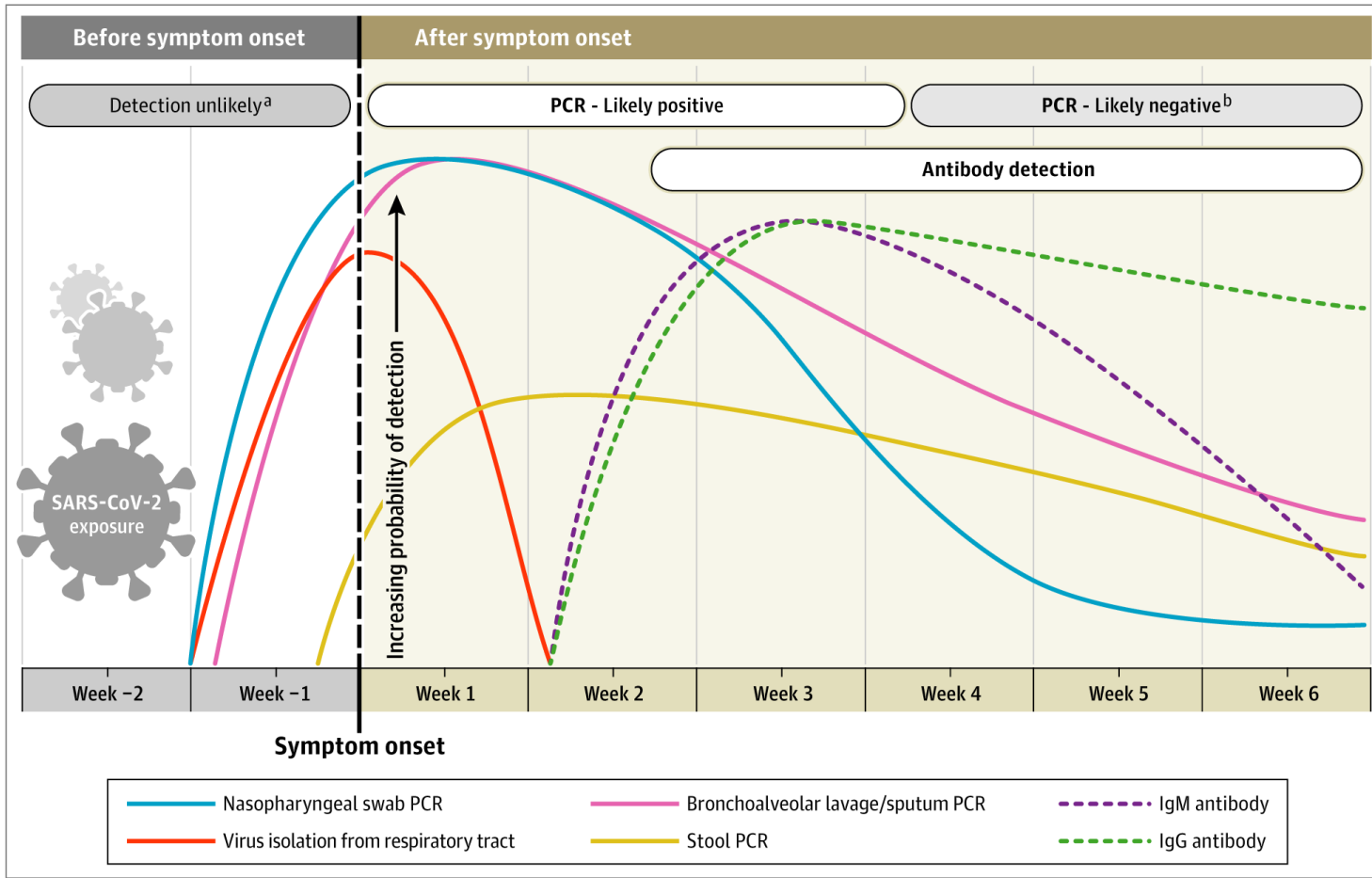


Use of Whole Genome Sequencing data for in silico specificity evaluation of the RT-qPCR assays used for SARS-CoV-2 detection.

Mathieu Gand, Kevin Vanneste, Isabelle Thomas, Steven Van Gucht, Arnaud Capron, Philippe Herman, Nancy H.C. Roosens and Sigrid C.J. De Keersmaecker

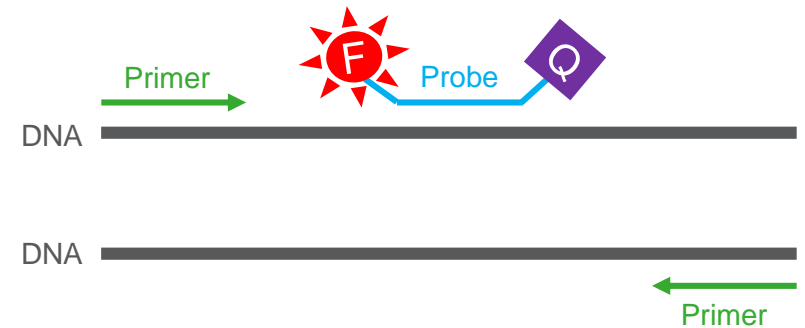
COVID-19 diagnosis



^a Detection only occurs if patients are followed up proactively from the time of exposure.

^b More likely to register a negative than a positive result by PCR of a nasopharyngeal swab.

Primary detection method: RT-qPCR



Specificity of RT-qPCR methods:

- Not extensively validated in the wet-lab
- Can be affected by virus mutations
- Mismatches affect test's sensitivity

Original Research | 18 August 2020

Variation in False-Negative Rate of Reverse Transcriptase Polymerase Chain Reaction-Based SARS-CoV-2 Tests by Time Since Exposure

Lauren M. Kucirka, MD, PhD, Stephen A. Lauer, PhD, Oliver Laeyendecker, PhD, MBA, Denali Boon, PhD,

SCREENED: polymerase Chain Reaction Evaluation through large-scale mining of genomic Data

ID	RT-qPCR tests
1	China CDC (China)*
2	Charité Hospital (Germany)*
3	Institut Pasteur (France)*
4	US CDC (USA)*
5	NIID, Japan*
6	HKU Med (Hong-Kong)*
7	NIH, Thailand*
8	Chan <i>et al.</i> , 2020
9	Lu <i>et al.</i> , 2020
10	Won <i>et al.</i> , 2020
11	Sigma-Aldrich 2020
12	Huang <i>et al.</i> , 2020

* WHO recommended assays

2569 representative SARS-CoV-2 genomes
2424 representative non SARS-CoV-2 genomes



Primers and probe sequences from an RT-qPCR assay

30 primers & probe sets

Bioinformatics tool
SCREENED

Whole Genome Sequence data

Amplicon clustering

Extraction of the amplified sequence

Check for mismatches between primers, probe and their template

No more than 2 mismatches
No mismatch in 3' end



Working document of Commission services

Current performance of COVID-19 test methods and devices and proposed performance criteria

Theoretical RT-qPCR outcome

In silico evaluation of RT-qPCR assays used for SARS-CoV-2 detection

ID	RT-qPCR tests	Target	Inclusivity
1	China CDC (China)*	ORF1ab	99,96%
		N	86,03%
2	Charité Hospital (Germany)*	RdRp-1	99,96%
		RdRp-2	100%
		E	100%
		N	99,81%
3	Institut Pasteur (France)*	RdRp_IP2	99,88%
		RdRp_IP4	100%
		E_Sarbeco	100%
4	US CDC (USA)*	N1	99,73%
		N2	99,96%
		N3	100%
5	NIID, Japan*	N	99,69%
6	HKU Med (Hong-Kong)*	ORF1b-nsp14	100%
		N	99,92%
7	NIH, Thailand*	N	99,73%
8	Chan et al., 2020	RdRp_hel	100%
		S	100%
		N	99,96%
9	Lu et al., 2020	ORF1a	100%
10	Won et al., 2020	RdRp	99,69%
		S	100%
		E	100%
		N	99,96%
11	Sigma-Aldrich 2020	N-1	99,69%
		N-2	99,69%
		ORF1ab-3	100%
		ORF1ab-4	100%
		S-5	99,57%
		S-6	99,57%
12	Huang et al., 2020	E	100%

Inclusivity:

- Tested on 2569 SARS-CoV-2 genomes
- Overall good inclusivity except for China CDC assay targeting N

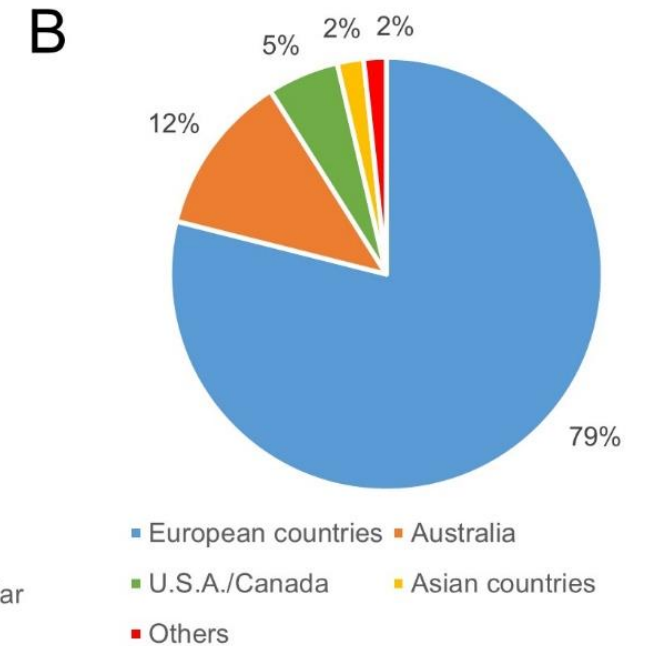
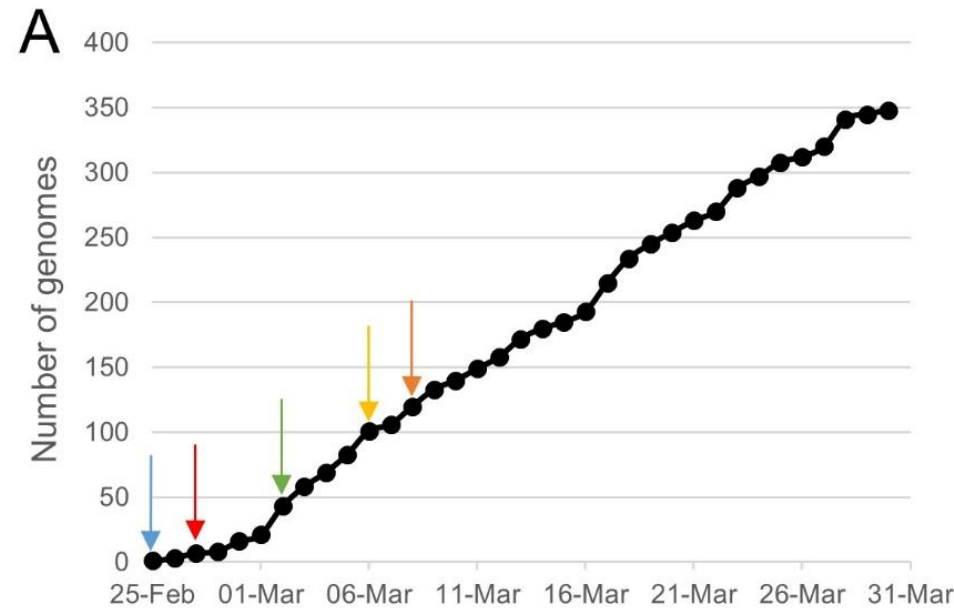
* WHO tests
 Green: 100%; Orange: > 99%; Red: < 99%

In silico evaluation of RT-qPCR assays used for SARS-CoV-2 detection

ID	RT-qPCR tests	Target	Inclusivity
1	China CDC (China)*	ORF1ab	99,96%
		N	86,03%
2	Charité Hospital (Germany)*	RdRp-1	99,96%
		RdRp-2	100%
		E	100%
		N	99,81%
3	Institut Pasteur (France)*	RdRp_IP2	99,88%
		RdRp_IP4	100%
		E_Sarbeco	100%
4	US CDC (USA)*	N1	99,73%
		N2	99,96%
		N3	100%
5	NIID, Japan*	N	99,69%
6	HKU Med (Hong-Kong)*	ORF1b-nsp14	100%
		N	99,92%
7	NIH, Thailand*	N	99,73%
8	Chan et al., 2020	RdRp_hel	100%
		S	100%
		N	99,96%
9	Lu et al., 2020	ORF1a	100%
10	Won et al., 2020	RdRp	99,69%
		S	100%
		E	100%
		N	99,96%
11	Sigma-Aldrich 2020	N-1	99,69%
		N-2	99,69%
		ORF1ab-3	100%
		ORF1ab-4	100%
		S-5	99,57%
	S-6	99,57%	
12	Huang et al., 2020	E	100%

Inclusivity:

- Tested on 2569 SARS-CoV-2 genomes
- Overall good inclusivity except for China CDC assay targeting N



Forward primer 5' GGGGAAGTTCTCCTGCTAGAAT 3'
 Mismatches 5' **AAC**GAACTTCTCCTGCTAGAAT 3'

* WHO tests
 Green: 100%; Orange: > 99%; Red: < 99%

In silico evaluation of RT-qPCR assays used for SARS-CoV-2 detection

ID	RT-qPCR tests	Target	Inclusivity	Exclusivity
1	China CDC (China)*	ORF1ab	99,96%	100%
		N	86,03%	100%
2	Charité Hospital (Germany)*	RdRp-1	99,96%	100%
		RdRp-2	100%	99,75%
		E	100%	100%
		N	99,81%	100%
3	Institut Pasteur (France)*	RdRp_IP2	99,88%	100%
		RdRp_IP4	100%	100%
		E_Sarbeco	100%	100%
4	US CDC (USA)*	N1	99,73%	100%
		N2	99,96%	100%
		N3	100%	100%
5	NIID, Japan*	N	99,69%	100%
6	HKU Med (Hong-Kong)*	ORF1b-nsp14	100%	100%
		N	99,92%	100%
7	NIH, Thailand*	N	99,73%	100%
8	Chan et al., 2020	RdRp_hel	100%	100%
		S	100%	100%
		N	99,96%	100%
9	Lu et al., 2020	ORF1a	100%	100%
10	Won et al., 2020	RdRp	99,69%	100%
		S	100%	100%
		E	100%	92,32%
		N	99,96%	99,92%
11	Sigma-Aldrich 2020	N-1	99,69%	100%
		N-2	99,69%	100%
		ORF1ab-3	100%	100%
		ORF1ab-4	100%	100%
		S-5	99,57%	100%
		S-6	99,57%	100%
12	Huang et al., 2020	E	100%	92,41%

Inclusivity:

- Tested on 2569 SARS-CoV-2 genomes
- Overall good inclusivity except for China CDC assay targeting N

Exclusivity:

- Tested on 2424 non SARS-CoV-2 genomes including:
 - SARS-CoV, MERS-CoV, and other coronaviruses from various host species
 - Other common respiratory viruses
 - Human reference genome
- **Blue arrows** = positive results with SARS-related coronavirus and batcoronavirus genomes according to SCREENED

* WHO tests
Green: 100%; Orange: > 99%; Red: < 99%

In silico evaluation of RT-qPCR assays used for SARS-CoV-2 detection

ID	RT-qPCR tests	Target	Inclusivity	Exclusivity
1	China CDC (China)*	ORF1ab	99,96%	100%
		N	86,03%	100%
2	Charité Hospital (Germany)*	RdRp-1	99,96%	100%
		RdRp-2	100%	99,75%
		E	100%	100%
		N	99,81%	100%
3	Institut Pasteur (France)*	RdRp_IP2	99,88%	100%
		RdRp_IP4	100%	100%
		E_Sarbeco	100%	100%
4	US CDC (USA)*	N1	99,73%	100%
		N2	99,96%	100%
		N3	100%	100%
5	NIID, Japan*	N	99,69%	100%
6	HKU Med (Hong-Kong)*	ORF1b-nsp14	100%	100%
		N	99,92%	100%
7	NIH, Thailand*	N	99,73%	100%
8	Chan et al., 2020	RdRp_hel	100%	100%
		S	100%	100%
		N	99,96%	100%
9	Lu et al., 2020	ORF1a	100%	100%
10	Won et al., 2020	RdRp	99,69%	100%
		S	100%	100%
		E	100%	92,32%
		N	99,96%	99,92%
11	Sigma-Aldrich 2020	N-1	99,69%	100%
		N-2	99,69%	100%
		ORF1ab-3	100%	100%
		ORF1ab-4	100%	100%
		S-5	99,57%	100%
		S-6	99,57%	100%
12	Huang et al., 2020	E	100%	92,41%

Inclusivity:

- Tested on 2569 SARS-CoV-2 genomes
- Overall good inclusivity except for China CDC assay targeting N

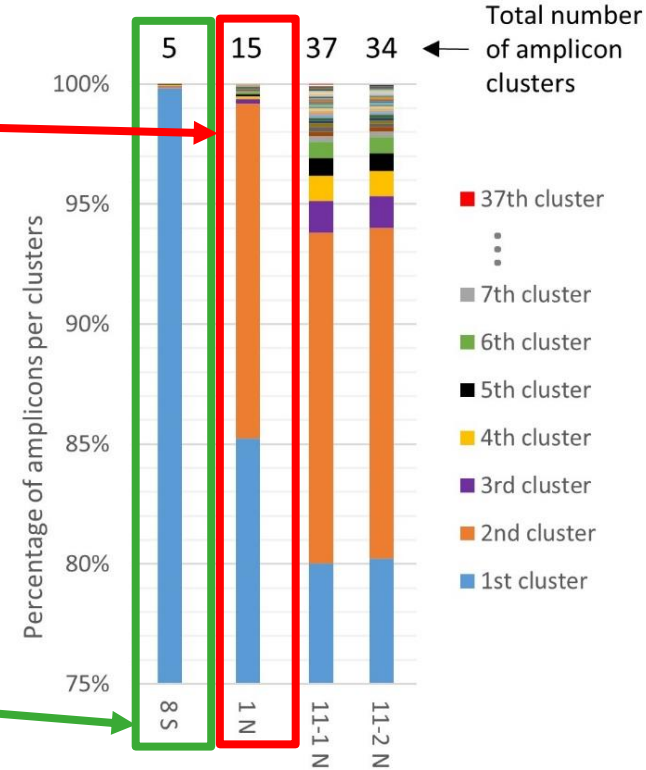
Exclusivity:

- Tested on 2424 non SARS-CoV-2 genomes including:
 - SARS-CoV, MERS-CoV, and other coronaviruses from various host species
 - Other common respiratory viruses
 - Human reference genome
- **Blue arrows** = positive results with SARS-related coronavirus and batcoronavirus genomes according to SCREENED

Best RT-qPCR assay: Assay 8 targeting the gene S

In silico evaluation of RT-qPCR assays used for SARS-CoV-2 detection

ID	RT-qPCR tests	Target	Inclusivity	Exclusivity
1	China CDC (China)*	ORF1ab	99,98%	100%
		N	86,03%	100%
2	Charité Hospital (Germany)*	RdRp-1	99,98%	100%
		RdRp-2	100%	99,75%
		E	100%	100%
3	Institut Pasteur (France)*	N	99,81%	100%
		RdRp_IP2	99,88%	100%
		RdRp_IP4	100%	100%
4	US CDC (USA)*	E_Sarbeco	100%	100%
		N1	99,73%	100%
		N2	99,96%	100%
5	NIID, Japan*	N3	100%	100%
		N	99,69%	100%
		ORF1b-nsp14	100%	100%
6	HKU Med (Hong-Kong)*	N	99,92%	100%
		N	99,73%	100%
8	Chan et al., 2020	RdRp_nel	100%	100%
		S	100%	100%
9	Lu et al., 2020	N	99,98%	100%
		ORF1a	100%	100%
10	Won et al., 2020	RdRp	99,69%	100%
		S	100%	100%
		E	100%	92,32%
		N	99,96%	99,92%
11	Sigma-Aldrich 2020	N-1	99,69%	100%
		N-2	99,69%	100%
		ORF1ab-3	100%	100%
		ORF1ab-4	100%	100%
		S-5	99,57%	100%
12	Huang et al., 2020	S-6	99,57%	100%
		E	100%	92,41%



Evaluated primers and probe sets

Best RT-qPCR assay: Assay 8 targeting the gene S

* WHO tests
Green: 100%; Orange: > 99%; Red: < 99%

Conclusions

- SCREENED: powerful tool for in silico specificity evaluation of RT-qPCR tests
 - can be used for PCR-like methods development as well as regular re-evaluation
 - save time and money as same evaluation in the wet-lab would be not feasible
- Real impact of the mismatches identified with SCREENED must be checked in the wet-lab
- Overall specificity of the tests was good but this can change in the future with the introduction of a treatment or a vaccine?

Thank you for your attention!

Contact

Mathieu Gand • mathieu.gand@sciensano.be

Full details of the study in:




Int. J. Mol. Sci. 2020, 21, 5585; doi:10.3390/ijms21155585



Article

Use of Whole Genome Sequencing Data for a First in Silico Specificity Evaluation of the RT-qPCR Assays Used for SARS-CoV-2 Detection

Mathieu Gand ¹, Kevin Vanneste ¹, Isabelle Thomas ², Steven Van Gucht ², Arnaud Capron ³, Philippe Herman ⁴, Nancy H. C. Roosens ^{1,†}  and Sigrid C. J. De Keersmaecker ^{1,*,†} 