National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport

Sensitivity and specificity of Wantai Ab and EUROIMMUN IgG/IgA SARS-CoV-2 ELISA tests in clinical patients

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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 30 April 2020, the FIND organization has listed 108 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 three ELISA kits.

Methods.

Three 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

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 2×10^4 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
healthy blood donors (the Netherlands)	42	42	30
acute EBV (the Netherlands)	10	10	10
acute CMV (the Netherlands)	10	10	10
Other HCoV (OC43; NL63; HKU1; 229E, all convalescent, the Netherlands)	20	20	3[a]
Total	**82**	**82**	**53**
Sensitivity panel[b]			
acute hospitalized patients (PCR-confirmed)	39	39	33
convalescent hospitalized patients (PCR-confirmed)	43	43	6
convalescent mild illness hospital workers (PCR-confirmed)	32	16	14
Total	**114**	**98**	**53**

[a]only hCoV-OC43.[b]Sera from confirmed SARS-CoV-2 patients were provided by (10)(2e) (10)(2e), ETZ (10)(2e) (ADRZ) and (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) (RIVM), (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 three 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 1-3 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
43/43	Convalescent hospitalized patients	10-15	100%	na
32/32	Convalescent mild illness hospital worker	>15	100%	na
89/98	Total cohort		91%	
75/75	**Total post onset symptoms > 10 days**	**>10**	**100%**	
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%**

Sensitivity Wantai Ab with virus neutralization as reference test.

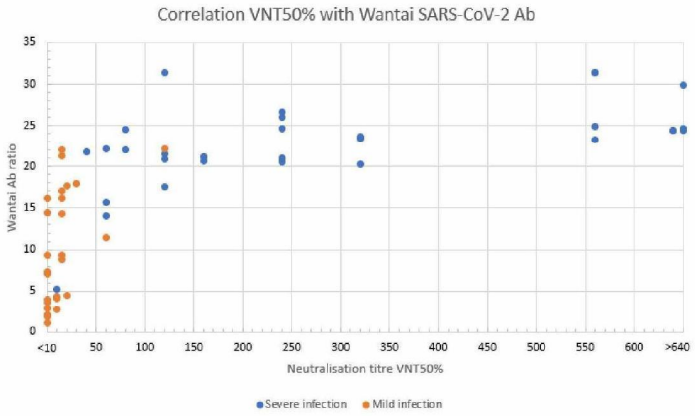
Secondly, the Wantai total Ab ELISA was analyzed for sensitivity with a SARS-CoV-2 virus neutralization test (VNT) as reference test. Of 46 sera with an established titer for neutralizing antibodies in a VNT scored at 50% neutralization (VNT50%), **100%** were observed positive in the ELISA. Of 80 sera 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.

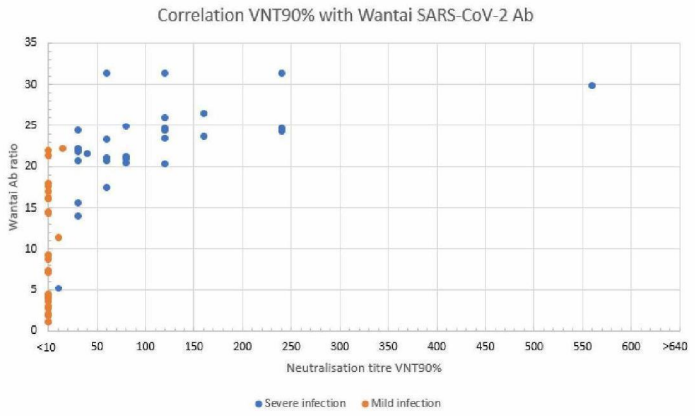
Lastly, the correlation between OD/C.O ratio in the Wantai total Ab ELISA and virus neutralization titers was analyzed in a cohort with PCR-confirmed, Wantai ELISA positive COVID-19 patients with mild disease (n=26) and severe disease (n=32). In the mild disease cohort, neutralizing antibodies were observed in 15/26 ELISA positive patients using a VNT50 (Figure 1A) and in only 2/26 using a VNT90 (Figure 1B). In the severe disease cohort, neutralizing antibodies were observed in 32/32 ELISA positive patients in both the VNT50 and VNT90.

Figure 1. Correlation Wantai Ab ELISA and virus neutralization test in mild and severe 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, all of the three ELISA tests fulfilled the sensitivity criteria set above. However, when analyzing sera from mild patients taken > 15 days post onset of symptoms, only the Wantai SARS-CoV-2 Ab ELISA fulfilled the preset criteria.

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 test did not reach the preset threshold for specificity. This was due to cross reactivity of sera from patients suffering from acute EBV and CMV infections, three out of ten samples were positive in EUROIMMUN IgA for both. Wantai Ab and EUROIMMUN IgG kits both had a specificity of 100%, 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.

30 April 2020,

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