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

A first analysis of the sensitivity and specificity of seven rapid SARS-COV-2 antibody tests in clinical patients, including analysis of correlation with neutralization and the (10)(1c) ELISA.

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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 and in the context of world-wide shortages for molecular testing, rapid diagnostic tests for detection of SARS-CoV-2 specific antibodies are currently overflowing the diagnostic market. As at 09 April 2020, the FIND organization has listed 155 rapid immuno-assays in different stages of validation and regulation on its website. The added value of these rapid immuno-assays 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 seven rapid SARS-CoV-2 antibody tests and compared the results with the outcomes of a virus neutralization test and a commercial ELISA (10)(1c) with apparent high specificity and sensitivity (data not shown).

Methods.
Seven rapid SARS-CoV-2 antibody tests 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. Additionally, tests were included that were delivered to the RIVM directly (without solicitation) and that were not triaged based on manufacturer's dossier. The following 7 tests were analyzed in this study:

Test	Certification	Antigen
(10)(1c)	CE-IVD	N
(10)(1c)	CE-IVD	N,S
	CE-IVD	N
	CE-IVD	N
	CE-IVD	not specified
	Unknown	not specified
	CE-IVD	N,S

The following 50 sera were used for the initial screening of the usefulness of the seven rapid immuno-assays:

specificity panel*	number
healthy blood donors (the Netherlands)	10
acute EBV (the Netherlands)	5
acute CMV (the Netherlands)	5
HCoV-OC43 (convalescent, the Netherlands)	5
Total	**25**
Sensitivity panel*	
acute hospitalized patients (PCR-confirmed)	8
convalescent hospitalized patients (PCR-confirmed)	6
mild illness hospital workers (PCR-confirmed)	11
Total	**25**

*due to the limited amount of tests available, the validation in this report is limited. Therefore, the data can be used only as a first screening of the utility of the tests. Tests with good performance can be selected for further, more in-depth validation once tests are available.
Furthermore the total number of validation sera used for the different tests varies between 38 and 50.

All tests were used according to manufacturer's instructions. Sera from confirmed SARS-CoV-2 patients were provided by (10)(2e) (10)(2e), EZT. CMV and EBV acute sera were provided by (10)(2e) (10)(2e) (LUMC), HCoV-OC43 convalescent sera were provided by (10)(2e) (10)(2e) (10)(2e) (RIVM). Serum from healthy blood donors were obtained through Sanquin.
The (10)(1c) ELISA as reference test was performed according to manufacturer's instructions. Virus neutralization test is an in-house test based on a Dutch SASR-CoV-2 isolate (unpublished).

Results.

The seven rapid immuno-assay tests were analyzed for an initial impression of sensitivity and specificity based on clinical samples from confirmed COVID-19 patients and from EBV/CMV/HCOV-OC43 patients/healthy individuals collected before 2019. In tables 1-7 the calculated specifics are depicted per test and are based on PCR-positivity as reference test (Corman et al., 2020).

Tables 1-7. Clinical sensitivity and specificity (%) for seven rapid immuno-assays.

(10)(1c)

A. IgG

Positive/ total no. samples	Cohort	Post onset symptoms (days)	Sensitivity	Specificity
2/8	Acute hospitalized patients	<10	25%	na
6/6	Convalescent hospitalized patients	10-15	100%	na
6/11	Mild illness hospital worker	>15	55%	na
14/25	**Total sensitivity cohort**		**56%**	
12/17	**Total sensitivity cohort > 10 days post onset symptoms**	**>10**	**71%**	
1/13	**Healthy blood donors (10), HCoV-OC43 (3)**			**92%**

B. IgM

Positive/ total no. samples	Cohort	Post onset symptoms (days)	Sensitivity	Specificity
3/8	Acute hospitalized patients	<10	38%	na
6/6	Convalescent hospitalized patients	10-15	100%	na
6/11	Mild illness hospital worker	>15	55%	na
15/25	**Total sensitivity cohort**		**60%**	
12/17	**Total sensitivity cohort > 10 days post onset symptoms**	**>10**	**71%**	
1/13	**Healthy blood donors (10), HCoV-OC43 (3)**			**92%**

(10)(1c)

A. IgG

Positive/ total no. samples	Cohort	Post onset symptoms (days)	Sensitivity	Specificity
3/8	Acute hospitalized patients	<10	38%	na
6/6	Convalescent hospitalized patients	10-15	100%	na
6/11	Mild illness hospital worker	>15	55%	na
15/25	**Total sensitivity cohort**		**60%**	
12/17	**Total sensitivity cohort > 10 days post onset symptoms**	**>10**	**71%**	
1/13	**Healthy blood donors (10), HCoV-OC43 (3)**			**92%**

B. IgM

Positive/ total no. samples	Cohort	Post onset symptoms (days)	Sensitivity	Specificity
4/8	Acute hospitalized patients	<10	50%	na
6/6	Convalescent hospitalized patients	10-15	100%	na
6/11	Mild illness hospital worker	>15	55%	na
16/25	**Total sensitivity cohort**		**64%**	
12/17	**Total sensitivity cohort > 10 days post onset symptoms**	**>10**	**71%**	
1/13	**Healthy blood donors (10), HCoV-OC43 (3)**	**>15**		**92%**

(10)(1c)

A. IgG

Positive/ total no. samples	Cohort	Post onset symptoms (days)	Sensitivity	Specificity
5/8	Acute hospitalized patients	<10	63%	na
6/6	Convalescent hospitalized patients	10-15	100%	na
7/11	Mild illness hospital worker	>15	64%	na
18/25	**Total sensitivity cohort**		**72%**	
13/17	**Total sensitivity cohort > 10 days post onset symptoms**	**>10**	**76%**	
1/13	**Healthy blood donors (10), HCoV-OC43 (3)**			**92%**

B. IgM

Positive/ total no. samples	Cohort	Post onset symptoms (days)	Sensitivity	Specificity
6/8	Acute hospitalized patients	<10	75%	na
6/6	Convalescent hospitalized patients	10-15	100%	na
7/11	Mild illness hospital worker	>15	64%	na
19/25	**Total sensitivity cohort**		**76%**	
13/17	**Total sensitivity cohort > 10 days post onset symptoms**	**>10**	**76%**	
1/13	**Healthy blood donors (10), HCoV-OC43 (3)**			**92%**

(10)(1c)

A. IgG

Positive/ total no. samples	Cohort	Post onset symptoms (days)	Sensitivity	Specificity
4/7	Acute hospitalized patients	<10	57%	na
6/6	Convalescent hospitalized patients	10-15	100%	na
6/10	Mild illness hospital worker	>15	60%	na
16/23	**Total sensitivity cohort**		**70%**	
12/16	**Total sensitivity cohort > 10 days post onset symptoms**	**>10**	**75%**	
0/25	**Healthy blood donors (10), HCoV-OC43 (5), EBV (5), CMV (5)**			**100%**

B. IgM

Positive/ total no. samples	Cohort	Post onset symptoms (days)	Sensitivity	Specificity
4/7	Acute hospitalized patients	<10	57%	na
2/6	Convalescent hospitalized patients	10-15	33%	na
6/10	Mild illness hospital worker	>15	60%	na
12/23	**Total sensitivity cohort**		**52%**	
8/16	**Total sensitivity cohort > 10 days post onset symptoms**	**>10**	**50%**	
0/25	**Healthy blood donors (10), HCoV-OC43 (5), EBV (5), CMV (5)**			**100%**

(10)(1c)

A. IgG

Positive/ total no. samples	Cohort	Post onset symptoms (days)	Sensitivity	Specificity
4/7	Acute hospitalized patients	<10	57%	na
6/6	Convalescent hospitalized patients	10-15	100%	na
6/10	Mild illness hospital worker	>15	60%	na
16/23	**Total sensitivity cohort**		**70%**	
12/16	**Total sensitivity cohort > 10 days post onset symptoms**	**>10**	**75%**	
0/25	**Healthy blood donors (10), HCoV-OC43 (5), EBV (5), CMV (5)**			**100%**

B. IgM

Positive/ total no. samples	Cohort	Post onset symptoms (days)	Sensitivity	Specificity
3/7	Acute hospitalized patients	<10	43%	na
1/6	Convalescent hospitalized patients	10-15	17%	na
0/10	Mild illness hospital worker	>15	0%	na
4/23	**Total sensitivity cohort**		**17%**	
1/16	**Total sensitivity cohort > 10 days post onset symptoms**	**>10**	**6%**	
0/25	**Healthy blood donors (10), HCoV-OC43 (5), EBV (5), CMV (5)**			**96%**

(10)(1c)

A. IgG

Positive/ total no. samples	Cohort	Post onset symptoms (days)	Sensitivity	Specificity
2/7	Acute hospitalized patients	<10	29%	na
6/6	Convalescent hospitalized patients	10-15	100%	na
0/10	Mild illness hospital worker	>15	0%	na
8/23	**Total sensitivity cohort**		**35%**	
6/16	**Total sensitivity cohort > 10 days post onset symptoms**	**>10**	**38%**	
0/25	**Healthy blood donors (10), HCoV-OC43 (5), EBV (5), CMV (5)**			**100%**

B. IgM

Positive/ total no. samples	Cohort	Post onset symptoms (days)	Sensitivity	Specificity
4/7	Acute hospitalized patients	<10	57%	na
6/6	Convalescent hospitalized patients	10-15	100%	na
2/10	Mild illness hospital worker	>15	20%	na
12/23	**Total sensitivity cohort**		**52%**	
8/16	**Total sensitivity cohort > 10 days post onset symptoms**	**>10**	**50%**	
0/25	**Healthy blood donors (10), HCoV-OC43 (5), EBV (5), CMV (5)**			**100%**

(10)(1c)

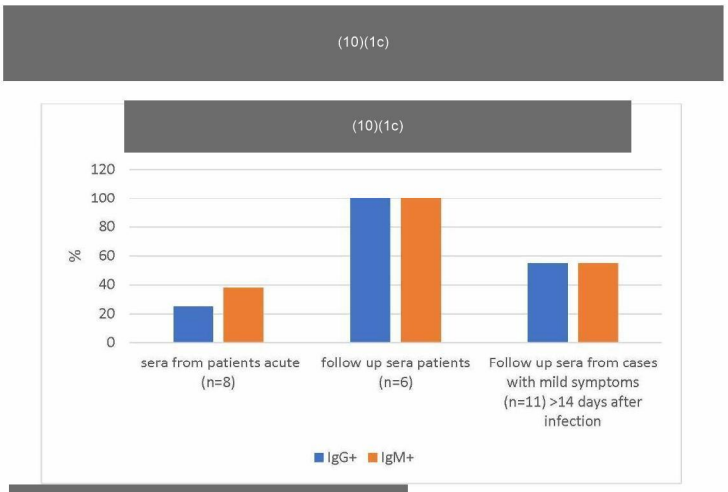
A. IgG

Positive/ total no. samples	Cohort	Post onset symptoms (days)	Sensitivity	Specificity
2/7	Acute hospitalized patients	<10	29%	na
4/6	Convalescent hospitalized patients	10-15	67%	na
0/10	Mild illness hospital worker	>15	0%	na
6/23	**Total sensitivity cohort**		**26%**	
4/16	**Total sensitivity cohort > 10 days post onset symptoms**	**>10**	**25%**	
0/25	**Healthy blood donors (10), HCoV-OC43 (5), EBV (5), CMV (5)**			**100%**

B. IgM

Positive/ total no. samples	Cohort	Post onset symptoms (days)	Sensitivity	Specificity
0/7	Acute hospitalized patients	<10	0%	na
0/6	Convalescent hospitalized patients	10-15	0%	na
1/10	Mild illness hospital worker	>15	10%	na
1/23	**Total sensitivity cohort**		**4%**	
1/16	**Total sensitivity cohort > 10 days post onset symptoms**	**>10**	**6%**	
0/25	**Healthy blood donors (10), HCoV-OC43 (5), EBV (5), CMV (5)**			**100%**

Figures 1 to 7 give a graphic representation of the performance of the tests (panels A) including the performance vs the (10)(1c) ELISA (panels B) and virus neutralization test. The panels depict the performance on PCR-confirmed COVID-19 patients



(10)(1c)

(10)(1c) ELISA Igtotal

		low pos*	high pos*	neg
IgG	pos	3	11	
	neg	4	5	2

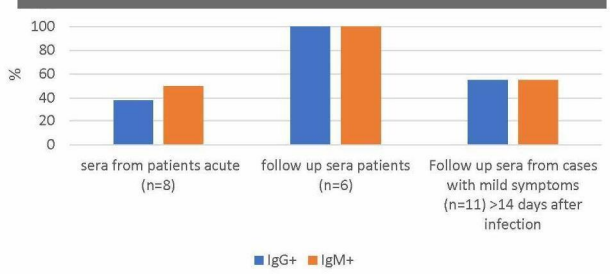
(10)(1c) ELISA Igtotal

		low pos*	high pos*	neg
IgM	pos	3	12	
	neg	4	4	2

**High positives in the (10)(1c) ELISA correlate with detectible titers in the virus neutralization assay. Low positives in the (10)(1c) ELISA did not correlate with detectable presence of neutralizing antibodies.*

(10)(1c)

(10)(1c)



(10)(1c)

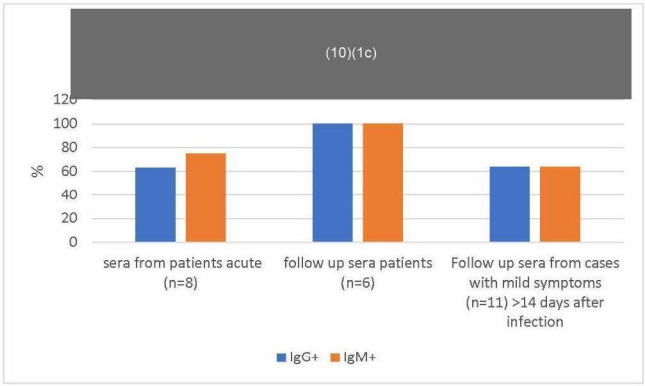
		(10)(1c) ELISA Igtotaal		
		low pos*	high pos*	neg
IgG	pos	3	12	
	neg	4	4	2

		(10)(1c) ELISA Igtotaal		
		low pos*	high pos*	neg
IgM	pos	3	13	
	neg	4	3	2

**High positives in the (10)(1c) ELISA correlate with detectible titers in the virus neutralization assay. Low positives in the (10)(1c) ELISA did not correlate with detectable presence of neutralizing antibodies.*

(10)(1c)

(10)(1c)



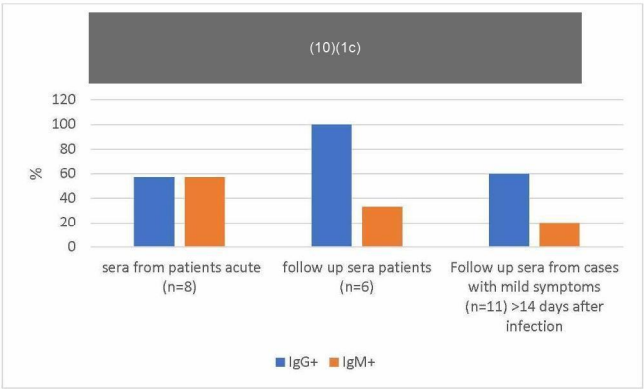
(10)(1c)

		(10)(1c) ELISA Igtotaal		
		low pos*	high pos*	neg
IgG	pos	4	14	
	neg	3	2	2

		(10)(1c) ELISA Igtotaal		
		low pos*	high pos*	neg
IgM	pos	3	16	
	neg	4		2

**High positives in the (10)(1c) ELISA correlate with detectible titers in the virus neutralization assay. Low positives in the (10)(1c) ELISA did not correlate with detectable presence of neutralizing antibodies.*

(10)(1c)



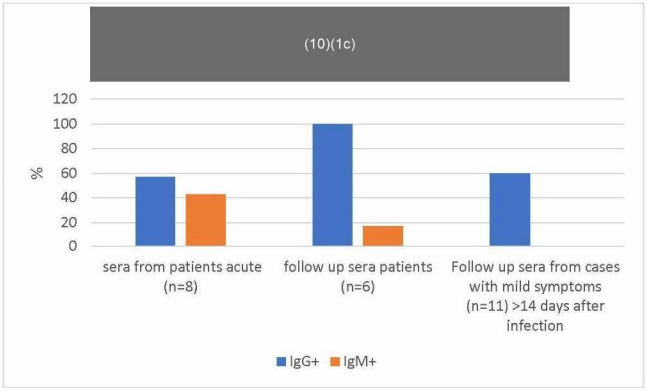
(10)(1c)

		(10)(1c) ELISA Igtotaal		
		low pos*	high pos*	neg
IgG	pos	3	13	
	neg	3	2	2

		(10)(1c) ELISA Igtotaal		
		low pos*	high pos*	neg
IgM	pos	2	6	
	neg	4	9	2

**High positives in the (10)(1c) ELISA correlate with detectible titers in the virus neutralization assay. Low positives in the (10)(1c) ELISA did not correlate with detectable presence of neutralizing antibodies.*

(10)(1c)



(10)(1c)

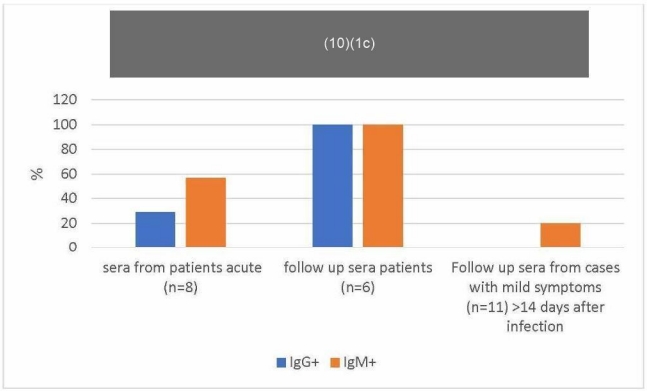
		(10)(1c) ELISA Igtotaal		
		low pos*	high pos*	neg
IgG	pos	3	13	
	neg	3	2	2

		(10)(1c) ELISA Igtotaal		
		low pos*	high pos*	neg
IgM	pos		4	
	neg	6	11	2

**High positives in the (10)(1c) ELISA correlate with detectible titers in the virus neutralization assay. Low positives in the (10)(1c) ELISA did not correlate with detectable presence of neutralizing antibodies.*

(10)(1c)

(10)(1c)



(10)(1c)

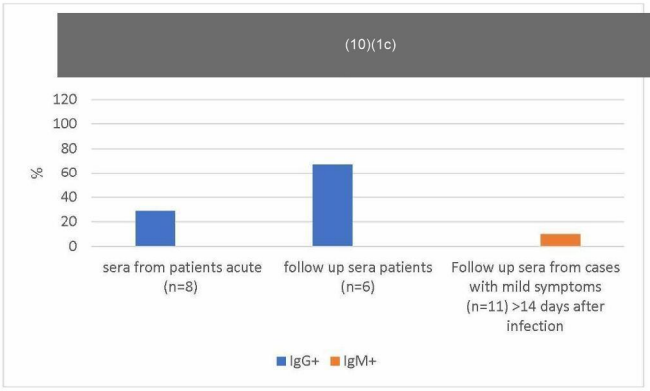
		(10)(1c) ELISA Igtotaal		
		low pos*	high pos*	neg
IgG	pos		8	
	neg	6	7	2

		(10)(1c) ELISA Igtotaal		
		low pos*	high pos*	neg
IgM	pos	1	11	
	neg	5	4	2

**High positives in the (10)(1c) ELISA correlate with detectible titers in the virus neutralization assay. Low positives in the (10)(1c) ELISA did not correlate with detectable presence of neutralizing antibodies.*

(10)(1c)

(10)(1c)



(10)(1c)

(10)(1c) ELISA Igtotaal

		low pos*	high pos*	neg
IgG	pos		6	
	neg	6	9	2

(10)(1c) ELISA Igtotaal

		low pos*	high pos*	neg
IgM	pos		1	
	neg	6	14	2

**High positives in the (10)(1c) ELISA correlate with detectible titers in the virus neutralization assay. Low positives in the (10)(1c) ELISA did not correlate with detectable presence of neutralizing antibodies.*

Discussion and conclusion.

Pre-setting the minimal performance required from rapid immuno-assays depends on the use of the rapid test. The seven rapid tests were judged based on the following minimal thresholds (expert opinion):
-individual patient diagnostics: specificity >98%; sensitivity >95% *for both IgG and IgM*
-individual status of having had a SARS-CoV-2 infection in specific (sub)populations, e.g. health care workers and caregivers of vulnerable persons to provide guidance in use of (types of) PPE: specificity >98%; sensitivity >80%. *Only IgG.*
-sero-epidemiological studies (e.g. collecting seroprevalence data as proxy for herd immunity, input in models): specificity >98%; sensitivity >90%. *Only IgG.*

Based on the first limited validation presented here, it can be concluded that based *on the overall data for samples taken > 10 days post onset symptoms* for PCR-confirmed COVID-19 cases none of the seven tests fulfilled the sensitivity criteria for IgG set above. When analyzing with sera taken 10-15 days post onset of symptoms from hospitalized patients, all tests but one (10)(1c) had a sensitivity of 100% for IgG. However, when analyzing with sera from mild patients taken > 15 days post onset of symptoms, a wide range of sensitivities for IgG were observed, varying from 0%-64%. In these cases none of the tests fulfilled the preset criteria. It should be noted that PCR was the reference test here.

Looking at specificity, (10)(1c) (10)(1c) and (10)(1c) tests did not reach the preset threshold for specificity of IgG. However, the result of 92% specificity was obtained as one of 13 samples in the specificity panel gave a false-positive result. To determine more precisely the specificity of these and the other four tests it is absolutely required that a larger specificity panel is tested.

When comparing the (10)(1c) performance of the rapid tests vs the (10)(1c) test and specifically those sera that were positive in both the (10)(1c) and the neutralization test (indicated as high pos for the (10)(1c) test), it was observed that the (10)(1c) test showed the best performance with 14 of 16 (87.5%) samples with a neutralization titer found positive. This was closely followed by (10)(1c) and (10)(1c) (both 13 of 15; 86.7%), (10)(1c) 12 of 16; 75%) and (10)(1c) (11 of 16; 68.7%).

Based on the data presented here we would advise to select the (10)(1c) (10)(1c) and (10)(1c) *for further validation* for *IgG detection*. For combined use for IgG and IgM testing the Intec test has the best qualifications to be validated further although in the current limited study not fulfilling the preset minimal standards for patient diagnostics. Further validation with larger well defined sample sets is an absolute necessity for a more precise determination of test specifics while test performances need to be interpreted in the light of the rapidly increasing information on antibody kinetics in different (sub) clinical patient cohorts.

None of the seven tests fulfilled the preset minimal requirements for test specificity and sensitivity for the three different operational contexts that were defined. These data underline the importance of extensive validation in the right (sub)populations and settings to avoid guidance of control efforts at individual and population level based on false diagnosis of individuals. Until extensive validation gives evidence that the accuracy of the tests is high in specific populations and settings, it is not appropriate to use rapid immuno-assays for clinical decision making, to guide dedicated measures for specific subpopulation and to guide general control measures.

VS 2.0 12 April 2020,

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