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

RIVM
Centre for Infectious Disease Control

Evaluation of NaGene COVID-2019 direct PCR kit for SARS-CoV-2 detection

Interim Report

Authors:
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Questions:

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Pro-Med Diagnostics

>95% sensitivity with low viral load

We want to understand from the RIVM data
1. Viral load and associated sensitivity (ie with dilutions, what happens to sensitivity). This would be for sample to swab for Nagene complete process (no in-house VTM, Nagene used as per manufacturer label)
2. Viral load and associated sensitivity (ie with dilution, what happens to sensitivity). This would be sample to GLY (VTM) to Nagene PB to PCR test with Nagene

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Main findings

The NaGene COVID2019 direct-PCR kit detects SARS-CoV-2 positive clinical specimens collected in GLY transport medium with a Ct value below approx. 31 in our in-house reference qRT-PCR.

The NaGene assay is partially inhibited by the currently used GLY transport medium compared to the dedicated Preservation Buffer supplied with the NaGene kit.

Using the NaGene kit in combination with GLY transport medium for routine screening during the corona outbreak will result in a roughly estimated percentage false negatives of 20-30% in comparison with our in-house qRT-PCR.

>95% sensitivity with low viral load

Efficiency and LOD95%

Using previously extracted SARS-CoV-2 RNA directly in the NaGene assay (10 µl) or the in-house E-gene qRT-PCR (5 µl) as reference the LOD95% values for the NaGene N-gene and ORF1ab-gene assays were a factor 10 and 20 higher than the reference (Table 1). **Amplification efficiency estimation indicated reduced efficiency compared to that for the in-house E-gene assay, which might have been caused by not including PB in the NaGene qRT-PCR reaction for which it has been optimized**.

Therefore we analyzed the amplification efficiency using two SARS-CoV-2 positive clinical specimens diluted in PB and subjected to the full NaGene protocol including non-extraction lysis to release virus RNA (Table 1).

Also here the NaGene assays showed reduced amplification efficiency compared to the in-house E-gene assay.

This does however not necessarily mean reduced clinical sensitivity.

Table 1. Determination of amplification efficiency and LOD95%.

Source material	Specimen diluted in	PCR assay	Efficiency			LOD95% (95% CI) dcopies/ml
			Slope	E	R^2	
SARS-CoV-2 RNA	Water with yeast RNA	NaGene ORF1ab	-2.161	190	0.76	19.3 (15.4-25.6)
		NaGene N	-2.295	173	0.91	8.0 (7.7-8.3)
		E-gene in-house	-3.269	102	0.99	0.92 (0.74-1.2)
SARS-CoV-2 positive clinical specimen 1	PB	NaGene ORF1ab	-2.189	186	0.98	NA
		NaGene N	-1.901	236	0.97	NA
	GLY, MagNApure extraction	E-gene in-house	-2.893	122	0.99	NA
SARS-CoV-2 positive clinical specimen 2	PB	NaGene ORF1ab	-2.744	128	0.79	NA
		NaGene N	-2.305	172	0.75	NA
	GLY, MagNApure extraction	E-gene in-house	-3.160	107	0.96	NA

PB = NaGene Preservation Buffer; NA = Not Applicable

Effect of transport medium on NaGene assay

To assess the possible negative effect of transport medium itself, we either diluted in GLY or in PB and determined the Ct value at each dilution in the NaGene assay (Fig. 2).
Dilution in GLY medium resulted in loss of signal much more rapidly compared to dilution in PB for both target genes.
Using PB, the NaGene assay appears a factor 10 to 1000 more sensitive than when using GLY medium, indicating GLY affected either the lysis or efficiency of the enzyme or both.

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We compared the Ct values of three clinical specimens with high viral load serially diluted in PB or GLY and then subjected to NaGene or extraction-base qRT-PCR respectively

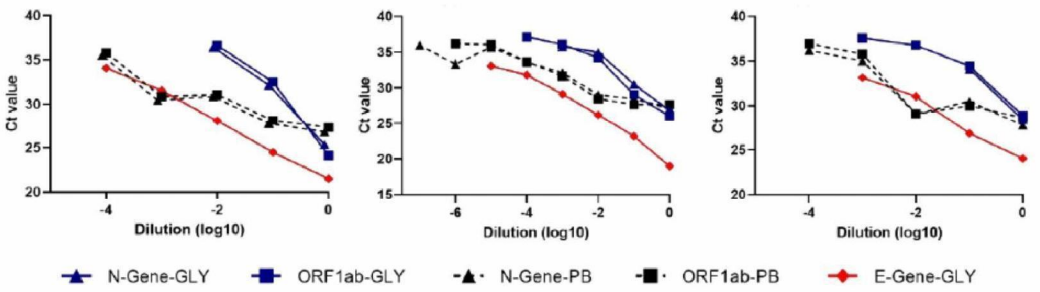

Figure 2. Negative effect of GLY transport medium in NaGene assay and comparison of NaGene assay with extraction-based in-house qRT-PCR.

Three SARS-CoV-2 positive clinical specimens collected in GLY were 10-fold serially diluted in either GLY (blue) or PB (black) as indicated and subsequently assessed for viral RNA by the NaGene assay or the in-house RT-PCR including viral RNA extraction (red). Blue and black lines compare the effect of transport medium and black and red lines compare relative sensitivity of the two strategies; NaGene assay and extraction-based in-house qRT-PCR.

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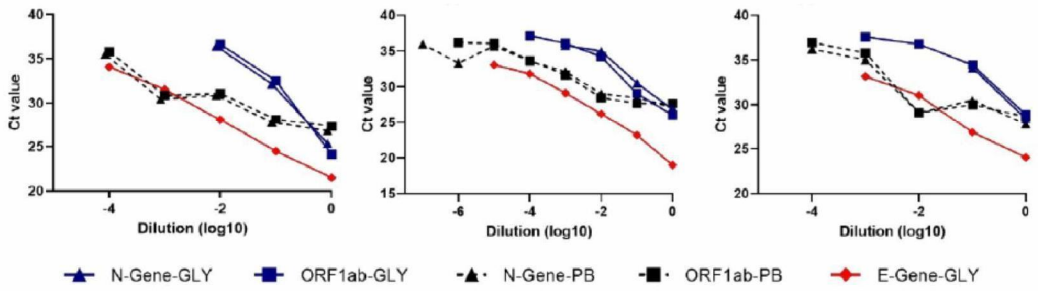
At higher dilutions, Ct values of the two methods approach each other, indicating diluting out the negative effect of GLY in PB in the NaGene assay.



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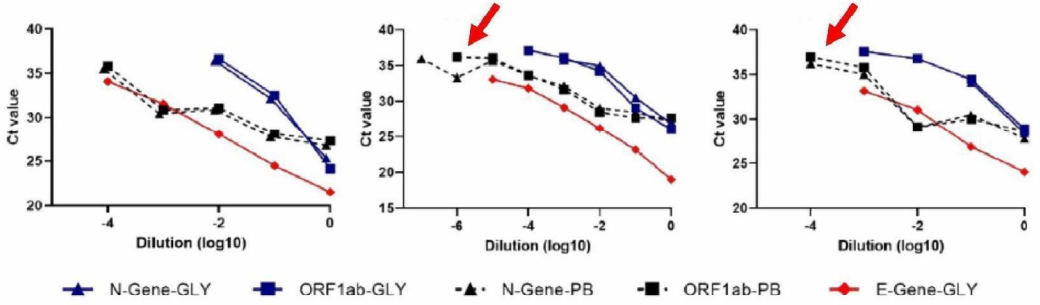
Finally, for two out of the three clinical specimens virus was detected at higher dilutions in the NaGene assay than in the extraction-based in-house qRT-PCR.
This indicates the NaGene assay is possibly more sensitive



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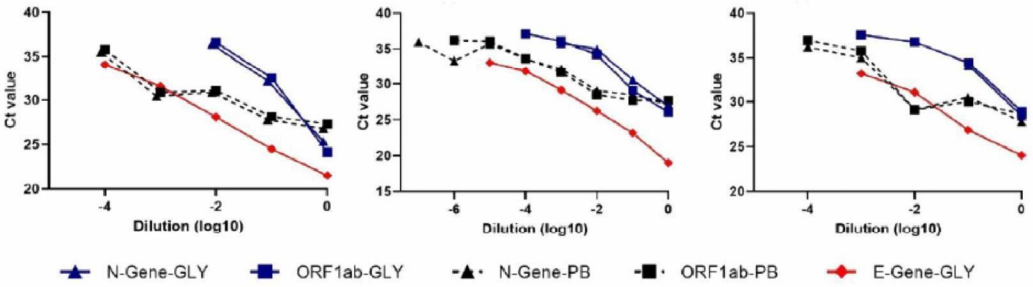
These later two experiments show the potency of the NaGene assay, but that it cannot be combined with specimens collected in GLY transport medium.
Using specimens collected in PB seems a prerequisite for equal performance with the routinely used extraction-based in-house qRT-PCR protocol.



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Influence of transport medium on sensitivity of NaGene assay in a clinical setting

We therefore initiated a small sampling comparison study in which of seven hospitalized COVID-19 patients the nasopharynx was sampled twice, once through the right and once through the left nostril with separate swabs which were collected either in GLY or in PB.

For the NaGene assay, all specimens collected in PB resulted in lower Ct values than those obtained upon collection in GLY (Fig. 3A).
More importantly, one patient was incorrectly classified as negative in the NaGene assay using the specimen collected in GLY, while SARS-CoV-2 was detected in the PB specimen

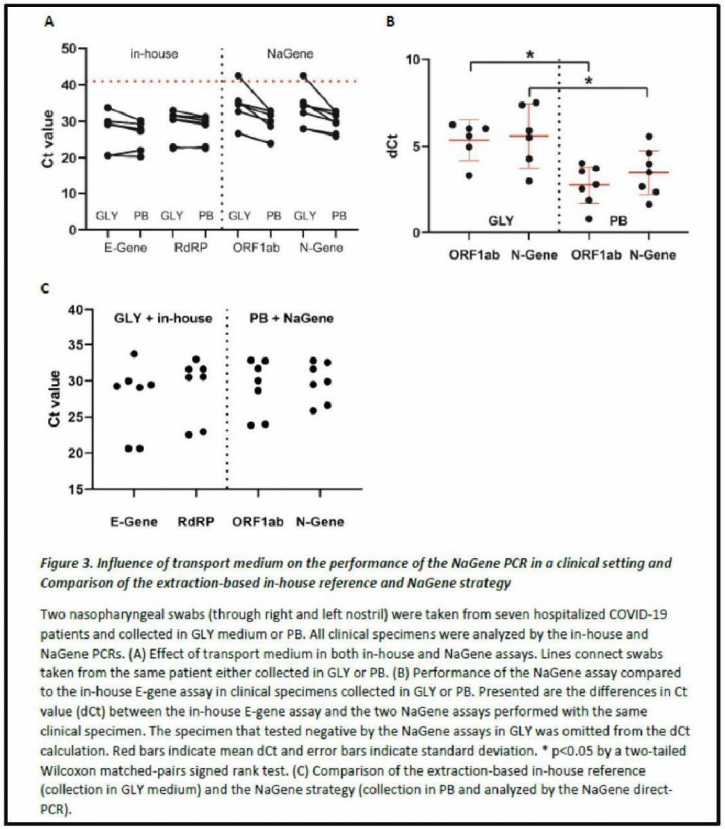
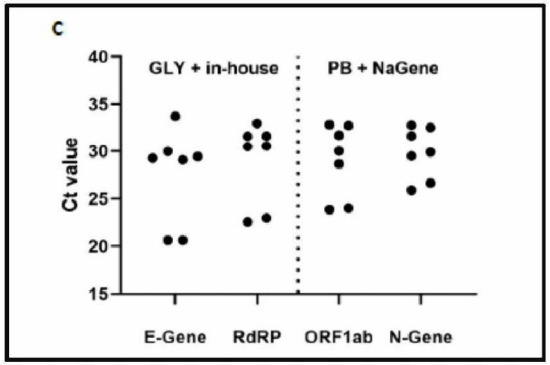


Figure 3. Influence of transport medium on the performance of the NaGene PCR in a clinical setting and Comparison of the extraction-based in-house reference and NaGene strategy

Two nasopharyngeal swabs (through right and left nostril) were taken from seven hospitalized COVID-19 patients and collected in GLY medium or PB. All clinical specimens were analyzed by the in-house and NaGene PCRs. (A) Effect of transport medium in both in-house and NaGene assays. Lines connect swabs taken from the same patient either collected in GLY or PB. (B) Performance of the NaGene assay compared to the in-house E-gene assay in clinical specimens collected in GLY or PB. Presented are the differences in Ct value (dCt) between the in-house E-gene assay and the two NaGene assays performed with the same clinical specimen. The specimen that tested negative by the NaGene assays in GLY was omitted from the dCt calculation. Red bars indicate mean dCt and error bars indicate standard deviation. * $p<0.05$ by a two-tailed Wilcoxon matched-pairs signed rank test. (C) Comparison of the extraction-based in-house reference (collection in GLY medium) and the NaGene strategy (collection in PB and analyzed by the NaGene direct-PCR).

Figure 3C shows that at higher Ct values all assays resulted in comparable Ct values. Only at lower Ct values the E-gene qRT-PCR generated lower Ct values than the NaGene assays.
This is however of less importance in a setting when either a negative or positive result is desired.
Interestingly, one specimen with a Ct value of ~34 by the E-gene in-house qRT-PCR



Interestingly, one specimen with a Ct value of ~34 by the E-gene in-house qRT-PCR was also detected in NaGene assay for both targets, **suggesting comparable clinical sensitivity**

More importantly, the NaGene protocol using specimens collected in PB showed similar performance as our standard extraction-based in-house qRT-PCR protocol.

Conclusion

Direct RT-qPCR may pose an attractive alternative to current extraction-based qRT-PCR used for laboratory confirmation of SARS-CoV-2 infection. A prerequisite for the application of direct qRT-PCR is that the sensitivity is not hampered too much as direct PCRs may be sensitive to inhibitory components of human origin or those present in transport medium. To prevent the latter the NaGene COVID-2019 PCR kit includes a dedicated collection and transport medium the Preservation Buffer (PB). NaGene therefore recommends to evaluate other transport media before being used, or to used purified RNA (removing however the benefit of using a non-extraction protocol). Our evaluation showed clearly that if GLY transport medium is used instead of PB the sensitivity of NaGene RT-PCR is reduced. When we diluted out the standard used GLY transport medium in a SARS-CoV-2 positive clinical specimen with PB, virus was detected at lower dilutions compared to dilution in GLY transport tmedium. As a result, clinical specimens in GLY with a Ct value above 31 as determined by the in-house SARS-CoV-2 qRT-PCR were incorrectly classified negative using the NaGene kit. Therefore, when the NaGene kit would be used during routine screening with the standard procedure of specimens collected in GLY transport medium in the Netherlands during the coronavirus outbreak this would result in an approximate percentage false negatives of 20-30%. Hence, NaGene non-extraction protocol is not suitable to be used with the current practice of collecting specimens in GLY transportmedium in The Netherlands.

To evaluate if collection of specimens in NaGene PB indeed improves the detection rate, a small sampling study was conducted in which we compared the influence of the two transport media in a clinical setting. In nasopharyngeal swab specimens obtained from hospitalized COVID-19 patients, Ct values were lower for the clinical specimens stored in PB compared to another clinical specimen taken from the same patient but collected in GLY medium. Collection in PB also significantly increased the sensitivity compared to the benchmark in-house E-gene qRT-PCR using GLY transport medium. The average Ct difference between E-gene using GLY specimen and NaGene within the same patient was 2.7 (SD 1.1) when the specimen was collected in PB whereas when collected in GLY transport medium the difference was 5.4 Ct (SD 1.1). Thus when considering implementation of the NaGene kit, it would be strongly advised to also implement collection of the swabs in PB. A direct comparison of the current testing strategy with the NaGene strategy including the use of PB showed that there were no remarkable differences between the two strategies. Although a samples with a high Ct (34) in the E-gene assay was also detected in the NaGene assay, more samples in this Ct range are required to better define the performance of the NaGene assay compared to the current approach with boarder-line positive specimens.

Logistically it may be a challenge to change the transport medium in routine diagnostics in The Netherlands. One benefit is that we here showed also that the extraction-based qRT-PCR is not affected by PB and therefore when GLY medium was replaced by PB both extraction-based PCR and the NaGene non-extraction protocol could be used.

In general, one should be cautious when applying the NaGene kit using other transport media, as is also outlined in the kit insert to evaluate first compatibility before being used. At least GLY transport medium appears to affect the sensitivity. However, in combination with the supplied Preservation Buffer the NaGene kit is an attractive alternative to extraction-based qRT-PCR for SARS-CoV-2 detection. Before being implemented for high-throughput use few issues should be addressed like centrifugation capacity and automation of the pipetting steps for adding specimen and the reagents mixture.

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Results

Specificity of NaGene assay

None of the non-coronavirus and non-SARS-COV-2 viral RNAs resulted in a positive signal in the NaGene assay, showing the assay is specific for SARS-CoV-2.

Supplementary table 1. Specificity of the NaGene kit

RNA from clinical specimens positive for indicated pathogen	NaGene	Pathogen-specific Ct
Influenza virus type A	neg	26.82
Influenza virus type A	neg	16.81
RSV A	neg	29.24
RSV B	neg	20.02
Rhinovirus	neg	16.64
Rhinovirus	neg	25.9
Negative control	neg	-
Negative control	neg	-
Human coronavirus viral RNA from cell culture		
HCoV OC43	neg	31.67
HCoV NL63	neg	27.2
SARS-CoV-1	neg	21.6
MERS-CoV	neg	34.16
HCoV 229E	neg	33.18