National Institute for Public Health
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Sensitivity and specificity of multiple ELISA tests in clinical patients; version 2

RIVM

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At the end of 2019, SARS-CoV-2 emerged in the human population. The subsequent growing pandemic spread of the virus is accompanied by high morbidity and mortality, and has an enormous negative impact on societal and economic circumstances world-wide. In response to this outbreak ELISA tests are currently overflowing the diagnostic market. As at 15 May 2020, the FIND organization has listed 106 manual or automated assays in different stages of validation and regulation on its website. The added value of these ELISA tests for individual patient diagnostics and their usefulness for epidemiological studies and to direct mitigation strategies, urgently needs to be established. Here, we took a first look at the clinical sensitivity and specificity of eight ELISA kits.

Methods.
Eight ELISA kits for detection of SARS-CoV-2 antibodies were included in the study. Selection was partially based on pre-study dossier analysis of data provided by the manufacturers that included test-specifics (antigen used), validation data on sensitivity and specificity in relation to type of cohort used and reliability of the manufacturer. The following three kits were analyzed in this report:

Test	Manufacturer	Certification	Antigen
Wantai SARS-CoV-2 Ab ELISA	Beijing Wantai Biological	CE-IVD	RBD
EUROIMMUN SARS-CoV-2 IgG	EUROIMMUN AG	CE-IVD	S1
EUROIMMUN SARS-CoV-2 IgA	EUROIMMUN AG	CE-IVD	S1
EDI™ Novel Coronavirus COVID-19 ELISA IgG	Epitope Diagnostics Inc	CE-IVD	COVID-19 recombinant protein
EDI™ Novel Coronavirus COVID-19 ELISA IgM	Epitope Diagnostics Inc	CE-IVD	COVID-19 recombinant protein
SARS-CoV-2 IgG ELISA kit	Creative Diagnostics	RUO	Whole virus lysate antigen
SARS-CoV-2 IgM ELISA kit	Creative Diagnostics	RUO	anti-μ chain monoclonal antibody
Platelia SARS-CoV-2 Total Ab	Bio-Rad Laboratories	CE-IVD	Nucleocapsid

All ELISA tests were used according to manufacturer's instructions.
Virus neutralization tests were performed as described previously with some modifications (Algaissi et al., 2020). Briefly, serial dilutions of heat-inactivated samples (30 min 56°C) were incubated with 100 TCID50 of SARS-CoV-2 virus for 1h at 35°C. African green monkey (Vero-E6) cells were added in a concentration of 2 x 10^4 cells per well and incubated for three days at 35°C in an incubator with 5% CO_2. The virus neutralization titer was defined as the reciprocal of the sample dilution that showed a 90% (VNT90) or 50% (VNT50) protection of virus growth. Samples with titers equal to ten and higher were defined as positive.
The following sera were used for the validation of the three ELISA kits with SARS-COV-2 RT-PCR (Corman et al., 2020) as reference test:

specificity panel	Number Wantai Ab	Number EUROIMMUN IgG	Number EUROIMMUN IgA	Other ELISAs[b]
healthy blood donors (the Netherlands)	42	42	30	40
acute EBV (the Netherlands)	10	10	10	10
acute CMV (the Netherlands)	10	10	10	10
Other HCoV (OC43; NL63; HKU1; 229E, all convalescent, the Netherlands)	20	20	3[a]	18
Total	**82**	**82**	**53**	**78**
Sensitivity panel[a]				
acute hospitalized patients (PCR-confirmed)	39	39	33	27
convalescent hospitalized patients (PCR-confirmed)	156	43	6	29
convalescent mild illness hospital workers (PCR-confirmed)	69	16	14	46
Total	**264**	**98**	**53**	**102**

[a]only hCoV-OC43.[b]EDI™ Novel Coronavirus COVID-19 ELISA IgG, EDI™ Novel Coronavirus COVID-19 ELISA IgM, Creative Diagnostics SARS-CoV-2 IgG ELISA kit, Creative Diagnostics SARS-CoV-2 IgM ELISA kit and Platelia SARS-CoV-2 Total Ab.Sera from confirmed SARS-CoV-2 patients were provided by (10)(2e) (10)(2e), ETZ (10)(2e) (10)(2e) (ADRZ) and (10)(2e) (10)(2e) (Izore). CMV and EBV acute sera were provided by (10)(2e) (10)(2e) (LUMC), common hCoV convalescent sera were provided by (10)(2e) (10)(2e) (10)(2e) (RIVM), (10)(2e) (10)(2e) (ADRZ). Serum from healthy blood donors were obtained through Sanquin.

For the validation of the sensitivity of the Wantai Ab ELISA against virus neutralization (VNT) as reference test, all serum samples of COVID-19 patients with a known neutralizing antibody titer (≥10) available at RIVM were analyzed versus the Wantai Ab ELISA. This included 46 sera with a titer in the VNT50 and 80 sera with a titer in the VNT90 (initially not all sera that were scored at 90% neutralization were scored at 50% as well, hence this discrepancy in numbers).

Results.

Specificity and sensitivity with RT-PCR as reference test.

The eight ELISA tests were analyzed for sensitivity and specificity based on clinical samples from PCR-confirmed COVID-19 patients and from EBV/CMV/other hCOV infected patients/healthy individuals collected before 2019. In tables 1-8 the calculated specifics are depicted per test and are based on PCR-positivity as reference test.

Tables 1-3. Clinical sensitivity and specificity (%) for three commercial ELISA tests.

1. Wantai SARS-CoV-2 Ab ELISA, Beijing Wantai Biological

Positive/ total no. samples	Cohort	Post onset symptoms (days)	Sensitivity	Specificity
30/39	Acute hospitalized patients	<10	77%	na
151/156	Convalescent hospitalized patients	10-15	97%	na
65/69	Convalescent mild illness hospital worker	>15	94%	na
246/264	Total cohort		93%	
216/225	**Total post onset symptoms > 10 days**	**>10**	**96%**	
0/82	**Healthy blood donors (42), EBV (10), CMV (10), other HCoV (20)**			**100%**

2. EUROIMMUN SARS-CoV-2 IgG, EUROIMMUN AG

Positive/ total no. samples	Cohort	Post onset symptoms (days)	Sensitivity	Specificity
15/39	Acute hospitalized patients	<10	38%	na
43/43	Convalescent hospitalized patients	10-15	100%	na
10/16	Convalescent mild illness hospital worker	>15	63%	na
68/98	Total cohort		69%	
53/59	**Total post onset symptoms > 10 days**	**>10**	**90%**	
0/82	**Healthy blood donors (42), EBV (10), CMV (10), other HCoV (20)**			**100%**

3. EUROIMMUN SARS-CoV-2 IgA, EUROIMMUN AG

Positive/ total no. samples	Cohort	Post onset symptoms (days)	Sensitivity	Specificity
24/33	Acute hospitalized patients	<10	72%	na
6/6	Convalescent hospitalized patients	10-15	100%	na
8/14	Convalescent mild illness hospital worker	>15	57%	na
38/53	Total cohort		72%	
14/20	**Total post onset symptoms > 10 days**	**>10**	**70%**	
9/53	**Healthy blood donors (30), EBV (10), CMV (10), HCoV-OC43 (3)**			**83%**

4. EDI™ Novel Coronavirus COVID-19 ELISA IgG, Epitope Diagnostics Inc

Positive/ total no. samples	Cohort	Post onset symptoms (days)	Sensitivity	Specificity
17/27	Acute hospitalized patients	<10	63%	na
27/29	Convalescent hospitalized patients	10-15	93%	na
39/46	Convalescent mild illness hospital worker	>15	85%	na
83/102	Total cohort		83%	
66/75	**Total post onset symptoms > 10 days**	**>10**	**88%**	
3/78	**Healthy blood donors (30), EBV (10), CMV (10), HCoV-OC43 (3)**			**96%**

5. EDI™ Novel Coronavirus COVID-19 ELISA IgM, Epitope Diagnostics Inc

Positive/ total no. samples	Cohort	Post onset symptoms (days)	Sensitivity	Specificity
16/27	Acute hospitalized patients	<10	59%	na
25/29	Convalescent hospitalized patients	10-15	86%	na
13/46	Convalescent mild illness hospital worker	>15	28%	na
54/102	Total cohort		53%	
38/75	**Total post onset symptoms > 10 days**	**>10**	**51%**	
1/78	**Healthy blood donors (30), EBV (10), CMV (10), HCoV-OC43 (3)**			**99%**

6. SARS-CoV-2 IgG ELISA kit, Creative Diagnostics

Positive/ total no. samples	Cohort	Post onset symptoms (days)	Sensitivity	Specificity
6/27	Acute hospitalized patients	<10	22%	na
21/29	Convalescent hospitalized patients	10-15	72%	na

24/46	Convalescent mild illness hospital worker	>15	52%	na
51/102	Total cohort		50%	
45/75	**Total post onset symptoms > 10 days**	**>10**	**60%**	
1/78	**Healthy blood donors (30), EBV (10), CMV (10), HCoV-OC43 (3)**			**99%**

7. SARS-CoV-2 IgM ELISA kit, Creative Diagnostics

Positive/ total no. samples	Cohort	Post onset symptoms (days)	Sensitivity	Specificity
14/27	Acute hospitalized patients	<10	52%	na
24/29	Convalescent hospitalized patients	10-15	83%	na
25/46	Convalescent mild illness hospital worker	>15	54%	na
63/102	Total cohort		62%	
49/75	**Total post onset symptoms > 10 days**	**>10**	**65%**	
2/78	**Healthy blood donors (30), EBV (10), CMV (10), HCoV-OC43 (3)**			**97%**

8. Platelia SARS-CoV-2 Total Ab, Bio-Rad laboratories

Positive/ total no. samples	Cohort	Post onset symptoms (days)	Sensitivity	Specificity
16/27	Acute hospitalized patients	<10	59%	na
27/29	Convalescent hospitalized patients	10-15	93%	na
40/46	Convalescent mild illness hospital worker	>15	87%	na
83/102	Total cohort		81%	
67/75	**Total post onset symptoms > 10 days**	**>10**	**89%**	
6/78	**Healthy blood donors (30), EBV (10), CMV (10), HCoV-OC43 (3)**			**92%**

Sensitivity Wantai Ab with virus neutralization as reference test.

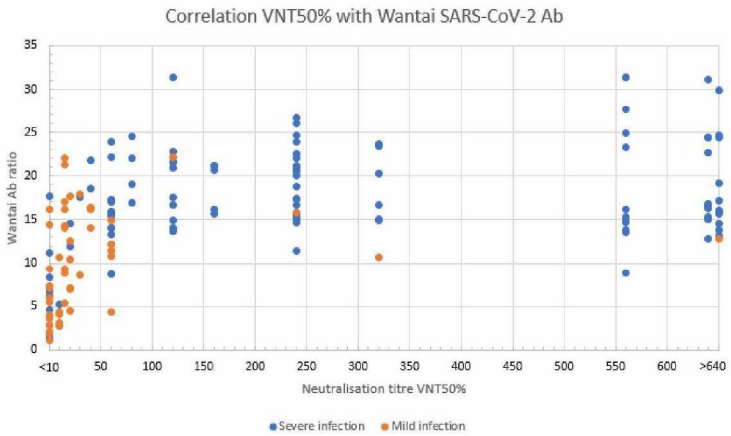
Secondly, the Wantai total Ab ELISA was analyzed for sensitivity with a SARS-CoV-2 virus neutralization test (VNT) as reference test. Of 155 sera with an established titer for neutralizing antibodies in a VNT scored at 50% neutralization (VNT50%), **100%** were observed positive in the ELISA. Of 155 sera with an established titer for neutralizing antibodies in a VNT scored at 90% neutralization (VNT90%), **99%** were positive in the Wantai ELISA.

Correlation positive result in Wantai Ab with presence of neutralizing antibodies.

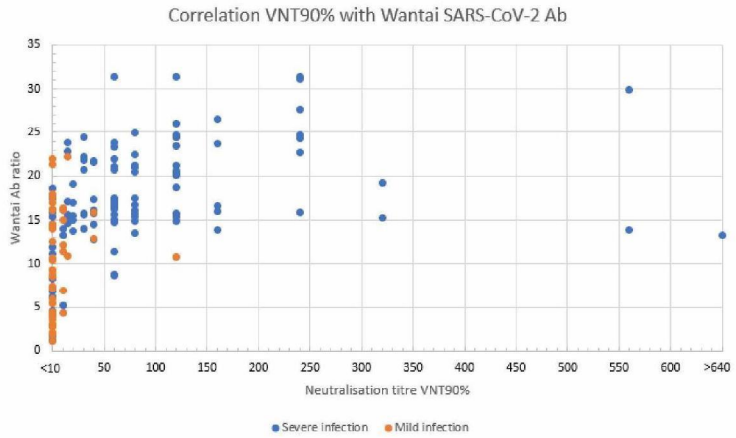
Lastly, the correlation between OD/C.O ratio in the Wantai total Ab ELISA and virus neutralization titers was analyzed in a cohort with PCR-confirmed, Wantai ELISA positive COVID-19 patients with mild disease (n=47) and severe disease (n=111). In the mild disease cohort, neutralizing antibodies were observed in 34/47 (72%) ELISA positive patients using a VNT50 (Figure 1A) and in 12/47 (26%) using a VNT90 (Figure 1B). In the severe disease cohort, neutralizing antibodies were observed in 102/111 (92%) ELISA positive patients in VNT50 and 95/111 (86%) in VNT90.

Figure 1. Correlation Wantai Ab ELISA and virus neutralization test in mild and severe laboratory confirmed COVID-19 patients

A. *vs VNT50*



B. vs VNT90%



Discussion and conclusion.

Pre-setting the minimal performance required from ELISA tests depends on the application. The three ELISA tests were judged based on the following minimal thresholds (expert opinion):

- individual patient diagnostics: specificity >98%; sensitivity >95%.
- sero-epidemiological studies (e.g. collecting seroprevalence data as proxy for herd immunity, input in models): specificity >98%; sensitivity >90%.

Based on the first validation data presented here with PCR as reference test, it can be concluded that based on the overall data for samples *taken > 10 days post onset symptoms* for PCR-confirmed *severe* COVID-19 cases, only Wantai Ab and EUROIMMUN IgG/IgA fulfilled the sensitivity criteria set above. However, when analyzing sera from mild patients taken > 15 days post onset of symptoms, none of the evaluated ELISAs fulfilled the preset criteria. The Wantai SARS-CoV-2 Ab is the only test that approaches the preset criteria for mild patients with a sensitivity of 94%. Because this percentage is based on a limited sample set (n=69), it needs to be tested with more samples to establish if it will fulfill the preset criterium.

Zooming in on the performance of the Wantai total Ab test, we observed a 100% sensitivity with virus neutralization as reference test. When looking at the correlation between a positive outcome in the Wantai total Ab test and the presence of virus neutralizing antibodies, we observed a good correlation in convalescent severe patients but not so much in convalescent mild patients.

Looking at specificity, the EUROIMMUN IgA, EDI IgG, Creative Diagnostics IgM and the Platelia total Ab tests did not reach the preset threshold for specificity. For EUROIMMUN IgA, this was due to cross reactivity of sera from patients suffering from acute EBV and CMV infections. This cross reactivity was also present in EDI IgG and Creative Diagnostics IgM, but also one healthy reacted in these tests. In the Platelia total Ab, there were cross reactions with sera from patients with EBV, CMV and other hCoV infections, and also with sera from two healthy blood donors.. EDI IgM and Creative Diagnostics IgG kits had a specificity of 99% and Wantai Ab and EUROIMMUN IgG kits both had a specificity of 100%, all four fulfilling the specificity criteria.

Further validation is necessary with larger well defined sample sets for a more precise determination of test specifics in relation with virus neutralization while test performances need to be interpreted in the light of the rapidly increasing information on antibody kinetics in different (sub) clinical patient cohorts.

These data presented here underline the importance of extensive validation in the right (sub)populations and settings to avoid guidance of clinical care and control efforts at individual and population level based on false diagnostic outcomes.

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