with an end volume of 9 ml. The 10-fold series are vortex for 1 minute at room temperature. For each SARS-CoV-2 Rapid-Ag test, 350 µl from each dilution is added to the buffer supplied by the manufacturer. After adding the dilution, the procedure is followed as described in the prescription supplied by the manufacturer. Interpretation of the resulting Ct values for the ORF1ab-gene fragment (FAM-labeled) and the N-gene (ROX-labeled) are performed according to the manufacturer's instructions (Appendix C). In summary, for the FAM channel, Ct values correspond with positive when Ct ≤ 37, and not positive otherwise and for the ROX channel, Ct values correspond with positive when Ct ≤ 35, and not positive otherwise (Appendix C), provided that the positive and negative control runs are pos and neg, respectively.

Appendix A describes a large run using 1398 clinical samples (Table 1 and Supplementary Confidential study report S1). As a follow up, samples S-001, S-002 and S-003 were processed by the Diano Bio-tech assay performance and shown to perform inferior to the alternative testing methods. A trouble shooting led to a new working hypothesis that the viral transport medium (VTM) could contain some *Interfering Substance*. Therefore, further experiments were designed and performed to document the impact of the transport medium. For this part of the study, we used patient samples S-004 and S-005.

7. RESULTS

7.1 Overview

In a preliminary an investigation using 1398 clinical samples the Diano Bio-tech Sars-CoV-2 assay has been compared with nine other rapid antigen tests (A-I in Table 1) as well as with the reference PCR test (cobas® SARS-CoV2 test on cobas® 6800 or cobas® 8800 or an in-house PCR assay as described by Kluytmans-van den Bergh et al. Details in Appendix A). In this study the Diano Bio-tech Sars-CoV-2 assay had the lowest sensitivity of all tests. This prompted the troubleshooting to hypothesize a possible *Interfering Substance* in the Viral Transport Medium (VTM). This led to part two of this study, where the VTM dilution buffer was replaced with physiological salt solution (PBS) described in the rest of this report, using 5 additional samples (S-001 – S-005).

Table 1. Results of the diluted SARS-CoV-2 stock read out by Diano reader. The dilution is done in triplicate in each SARS-CoV-2 Rapid-Ag test. The table shows how often the SARS-CoV-2 Rapid-Ag test has become positive. Colored boxes show the categorization of the SARS-CoV-2 Rapid-Ag test in sensitivity levels. (reproduced from Appendix A).

Dilution	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	Sensitivity level:
TCID50/ml	3.16E+04	3.16E+03	3.16E+02	3.16E+01	3.16E+00	3.16E-01	3.16E-02	3.16E-03	
E-gene Copies/ml	4.98E+06	4.98E+05	4.98E+04	4.98E+03	4.98E+02	4.98E+01	4.98E+00	4.98E-01	
Ct-value E-gene qRT-PCR	10.86	14.43	17.77	20.97	24.02	27.34	30.18	34.29	
Test A	(3/3)	(3/3)	(3/3)	(3/3)	(3/3)^	(0/3)	(0/3)	(0/3)	1
Test B	(3/3)	(3/3)	(3/3)	(3/3)	(3/3)^	(0/3)	(0/3)	(0/3)	
Test C	(3/3)	(3/3)	(3/3)	(3/3)	(3/3)^	(0/3)	(0/3)	(0/3)	
Test D	(3/3)	(3/3)	(3/3)	(3/3)	(3/3)^	(0/3)	(0/3)	(0/3)	
Test E	(3/3)	(3/3)	(3/3)	(3/3)	(3/3)^	(0/3)	(0/3)	(0/3)	
Test F	(3/3)	(3/3)	(3/3)	(3/3)	(0/3)	(0/3)	(0/3)	(0/3)	2
Test G	(3/3)	(3/3)	(3/3)	(3/3)	(0/3)	(0/3)	(0/3)	(0/3)	
Test H	(3/3)	(3/3)	(3/3)	(1/3)	(0/3)	(0/3)	(0/3)	(0/3)	3
Test I	(3/3)	(3/3)	(3/3)	(3/3)^	(0/3)	(0/3)	(0/3)	(0/3)	
Diano Bio-tech Detection kit for 2019-novel Coronavirus antigen*	(3/3)	(3/3)	(3/3)	(0/3)	(0/3)	(0/3)	(0/3)	(0/3)	4
Test K	(3/3)	(3/3)	(3/3)	(0/3)	(0/3)	(0/3)	(0/3)	(0/3)	

During the second part of the protocol, a total of 5 clinical samples have been used (S-001 - S-005). Each sample was tested by the Diano Bio-tech Sars-CoV-2 assay as well as by PCR for two independent targets read by rox-1 and fam-1, respectively.

Table 2. Results Samples S-001, S-002 and S-003. Pos

Sample ID	Relative Dilution	PCR fam-1	PCR rox-1	Diano Bio-tech Sars-CoV-2 assay	See
S-001	0x	Pos	Pos	Pos	Table S1
S-001	1x	Pos	Pos	Pos	Table S1
S-001	2x	Pos	Pos	Pos	Table S1
S-001	3x	Pos	Pos	-	Table S1
S-001	4x	Pos	Pos	-	Table S1
S-001	5x	Pos	Pos	-	Table S1
S-001	6x	-	-	-	Table S1
S-001	7x	-	-	-	Table S1
S-002	0x	Pos	Pos	Pos	Table S2
S-002	1x	Pos	Pos	Pos	Table S2
S-002	2x	Pos	Pos	Pos	Table S2
S-002	3x	Pos	Pos	-	Table S2
S-002	4x	Pos	Pos	-	Table S2
S-002	5x	Neg	Pos	-	Table S2
S-002	6x	-	Neg	-	Table S2
S-002	7x	-	-	-	Table S2
S-003	0x	Pos	Pos	Pos	Table S3
S-003	1x	Pos	Pos	Pos	Table S3
S-003	2x	Pos	Pos	Pos	Table S3
S-003	3x	Pos	Pos	-	Table S3
S-003	4x	Pos	Pos	-	Table S3
S-003	5x	Neg	Neg	-	Table S3
S-003	6x	Neg	-	-	Table S3
S-003	7x	-	-	-	Table S3

Table 3. Results Samples S-004, and S-005

Sample ID	Relative Dilution	PCR fam-1	PCR rox-1	Diano Bio-tech Sars-CoV-2 assay	See
S-004	0x	Pos	Pos	Pos	Table S4
S-004	1x	Pos	Pos	Pos	Table S4
S-004	2x	Pos	Pos	Pos	Table S4
S-004	3x	Pos	Pos	Pos	Table S4
S-004	4x	Neg	Pos	Grey zone	Table S4
S-004	5x	-	-	Neg	Table S4
S-004	6x	-	-	Neg	Table S4
S-004	7x	-	-	Neg	Table S4
S-005	0x (=1:100)	Pos	Pos	Pos	Table S5
S-005	1x (= 1:125)	Pos	Pos	Pos	Table S5
S-005	2x (= 1:150)	Pos	Pos	Pos	Table S5
S-005	3x (= 1:200)	Pos	Pos	Pos	Table S5
S-005	4x (= 1:250)	Pos	Pos	Grey zone	Table S5
S-005	5x (= 1:300)	Pos	Pos	Neg	Table S5
S-005	6x (= 1:500)	Pos	Pos	Neg	Table S5
S-005	7x (= 1:600)	Pos	Pos	Neg	Table S5
S-005	8x (= 1:750)	Pos	Pos	Neg	Table S5

7.2 Limit of detection

Sensitivity in terms of limiting dilutions to obtain a first estimate for the limit of detection (LOD) of the Diano Bio-tech Sars-CoV-2 assay were performed with Samples S-001, 2 and 3. Each sample was selected because it had been (PCR) positive during routine clinical testing. Limiting dilutions have been made until no, or a negative PCR test result was obtained. The three samples S-001, S-002 and S-003 all resulted in a valid (i.e. either pos or neg) PCR down to 5 x dilutions, while the the Diano Bio-tech Sars-CoV-2 assay did not result in a valid result (i.e. pos or neg) for dilutions more than 3x (Table 2), indicating – as expected - a higher sensitivity of PCR as compared with Ag testing. But similar as the study in Appendix A, further

dilutions were expected to result in valid assay results, suggesting some problem with the method (in particular the compatibility of the dilution buffer with fluorescence measurement).

Therefore, a further limit of detection (LOD) of the Diano Bio-tech Sars-CoV-2 assay was performed with Samples S-004 and S-005, but now diluted in physiological salt (PBS). These samples have been selected because they had tested positive by PCR during routine clinical testing. Eight limiting dilutions have been prepared for S-004 (0x, 1x, 2x, 3x, 4x, 5x, 6x, 7x) and Nine for S-005 (100x, 125x, 150x, 200x, 250x, 300x, 500x, 600x and 750x). PCR results had valid results up until, and including the 4x (S-004) and 8x (S-005) dilution respectively while the Diano Bio-tech Sars-CoV-2 assay resulted in 'Grey Scores' at 4x for both samples (Table 3).

8. Conclusions

The design of the study (serial dilutions only using a small set of positive samples) is inadequate for sensitivity and specificity calculations. This study can however inform about LOD and dilution buffer impact (interfering substance).

The current study was performed to obtain preliminary information about the limit of detection (LOD) for the Diano Bio-tech Sars-CoV-2 assay. Three biobanked, PCR-positive clinical samples have been serially diluted in VTM and all three show loss of positivity of the Diano Bio-tech Sars-CoV-2 assay beyond dilution 3x. Diano Bio-tech Sars-CoV-2 assay results for dilutions of S-001, 2 and 3 only resulted in positive or invalid (Orange cells in Table 1) results, but not in 'negative' or 'grey zone'. This observation triggered an unplanned evaluation trough troubleshooting after sample S-001, 2, and 3. This led to the hypothesis that the VTM transport medium used for storage and all subsequent dilutions could be suboptimal or incompatible with the Diano Bio-tech Sars-CoV-2 assay, especially since it depends on fluorescent detection of the immunoaffinity antibodies. Thus, a second study was performed with Samples S-004 and S-005 that tried to avoid using the VTM buffer during serial dilutions but could not prevent the VTM buffer from being part of the original biobanking procedure.

Comparing between the first part of the study using S-001/S-003 and the latter part of the study using S-004/S-005 we observe no further invalid results (Table 1; Orange) and do get "grey zone" (these are considered negative as per IFU) and "negative" results. Furthermore, although the two parts of the study used different patient samples, we observed no positive Diano Bio-tech Sars-CoV-2 assay below 2x dilution when the VTM buffer was used while both S-004 and S-005 were positive in the 3x dilution, indicative for a possible higher LOD after changing the dilution buffer. For comparison, the PCR results in this report are PCR runs that, similar to the Diano Bio-tech Sars-CoV-2 assay, have been performed after freeze thaw of the

clinical biobanked samples. For all five clinical samples the observed LOD for PCR is lower than for the Diano Bio-tech Sars-CoV-2 assay. This difference in sensitivity between immunologic testing and PCR is expected and has been widely reported.

The current results are a clear indication that the Diano Bio-tech Sars-CoV-2 assay might be as sensitive as other commercial assays for the same purpose and reach the WHO required limit of sensitivity ≥80% (as calculated compared to PCR-positives). But, to obtain the true limit of detection for the Diano Bio-tech Sars-CoV-2 assay it is important to perform a larger study using preferably, fresh (never frozen) human swab samples collected and diluted as per manufacturer's instructions, tested alongside one or multiple alternative (immunologic point of care) testing methods.

9. Supplementary Data

Table S1: PCR Ct results for sample S-001

21-Jan-2021						
Well	Target	Sample ID	Dilution	Ct	PCR Result	Diano Biotech result
A3	fam-1	S-001	0x	21.2	P(+)	Pos
A3	rox-1	S-001		20.25	P(+)	
B3	fam-1	S-001	1x	23.55	P(+)	Pos
B3	rox-1	S-001	1x	22.33	P(+)	
C3	fam-1	S-001	2x	26.09	P(+)	Pos
C3	rox-1	S-001	2x	24.76	P(+)	
D3	fam-1	S-001	3x	30.47	P(+)	-
D3	rox-1	S-001	3x	28.19	P(+)	
E3	fam-1	S-001	4x	34.15	P(+)	-
E3	rox-1	S-001	4x	31.97	P(+)	
F3	fam-1	S-001	5x	36.94	P(+)	-
F3	rox-1	S-001	5x	35.86	P(+)	
G3	fam-1	S-001	6x	-	-	-
G3	rox-1	S-001	6x	-	-	
H3	fam-1	S-001	7x	-	-	-
H3	rox-1	S-001	7x	-	-	

Reference values: Pos Control fam_1 = 26.59 and 26.07 (duplicate); Pos Control rox_1 = 24.48 and 24.35 (duplicate); Neg Controls no values detected.

Table S2: PCR Ct results for sample S-002

21-Jan-2021						
Well	Target	Sample ID	Dilution	Ct	PCR Result	Diano Biotech result
A5	fam-1	S-002		21.16	P(+)	Pos
A5	rox-1	S-002		20.1	P(+)	
B5	fam-1	S-002	1x	22.84	P(+)	Pos
B5	rox-1	S-002	1x	21.89	P(+)	
C5	fam-1	S-002	2x	25.05	P(+)	Pos
C5	rox-1	S-002	2x	24.02	P(+)	
D5	fam-1	S-002	3x	29.93	P(+)	-
D5	rox-1	S-002	3x	28.01	P(+)	
E5	fam-1	S-002	4x	34.18	P(+)	-
E5	rox-1	S-002	4x	31.56	P(+)	
F5	fam-1	S-002	5x	39.35	N(-)	-
F5	rox-1	S-002	5x	35.88	P(+)	
G5	fam-1	S-002	6x	-	-	-
G5	rox-1	S-002	6x	38.6	N(-)	
H5	fam-1	S-002	7x	-	-	-
H5	rox-1	S-002	7x	-	-	

Reference values: Pos Control fam_1 = 26.59 and 26.07 (duplicate); Pos Control rox_1 = 24.48 and 24.35 (duplicate); Neg Controls no values detected.

Table S3: PCR Ct results for sample S-003

21-Jan-2021						
Well	Target	Sample ID	Dilution	Ct	PCR Result	Diano Biotech result
A7	fam-1	S-003		20.94	P(+)	Pos
A7	rox-1	S-003		19.98	P(+)	
B7	fam-1	S-003	1x	23.01	P(+)	Pos
B7	rox-1	S-003	1x	22.16	P(+)	
C7	fam-1	S-003	2x	25.1	P(+)	Pos
C7	rox-1	S-003	2x	24.12	P(+)	
D7	fam-1	S-003	3x	29.76	P(+)	-
D7	rox-1	S-003	3x	28.25	P(+)	
E7	fam-1	S-003	4x	34.02	P(+)	-
E7	rox-1	S-003	4x	31.64	P(+)	
F7	fam-1	S-003	5x	38.97	N(-)	-
F7	rox-1	S-003	5x	37.12	N(-)	
G7	fam-1	S-003	6x	40.35	N(-)	-
G7	rox-1	S-003	6x	-	-	
H7	fam-1	S-003	7x	-	-	-
H7	rox-1	S-003	7x	-	-	

Reference values: Pos Control fam_1 = 26.59 and 26.07 (duplicate); Pos Control rox_1 = 24.48 and 24.35 (duplicate); Neg Controls no values detected.

Table S4: PCR Ct results for sample S-004

27-Jan-2021						
Well	Target	Sample ID	Dilution	Ct	PCR Result	Diano Biotech result
A10	fam-1	S-004		22.56	P(+)	Pos
A10	rox-1	S-004		20.78	P(+)	
B10	fam-1	S-004	1x	24.46	P(+)	Pos
B10	rox-1	S-004	1x	23.48	P(+)	
C10	fam-1	S-004	2x	26.54	P(+)	Pos
C10	rox-1	S-004	2x	25.31	P(+)	
D10	fam-1	S-004	3x	32.38	P(+)	Pos
D10	rox-1	S-004	3x	29.95	P(+)	
E10	fam-1	S-004	4x	37.81	N(-)	Grey Zone
E10	rox-1	S-004	4x	33.96	P(+)	
F10	fam-1	S-004	5x	-	-	Neg
F10	rox-1	S-004	5x	-	-	
G10	fam-1	S-004	6x	-	-	Neg
G10	rox-1	S-004	6x	-	-	
H10	fam-1	S-004	7x	-	-	Neg
H10	rox-1	S-004	7x	-	-	

Reference values: Pos Control fam_1 = 25.62; Pos Control rox_1 = 24.07; ; Neg Controls no value detected

Table S5: PCR Ct results for sample S-005

28-Jan-2021						
Well	Target	Sample ID	Dilution	Ct	PCR Result	Diano Biotech result
A9	fam-1	S-005	onverdund 100x	27.15	P(+)	Pos
A9	rox-1	S-005	onverdund 100x	24.36	P(+)	
A10	fam-1	S-005	125x	27.71	P(+)	Pos
A10	rox-1	S-005	125x	24.59	P(+)	
B10	fam-1	S-005	150x	28.2	P(+)	Pos
B10	rox-1	S-005	150x	24.83	P(+)	
C10	fam-1	S-005	200x	29.13	P(+)	Pos
C10	rox-1	S-005	200x	25.48	P(+)	
D10	fam-1	S-005	250x	28.05	P(+)	Grey Zone
D10	rox-1	S-005	250x	24.79	P(+)	
E10	fam-1	S-005	300x	29.46	P(+)	Neg
E10	rox-1	S-005	300x	26.07	P(+)	
F10	fam-1	S-005	500x	31.11	P(+)	Neg
F10	rox-1	S-005	500x	26.82	P(+)	
G10	fam-1	S-005	600x	30.44	P(+)	Neg
G10	rox-1	S-005	600x	26.95	P(+)	
H10	fam-1	S-005	750x	32.4	P(+)	Neg
H10	rox-1	S-005	750x	28.13	P(+)	

Reference values: Pos Control fam_1 = 27.26; Pos Control rox_1 = 24.3; Neg Controls no value detected

10. Appendix A: Confidential Evaluation Diano SARS Ag test_final.pdf

11. Appendix B: Instruction For Use-1_20201224184600.pdf

12. Appendix C: Zeesan-IFU-SARS-CoV-2-Test-Kit-Real-time-PCR20200316.pdf