Clinical sensitivity and specificity of three rapid SARS-CoV-2 Antibody (IgM/IgG) Tests on a hospitalized patient cohort: InTec, Cellex and OrientGene

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1. Background

Serological detection of COVID-19 is key to see whether infection has already taken place however whether this also correlate with protection we still do not know. In addition to serological testing there is big urgency to have validated rapid diagnostic test (RDT) ready to be rolled out if found to be suitably sensitive and specific to test large populations quickly. It should be noted that there are limited data on whether immune responses will be the same in all patients independent of severity of illness.
There are countless RDTs developed/in development and offered to diagnostic laboratories. We have used the following criteria to consider inclusion of a RDT in our validation as the capacity of testing and clinical samples are limited:

1. A wide range of diagnostic tests are commercially available for SARS-CoV-2 (list collated by FIND - https://www.finddx.org/covid-19/pipeline/?section=immunoassays#diag_tab), some of which have received authorizations for use by various national regulatory agencies like CE marking or FDA approval. Checking whether the company had a product already prequalified in the WHO PQ scheme can ensure high QC in place.
2. Due to the pandemic situation high and continuous quantities should to be available within a short period eg a week.
3. Manufacturer should provide all paperwork for their validation studies.
4. Manufacturer should provide relevant details about the test details eg antigen used for a serological assay.
5. Specificity and sensitivity should be within an acceptable range; and it is important to check on which population the validation was done eg hospitalized patients, ambulant patients. Relevant controls should have been included eg healthy population and other infections with potential differential diagnosis and cross-reactive nature.
6. Right to share and publish data from validation/comparisons should be clarified.

We have selected InTec, Cellex and OrientGene tests as they fulfilled most criteria, and were available in big quantities enough to perform validation on a bigger sample set. InTec utilizes the N antigen, Cellex a combination of N and S; this information is not provided by OrientGene.

2. Purpose

This study was conducted at Erasmus MC viroscience, Rotterdam, NL between March 3, 2020 to analyze the clinical sensitivity and specificity of the following rapid tests:

1, *Rapid SARS-CoV-2 Antibody (IgM/IgG) Test* of InTec Product, Inc.

2, qSARS-CoV-2 IgG/IgM Cassette Rapid Test (GICA) of Cellex Inc.

3, COVID-19 IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma) Orient Gene / Healgen

3. Sample and reagent

- 93 serum samples from 24 PCR (1) confirmed COVID-19 patients at various time point post symptom onset.
- 1, kits of *Rapid SARS-CoV-2 Antibody (IgM/IgG) Test* of lot S2020021505. Expiry date: 14-08-2020
- 2, qSARS-CoV-2 IgG/IgM Cassette Rapid Test (GICA) of Cellex Inc. Test lot 20200311WI5513C-3. Expiry date: 3-9-2022
- 3, COVID-19 IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma) Orient Gene / Healgen. Test lot 2003260; Expiry date: 2022-03

Table 1. Sample panel used to validate the sensitivity and specificity of the antibody RDT for SARS-CoV-2

		Sensitivity		
Country	**Sample source**	**Infection**	**No. samples**	**post symptom onset range**
Netherlands	RT-PCR confirmed SARS-CoV-2	Mild/moderate	15	1-24
		Severe	78	1-43
		Specificity		
Netherlands	Healthy blood donors	NA	11	NA
Netherlands	Non-CoV respiratory infections*	Adeno virus	1	2-4weeks
		HMPV	3	2-4weeks
		Flu A	4	2-4weeks
		Flu B	4	2-4weeks
		RSV A	4	2-4weeks
		RSV B	4	2-4weeks
		CMV	2	2-4weeks
		EBV	3	2-4weeks
		Myco	1	2-4weeks
		Rhino virus	2	2-4weeks
Netherlands	hCoV infections	HCoV 229E	(6).	2-4weeks
		HCoV-NL63	(7).	2-4weeks
		HCoV-OC43	(9).	2-4weeks
		MERS	(3).	2-4weeks

* numbers were limited due to RDT kit availability

Equipment:

- Disposables including tips for the pipette
- Manual pipette
- Timer

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4. Test principle

The tests were operated according to the test inserts. Samples were collected from COVID-19 suspected patients at Erasmus MC for diagnostic purpose and following PCR confirmation residual sera/plasma was used for this RDT evaluation. Patients were mostly moderate/seriously ill (15 ICU, 7 moderate, 2 mild).
Each sample was tested by one test and interpreted by two operators in parallel from Erasmus MC.

5. Test results and data analysis

Various samples showed (false)negative results with the tests compared to RT-PCR results (**Table 3, 4, 5**) and $PRNT_{50}$ neutralization (**Table 6**).

Possible reasons for the (false)negative results could be:

- The patients were at the very early infection stage, there was no antibody generated.
- Antibody concentration is below the limit of detection of the test.
- Neither IgG nor IgM to SARS-CoV-2 is present in the patient's sample that react with specific antigens utilized in the assay configuration.
- Extremely high concentrations of IgM and IgG which could have caused hook or prozone effect but it is unlikely in polyclonal responses.
- Unknown interference.

Table 3a Clinical sensitivity/specificity of the InTec test on SARS-CoV-2/other samples collected after 7 days from the symptom onset

		PCR		Total
		Positive	Negative	
Rapid SARS-CoV-2 Antibody (IgM/IgG) Test	Positive	67	17	84
	Negative	4	47	51
Total		71	64	135

The sensitivity on samples collected after 7 days from the symptom onset is 94.67% (95%CI: 86.90% to 98.53%) and the specificity is 79.01% (68.54% to 87.27%).

Table 3b Overall clinical sensitivity/specificity of InTec test on SARS-CoV-2/other samples

		PCR		Total
		Positive	Negative	
Rapid SARS-CoV-2 Antibody (IgM/IgG) Test	Positive	83	17	100
	Negative	10	47	57
Total		93	64	157

The sensitivity on all collected samples is 90.29% (95%CI:82.87% to 95.25%) and the specificity is 79.01% (68.54% to 87.27%).

Table 3c Overall clinical sensitivity/specificity of the IgG test on SARS-CoV-2/other samples

	1-7		**7- 14**		**>14**		
PCR	**IgG +**	**IgG -**	**IgG +**	**IgG -**	**IgG +**	**IgG -**	**Total**
	13	9	42	4	24	1	
Positive	22		46		25		93
Negative	Days since symptom onset not taken into account						
			IgG +	**IgG -**			
			9	55			
			64				64
							157

The sensitivity of IgG on all collected samples is 86.11% (95%CI: 78.13% - 92.01%%) and the specificity is 87.67% (77.88% -94.20%).

Table 3d Overall clinical sensitivity/specificity of the IgM test on SARS-CoV-2/other samples

	1-7		**7- 14**		**>14**		
PCR	**IgM +**	**IgM -**	**IgM +**	**IgM -**	**IgM +**	**IgM -**	**Total**
	12	10	40	6	20	5	
Positive	22		46		25		93
Negative	Days since symptom onset not taken into account						
			IgM +	**IgM -**			
			13	51			
			64				64
							157

The sensitivity of IgM on all collected samples is 81.58% (95%CI: 73.23% to 88.22%%) and the specificity is 83.12% (72.86% to 90.69%).

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Table 4a Clinical sensitivity/specificity of the Cellex test on SARS-CoV-2/other samples collected after 7 days from the symptom onset

		PCR		Total
		Positive	Negative	
Rapid SARS-CoV-2 Antibody (IgM/IgG) Test	Positive	62	3	65
	Negative	9	41	50
Total		71	44	115

The sensitivity on samples collected after 7 days from the symptom onset is 88.75% (95%CI:79.72-94.72%) and the specificity is 93.62% (82.46% to 98.66%).

Table 4b Overall clinical sensitivity/specificity of the Cellex test on SARS-CoV-2/other samples

		PCR		Total
		Positive	Negative	
Rapid SARS-CoV-2 Antibody (IgM/IgG) Test	Positive	69	3	72
	Negative	24	41	65
Total		93	44	137

The sensitivity on all collected samples is 79.49% (95%CI:71.03% to 86.39%) and the specificity is 93.62% (82.46% to 98.66%).

Table 4c Overall clinical sensitivity/specificity of the IgG test on SARS-CoV-2/other samples

	1-7		**7- 14**		**>14**		
PCR	**IgG +**	**IgG -**	**IgG +**	**IgG -**	**IgG +**	**IgG -**	**Total**
	7	15	34	12	24	1	
Positive	22		46		25		93
Negative	Days since symptom onset not taken into account						
			IgG +	**IgG -**			
			2	42			
			44				44
							137

The sensitivity of IgG on all collected samples is 76.86% (95%CI: 68.32%-84.04%) and the specificity is 95.65% (85.16% to 99.47%).

Table 4d Overall clinical sensitivity/specificity of the IgM test on SARS-CoV-2/other samples

	0- 7		**7- 14**		**>14**		
PCR	**IgM +**	**IgM -**	**IgM +**	**IgM -**	**IgM +**	**IgM -**	**Total**
	7	15	38	8	24	1	
Positive	22		46		25		93
Negative	Days since symptom onset not taken into account						
			IgM +	**IgM -**			
			3	41			
			44				44
							137

The sensitivity of IgM on all collected samples is 79.49% (95%CI: 71.03% to 86.39%) and the specificity is 93.62% (82.46% to 98.66%.)

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Table 5a Clinical sensitivity/specificity of the OrientGene test on SARS-CoV-2/other samples collected after 7 days from the symptom onset

		PCR		Total
		Positive	Negative	
Rapid SARS-CoV-2 Antibody (IgM/IgG) Test	Positive	60	0	60
	Negative	9	9	18
Total		69	9	78

The sensitivity on samples collected after 7 days from the symptom onset is 88.46% (79.22%-94.59%) and the specificity is 100% (66.37%-100%) however specificity is based on too few samples due to limited access to tests.

Table 5b Overall clinical sensitivity/specificity of the OrientGene test on SARS-CoV-2/other samples

		PCR		Total
		Positive	Negative	
Rapid SARS-CoV-2 Antibody (IgM/IgG) Test	Positive	72	0	72
	Negative	18	9	27
Total		90	9	99

The sensitivity on all collected samples is 83.33% (74.94% - 89.81%) and the specificity is 100% (66.37%-100%) however specificity is based on too few samples due to limited access to tests.

5c Overall clinical sensitivity/specificity of the IgG test on SARS-CoV-2/other samples

	1-7		**7- 14**		**>14**		
PCR	**IgG +**	**IgG -**	**IgG +**	**IgG -**	**IgG +**	**IgG -**	**Total**
	10	11	32	12	25	0	
Positive	21		44		25		90
Negative	Days since symptom onset not taken into account						
			IgG +	**IgG -**			
			0	9			
			9				9
							99

The sensitivity of IgG on all collected samples is 79.65% (95%CI: 71.04% -86.64%), specificity is 100% (66.37% -100%).

Table 5d Overall clinical sensitivity/specificity of the IgM test on SARS-CoV-2/other samples

	1-7		**7- 14**		**>14**		
PCR	**IgM +**	**IgM -**	**IgM +**	**IgM -**	**IgM +**	**IgM -**	**Total**
	12	9	34	10	18	7	
Positive	21		44		25		90
Negative	Days since symptom onset not taken into account						
			IgM +	**IgM -**			
			0	9			
			9				9
							99

The sensitivity of IgM on all collected samples is 77.59% (68.91% to 84.81%), 100.00% (66.37%- 100%).

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7. Correlation with neutralization

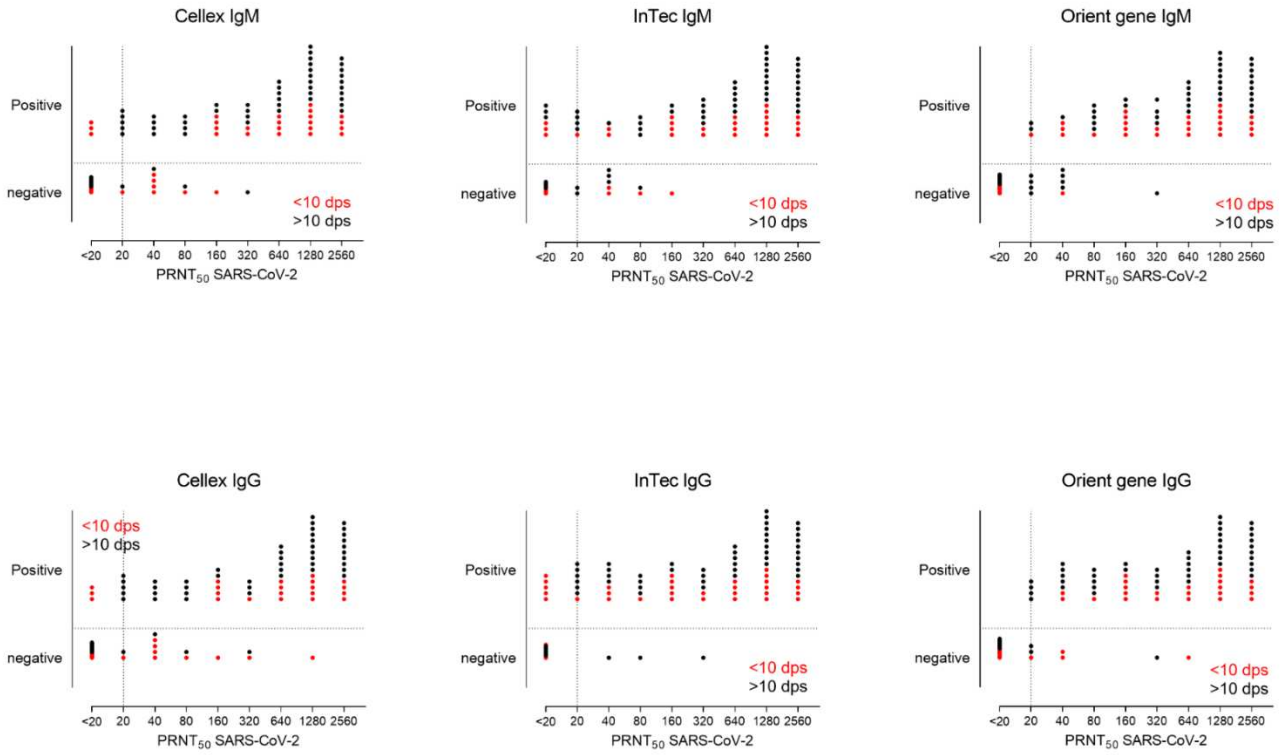

Table 6. Sensitivity and specificity of the three tested RDTs (CI 95%) compared to $PRNT_{50}$ results, whole sample set and samples >10 days post symptom onset (borderline $PRNT_{50}$ values counted as positive).

	Cellex		InTec		Orient gene	
	IgM	**IgG**	**IgM**	**IgG**	**IgM**	**IgG**
Sensitivity-overall	**87.36%** (78.50% to 93.52%)	**84.44%** (75.28% to 91.23%)	**88.37%** (79.65% to 94.28%)	**95.00%** (87.69% to 98.62%)	**89.41%** (80.85% to 95.04%)	**91.57%** (83.39% to 96.54%)
Sensitivity >10 DPO	**98.08%** (89.74% to 99.95%)	**96.23%** (87.02% to 99.54%)	**91.07%** (80.38% to 97.04%)	**98.08%** (89.74% to 99.95%)	**86.44%** (75.02% to 93.96%)	**94.44%** (84.61% to 98.84%)
Specificity - overall	**80.95%** (58.09% to 94.55%)	**85.00%** (62.11% to 96.79%)	**73.91%** (51.59% to 89.77%)	**77.27%** (54.63% to 92.18%)	**100.00%** (80.49% to 100.00%)	**100.00%** (80.49% to 100.00%)

8. Conclusion

According to the test results of the 93 samples from PCR confirmed COVID-19 patients, the sensitivity/specificity of the tests are:

- **InTec *Rapid SARS-CoV-2 Antibody (IgM/IgG) Test*** has an overall sensitivity of 90.29% (95% CI: 82.87% to 95.25%) and specificity of 79.01% (68.54% to 87.27%). The sensitivity on samples collected after 7 days from the symptom on set is 94.67% (95%CI:86.90% to 98.53%), specificity is 79.01% (68.54% to 87.27%).
- **Cellex qSARS-CoV-2 IgG/IgM Cassette Rapid Test (GICA)** has an overall sensitivity of 79.49% (95%CI:71.03% to 86.39%) and specificity of 93.62% (82.46% to 98.66%). The sensitivity on samples collected after 7 days from the symptom on set is 88.75% (95%CI:79.72-94.72%), specificity is 93.62% (82.46% to 98.66%).
- **Orient Gene/Healgen COVID-19 IgG/IgM Rapid Test Cassette** has an overall sensitivity of 83.33% (74.94% to 89.81%) and specificity of 100.00% (66.37%-100%). The sensitivity on samples collected after 7 days from the symptom on set is 88.46% (79.22% to 94.59%), specificity is 100% (66.37%-100.00%).

Compared to RT-PCR, InTec product showed the highest overall sensitivity followed by OrientGene and Cellex. Samples >7 days post symptom onset were detected more often.

Caveat of the specificity owing to limited availability of test kits, the validation was limited on this point. However based on observations above we would recommend further testing on all three tests.
For neutralization, samples taken >10 days post onset correlate well with neutralization activity with all tests.
Evaluation should also be carried out with samples from asymptomatic/mild population.

9. References

1, https://eurosurveillance.org/content/10.2807/1560-7917.ES.2020.25.3.2000045

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Summary

93 Samples tested from confirmed positive donors, who were hospitalized, and 44 negatives from healthy blood donors.

- Non-human coronavirus infections tested for specificity:
- Adenovirus
- Human Metapneumovirus (HMPV)
- Influenza A
- Influenza B
- Respiratory Syncytial Virus A
- Respiratory Syncytial Virus B
- Cytomegalovirus (CMV)
- Epstein-Barr
- Mycoplasma
- Rhinovirus
- Other human coronavirus [HCoV] strains were also tested for specificity:
- HCov 229E (alpha coronavirus)
- HCoV-NL63 (alpha coronavirus)
- HCoV-OC43 (beta coronavirus)
- MERS-CoV
- Healgen Sensitivity 83.33% and Specificity 100%

Overall Clinical Sensitivity/Specificity

Company/Product	**Healgen**	**Cellex**	**InTec**
Overall Sensitivity %	**83.33**	**79.49**	**90.29**
Overall Specificity %	**100**	**93.6**	**79.01**

Sensitivity: Probability the test will be positive when disease is present.
Specificity: Probability will be negative when the target disease is not present.

Conclusion

Our Healgen (Orient Gene) COVID-19 Rapid Test performed ***better*** than Cellex which has been granted FDA Emergency Use Authorization (EUA).

Healgen's (OrientGene's) product was the only test with *100%* specificity and demonstrates *no* cross-reactivity with the listed human coronavirus strains.