Rapid, sensitive and specific SARS coronavirus-2 detection: a multi-center comparison between standard qRT-PCR and CRISPR based DETECTR.

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Key points

SARS-CoV-2 detection using DETECTR is for 95% in concordance with qRT-PCR.

DETECTR is highly specific for SARS-CoV-2 and equally sensitive compared to qRT-PCR.

DETECTR-point of care and DETECTR-high throughput represent independent alternatives to qRT-PCR platforms for SARS-CoV-2 detection.

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Abstract

Recent advances in CRISPR-based diagnostics suggest that DETECTR, a combination of isothermal reverse transcriptase loop mediated amplification (RT-LAMP) and subsequent Cas12 bystander nuclease activation by amplicon targeting ribonucleoprotein complexes, could be a faster and cheaper alternative to qRT-PCR without sacrificing sensitivity/specificity.

Here we compare DETECTR with qRT-PCR to diagnose COVID-19 on 378 patient samples.

Patient sample dilution assays suggest a higher analytical sensitivity of DETECTR compared to qRT-PCR, however, this was not confirmed in this large patient cohort, were we report 95% reproducibility between the two tests. These data showed that both techniques are equally sensitive in detecting SARS-CoV-2 providing additional value of DETECTR to the currently used qRT-PCR platforms. For DETECTR, different gRNAs can be used simultaneously to obviate negative results due to mutations in N-gene. Lateral flow strips, suitable as a point of care test (POCT), showed a 100% correlation to the high-throughput DETECTR assay. Importantly, DETECTR was 100% specific for SARS-CoV-2 relative to other human coronaviruses.

As there is no need for specialized equipment, DETECTR could be rapidly implemented as a complementary technically independent approach to qRT-PCR thereby increasing the testing capacity of medical microbiological laboratories and relieving the existent PCR-platforms for routine non-SARS-CoV-2 diagnostic testing.

Key words: COVID-19, SARS-CoV-2, DETECTR, qRT-PCR,

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Introduction

SARS Coronavirus-2 (SARS-CoV-2), the causative agent for coronavirus disease 2019 (COVID-19), emerged in December 2019 in Wuhan, China and caused a pandemic. As of July 19th 2020, over 14 million confirmed SARS-CoV-2 infections and more than 600.000 COVID-19 related deaths have been reported worldwide. To curb this epidemic, effective prevention and control measures including the early identification of SARS-CoV-2 infected individuals, are crucial. Outbreak management is hampered by the high transmissibility and broad spectrum of clinical features of SARS-CoV-2. Severe illness marked by pneumonia, acute respiratory distress syndrome (ARDS) and the need for mechanical ventilation is strongly skewed towards people over 70 years old and those with underlying diseases. Many others experience only mild to moderate symptoms such as fever, fatigue, (dry) cough and/or dyspnoea or do not have complaints at all[1].

Infection surveillance and notification play an important role in outbreak prevention and control. As many infections may go unnoticed, large-scale availability of reliable diagnostic tests also for those with mild symptoms is of critical importance to protect especially those at highest risk of developing severe illness. Accurate monitoring of the SARS-SoV-2 epidemic curve helps estimating future disease burden and serves as an important societal impact parameter for pre-emptive policy making e.g. with regards to the justification of less or more restrictive quarantine measures and prevention of health-care system overflow[2–4]. Reverse transcriptase polymerase chain reaction (RT-PCR) is the current diagnostic standard for the detection of SARS-CoV-2. Despite its high sensitivity and specificity, qRT-PCR requires (expensive) specialized equipment, trained staff, and has a relative long turn-around-time (TAT; 2-4 hours). In the Netherlands, the strong dependence on qRT-PCR caused a shortage of reagents and consumables during the pandemic, which limited the test-capacity and resulted in possibly suboptimal outbreak management.

Isothermal reverse transcriptase loop mediated isothermal amplification (RT-LAMP) in combination with Cas12 detection does not need expensive specialized equipment, is highly sensitive and specific,

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has a short TAT and is easy to implement and therefore could be used as an alternative for qRT-PCR [5,6]. This technology is termed DNA Endonuclease-Targeted CRISPR Trans Reporter (DETECTR). The single strand DNA nuclease activity of Cas12 can generate a high-throughput SARS-CoV-2 point-of-care test (POCT) without aspecific amplification as observed with RT-LAMP using intercalating fluorescent dyes or turbidity readouts [5,7], review see [8,9]. Since DETECTR depends on both signal amplification by RT-LAMP and reporter degradation after Cas12-dependent amplicon recognition (Figure 1), the assay produces a binary readout and is potentially more sensitive and specific compared to qRT-PCR [5,6]. A direct comparison between qRT-PCR and this novel DETECTR assay on a large patient cohort has not yet been performed. In the Netherlands, patients suspected of COVID-19 are admitted under strict isolation procedures to prevent nosocomial transmission of SARS-CoV-2 within the hospital. Unnecessary isolation measures pose a significant burden on the nursing staff as well as on the capacity and costs of the hospital. A rapid highly sensitive SARS-CoV-2 assay, preferably suitable as a POCT, would be of added value for (rapid) clinical decision-making and the optimization of patient flow within the hospital. In this manuscript we describe the development of an in-house SARS-CoV-2 DETECTR assay, compare its performance with routine diagnostic qRT-PCR on almost 400 patient samples of three Dutch hospitals, thereby providing a first field test of this novel Cas12-mediated SARS-CoV-2 detection tool.

Materials and methods

All specific information on reagents and relevant concentrations are listed in supplemental tables 1.0-1.6

RT-Lamp reaction

Primers (supplementary Table 1.1) were dissolved in ultrapure water to a final concentration of 100 µM and prepared in 10x primer master mixes (supplementary Table S1.2). For isothermal amplification, 15 µl of complete RT lamp reaction mix was prepared on ice (supplementary Table S1.3) and incubated with 10 µl of isolated RNA or DNA CTRL plasmid at 62°C.

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RT-(PCR)-Cas12

RNA extracts derived from COVID-19 positive patients were run in a reverse transcriptase (RT) reaction according to table 1.6 and hence amplified with or without PCR. Next, qRT-PCR as well as RT products were incubated with N-gene RNPs and analyzed via HT-detection as described below.

RNP formation including reporter probe

RNPs were formed by incubating LbCas12 (supplementary Table S1.4) with targeting Guide RNAs in a RNP reaction mix for 30 min at 37°C (supplementary Table S1.5) and subsequently, probe 1,2 or 3 was added in a final concentration of 100 nM (probe 1 and 3) or 500 nM (probe 2).

High throughput (HT) detection

2,5 µl of RT-LAMP reaction mix was incubated with 22,5 µl of RNP complex containing probe 1 or 3, at 37°C for 10 minutes in chimney multi-well plates covered with seals. Readout was performed after 10 minutes of incubation, unless indicated differently in the figure legends, at 37°C in a Biotek Synergy 2 plate reader using a 485/20 excitation and a 528/20 emission filter.

lateral chip assay

2 µl of RT-LAMP reaction mix was incubated with 20 µl of RNP complex containing probe 2, at 37°C for 10 minutes. Next, 80 µl NEBuffer2.1 (1x concentrated) was added. Lateral flow strips were incubated for 2 minutes at RT allowing liquid to migrate. Readout was performed visually.

Statistics

All data was first tested for normality by the Shapiro Wilk test (p=0,05). Data with a gaussian distribution was analyzed with an unpaired two-sided student's t-test in case of the comparison of 2 samples or an one-way ANOVA with a Dunnett's post-test in case of 3 samples or more. Data which did not follow a gaussian distribution and contained 3 groups or more, was analyzed with a Kruskall-

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Wallis test followed by a Dunnett's post-test. All statistics were analyzed in Graphpad Prism version 8.0.2.

Patient samples

The majority of patient samples were nasopharyngeal swabs in transport medium, the remainder were either broncheo-alvealar lavage (BAL) or sputum. Extensive description of RNA isolation and qPCR methods can be found in supplemental methods.

Results

Both (RT-)LAMP and Cas12-RNPs can be used to detect RNA/DNA, while the combination potentially increases sensitivity and specificity [6]. We compared the sensitivity of RT-LAMP, RT-Cas12-RNP and DETECTR (combination RT-LAMP/RT-Cas12-RNP, Figure 2A-C; supplemental figure 1A-C). We show that using solely RT-LAMP (figure 2A) or RT-Cas12-RNP (figure 2B and Supplemental figure 1D) did not match the sensitivity of DETECTR (figure 2C, 1E). Of note, the limit of detection (LOD) for RT-LAMP was similar to previously reported [10]. RT followed by Cas12-RNP was not sufficient to detect SARS-CoV-2 RNA in samples with high SARS-CoV-2 viral load (qRT-PCR, Cq-value<20)(Figure 2B). This emphasizes the importance of a separate amplification step (PCR or LAMP) prior to Cas12 detection (Figure 2B-C and Supplemental figure 1D). The added value of Cas12-RNP shows in the improved signal-to-noise ratio, which eases interpretation, compared to RT-LAMP alone (figure 2D). Taken the very large increase in signal to noise ratio (>15 FC) of positive versus negative samples (data distribution is shown in figure 5A (right panel), any plate reader, able to measure the indicated emitted wavelength, will result in similar signal to noise ratio albeit with slightly different fluorescence intensities and fold change values. Interestingly, Cas12-RNP by itself also displays a dependency on target concentration (figure 2B; supplemental figure 2A). This suggests that the RT-LAMP reaction is required to allow sufficient amplification of Cas12-RNP target DNA to allow efficient probe degradation. To investigate the effect of probe length on assay performance, we tested a wide range of SARS-CoV-2 N gene DNA (range 10^{-7} to 10^{-16}M) using probes of 8 and 12

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nucleotides (nt). The use of a 12 nt probe increased the signal to noise ratio but not the sensitivity of the test (Supplemental Figure 2A-B). The plateau of the fluorescent signal using DETECTR is reached after 10 minutes. However, >75% of the maximum fluorescence is reached within 5 minutes, suggesting that the assay can be performed faster if required (Supplemental Figure 2C). Longer incubation does not increase the fluorescent signal (Supplemental Figure 2D). However, plates can be re-measured or stored for at least three days without significant loss of signal when stored at room temperature in ambient light (Supplemental Figure 2D). In conclusion, our DETECTR data confirm short turn-around-times (<30 minutes including RT-LAMP), signal robustness and ease of result interpretation.

In a pilot experiment we blindly tested a small cohort of patient samples including four positive, four negative and four samples with not interpretable (NI) qRT-PCR results. SARS-CoV-2 RNA was detected in all 4 qRT-PCR positive samples plus 2 qRT-PCR NI samples (Figure 3A-B). Human RNAse P RNA, used as an internal control, was detected in all 12 samples. Hence, DETECTR results were consistent with qRT-PCR, and provided a clear-cut positive (n=2) or negative (n=2) test result for the samples with NI qRT-PCR results. The analytical sensitivity of DETECTR was compared to qRT-PCR using log-scale dilutions of SARS-CoV-2 RNA extracted from patient samples. DETECTR proved 10-100 times more sensitive in 3 out of 4 experiments (Figure 4A, supplemental figure 2E and F). Of note, the observed analytical sensitivity of both tests does not necessarily equal their clinical sensitivity as (potential) inhibitory factors present in patient material have also been diluted. As the Cas12-RNP complex is single nucleotide sensitive [11,12], mutations within the gRNA recognition site may prevent Cas12 detection. Using a dual target approach with gRNAs that anneal to distinct parts of the RT-LAMP generated amplicon could prevent escape from Cas12 detection (supplemental figure 1B). DETECTR results with gRNA1, gRNA2 and combined gRNA1/gRNA2 yielded similar results (Figure 4B). As the risk of aberrant viral variants increases with the ongoing worldwide epidemic, the use of multiple gRNAs is highly recommended. Strong homology within the N-gene of SARS-CoV-2 and other human coronaviruses may compromise the specificity of DETECTR. N-gene homology with

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other human coronaviruses varies between 50.1% and 88.2%. The highest concordance is seen with SARS-CoV-1, with a maximum homology of 86.7% in the regions used for the development of RT-LAMP primers and Cas12 gRNA recognition sites. We analyzed 22 samples of patients infected with other human coronaviruses; 22/22 samples tested negative for SARS-CoV-2 and positive for the RNase P housekeeping gene with DETECTR suggesting a specificity of 100% (Figure 4C).

Finally, we tested our DETECTR assay on 378 patient samples derived from three hospitals in the Netherlands. The cohort consisted of RNA extracted from clinical samples of patients that were diagnosed SARS-CoV-2 positive or SARS-CoV-2negative based on routine qRT-PCR. Our DETECTR assay showed 94.9% (+/- 1.8%/0.8%) concordance with qRT-PCR (figure 5A, 5B), with minor differences between the three centers (Supplemental figure 3; A=94.1%; B=96.7%; C=94.7%). DETECTR positive but qRT-PCR negative samples (n=10) were mainly found in center A (n=9); all 9 samples from this hospital also showed a SARS-CoV-2 band pattern on gel indicating specific product amplification and suggesting they were missed by qRT-PCR (supplemental figure 3D). The DETECTR+/PCR- negative sample of center B was later confirmed SARS-CoV-2 positive by Center B, albeit with Cq-value >35. In total, we found 11 PCR+/DETECTR- samples, 7/11 samples had Cq-values >30 (figure 2B; supplemental Figure 3A-C), but the other four had Cq values of 20,74; 29,78; 29,28 and 28. Re-analysis with an alternative gRNA that anneals to a different part of the N-gene (supplementary Table S1.1) did not yield positive test results, indicating that a mutation within the binding region of the gRNA is unlikely to explain the false negative DETECTR results. An equal number of clinical SARS-CoV-2 positive samples was missed by qRT-PCR (10) and DETECTR (11) indicating similar sensitivity of both approaches in clinical samples. Interestingly screening of patient samples with a non-interpretable qRT-PCR result yielded positive detection of SARS-CoV-2 in 9/19 patient samples (figure 5C), indicating that DETECTR can be used as a fast confirmatory test for samples yielding a NI result in qRT-PCR. Altogether, the overall concordance of around 95% in clinical sensitivity, shows that DETECTR can be used as a specific, fast and reliable technique for patient samples.

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Most DETECTR results were obtained using a high throughput 96/384 wells spectrophotometer to detect the cleaved fluorescent probe. A major advantage of DETECTR is that it can be used as an individual POCT using lateral flow strips for read out. Individual lateral flow results (n=40) were 100% concordant with the high throughput results (supplemental figure 4). To confirm robust signals in 'difficult' clinical samples, we analyzed 8 samples with not interpretable qRT-PCR results using spectrophotometric and lateral flow detection. Again, fully concordant results: SARS-Cov-2 positive (n=4) and SARS-CoV-2 negative (n=4) (Supplemental Figure 4C). The binary readout is easy to interpret, irrespective of readout method or Cq-value. Therefore, DETECTR POC tests could be used in low-resource countries/regions or as a fast and reliable equipment independent confirmation test to confirm ambiguous qRT-PCR samples.

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Discussion

In summary, here we compared DETECTR with qRT-PCR for SARS-CoV-2 diagnosis in a large patient cohort over multiple hospitals and report a 95% accordance. These data are in line with recently published studies where cohorts were tested in a single institute [5,13].These data are in line with a recently published study where only a small cohort (83 samples) was tested derived from a single hospital In addition, our data suggest that a 12nt probe is superior over a 8nt probe and we suggest to use a double guide approach to prevent escape from DETECTR due to mutations within amplicons. Overall, DETECTR has comparable sensitivity and superior specificity to qRT-PCR. Our results show that DETECTR represents a reliable, cheap, fast and technically independent alternative to complement qRT-PCR platforms. The low-demand on facility equipment, especially concerning the POCT, makes DETECTR especially suitable for resource low countries/regions. In this paper we

show a LOD for RT-LAMP at 500 copies and for DETECTR at 50 copies. It is however important to note that we have defined the LOD on N-gene plasmids instead of synthetic SARS-CoV-2 RNA. This makes the comparison between RT-LAMP and DETECTR independent of reverse transcriptase efficiency. However, it may not accurately display the LOD of RNA samples, since efficiency to convert RNA to DNA by reverse transcriptase also depends on secondary RNA-structure and sample matrix. Other studies have however shown a similar LOD for RT-LAMP as reporter here after spiking synthetic viral RNA into different matrices, such as mucin or blood [10]. A current limitation of DETECTR is the dependence on three separate reactions, namely RNA isolation, RT-LAMP amplicon amplification and Cas12 mediated reporter degradation. The latter has to be considered a step back in comparison to qRT-PCR, where post amplification handling, a major risk in causing false positive results by contamination, could be removed from the workflow. Further research should focus on integrating all DETECTR steps, including RNA-isolation, into the same reaction tube without post amplification processing. In the current study, the extracted RNA used as input for qRT-PCR was also used for DETECTR. Excitingly, in a recent paper published during the review process, the use of a heat stable Cas12 from *Alicyclobacillus acidiphilus* potentially makes combining the RT-LAMP and Cas12 reactions in one tube possible, which was verified in a test panel including 200 positive patient samples[13]. In addition, these authors showed that RT-LAMP multiplexing of various internal control amplicons together with the viral amplicon in one reaction may be possible, further adding to the robustness of assay results. Of note, onestep RT-LAMP approaches including various RNA extractions have been developed, e.g. for Zika virus[14,15], and compatibility with DETECTR will need to be determined. However, Joung et al. showed that RNA isolation may need to be carried out separately from the RT-LAMP and Cas12 reactions to maintain optimal sensitivity [13]. Importantly, as detection is not compromised upon diluting patient material 10-100 times, the technique may allow the implementation of pooled sample approaches in low-prevalence regions/countries significantly increasing testing capacity (e.g. 20 samples without loss of detection). However, it must be noted that in this patient cohort DETECTR and qRT-PCR were performing on parity. The clinical

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sensitivity of DETECTR could be lower despite its higher analytical sensitivity (Figure 1F, 1H) due to the matrix of clinical samples having a more profound inhibitory effect on DETECTR technology. Importantly, once implemented the suggested approach can be easily diverted to screen other existing or emerging pathogens or any other platform that requires identification based on specific DNA/RNA [6,11,12]. The DETECTR test helps to optimize diagnostic strategies for both bedside and high-throughput settings leading to an increase in testing capacity and improved diagnostic evaluation, ultimately leading to better determination of endemic progression facilitating governmental policy decisions.

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Authorship contributions

EB and HV performed the RT-LAMP, DETECTR assays. MC, TvdL, EC and JS collected the cohort material, isolated RNA and performed the qRT-PCR on validated platforms. EvdA supervised the study. All authors contributed in writing the manuscript, which was critically reviewed by all authors.

Conflict of interest

The authors report no conflict of interest

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Figure legends

Figure 1: Graphic representation of DETECTR assay

RNA is converted to cDNA and amplified in one reaction mix for 20 minutes using RT-LAMP. Next, Cas12 RNPs are added that recognize and cleave SARS-CoV-2 amplified products leading to activation of Cas12. Activated Cas12 destroys the single stranded linker DNA between quencher and probe leading to fluorescence that can be detected on indicated platforms. ***=p<0.001; ****=p<0.0001 using ANOVA and Dunnett's post-test

Figure 2: Combining RT-LAMP and Cas12 improves sensitivity of SARS-CoV-2 detection

(A-D) Specific N-gene plasmid copy concentrations (A,C) or confirmed RNA from SARS-CoV-2 positive individuals with indicated range of qRT-PCR cq-values (B) were run in a RT-LAMP reaction (A), RT-Cas12 (B) or RT-LAMP-Cas12 (DETECTR, C) assays using an amplicon within the N-gene and a gRNA annealing to that amplicon (supplemental figure 1). Note that whereas the RT-LAMP reaction results in a fluorescence signal proportional to the input (A), the combination of RT-LAMP/Cas12-RNP results in a binary test outcome with a high signal to noise ratio (24 times on average) due to the degradation of the reporter probe, which depends on the induced nuclease activity of Cas12 (C). D) shows the fold change in fluorescence normalized to the negative control.

Figure 3: DETECTR accurately identifies SARS-CoV-2 in clinical samples

A) a cohort of 12 patient RNA isolates, including 4 RT-PCR positive, 4 RT-PCR and clinically negative and 4 NI were screened with DETECTR (the convention of a NI (not interpretable) result can be found in material and methods). Bars represent the average of a duplicate and error bars the SD (N-gene (red) and internal control RNAseP (black)). B) shows the comparison of qRT-PCR result and DETECTR fluorescence signal (red positive samples; grey negative samples; white NI.

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Figure 4: RT-LAMP/Cas12 is a specific and sensitive test to detect SARS-CoV-2.

A) Log scale dilutions of SARS-CoV-2 positive patient samples were tested with qRT-PCR and DETECTR in indicated log dilutions were + indicates a positive result and – a negative test result. (B) Two gRNAs with distinct annealing sites were tested in the DETECTR assay. The dot plot shows the fluorescence signal of positive (red) and negative samples (black). Numbers indicate the number of negatives and positives samples analyzed. C) A collection of non-SARS-CoV-2 corona strains samples confirmed by qRT-PCR were found to be negative using SARS-CoV-2 specific DETECR. ****=p<0.0001 using two-sided unpaired T-test.

Figure 5 High level concordance between qRT-PCR and DETECTR.

A) 378 qRT-PCR confirmed SARS-CoV-2 tested samples from different centers were compared to the results obtained using DETECTR. The matrix displays the results from the DETECTR assay (vertical) compared to the qRT-PCR results (horizontal). The graph shows the fluorescence intensity (left graph) and fold change fluorescence signal normalized to a negative control (right graph) with each dot representing a DETECTR test on RNA from a different individual. B) Subclassification based on qRT-PCR cq-values compared to DETECR result. C) DETECTR on 19 samples that gave an NI results by qRT-PCR (convention of NI can be found in Material and Methods). Orange bars: N-gene DETECTR; black bars: RNaseP control DETECTR. ****=p<0.0001 using Kruskal-Wallis test, followed by a Dunnett's post-test, comparing all groups with the 'True negatives' group.

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Table 1.0

	Vendor	cat
Oligos, probes, sgRNA	IDT	na
Ultra pure DNAse and Rnase free water	invitrogen	10977-035
NaCl	Merck	106404
Sodium acetate	Sigma Aldrich	S8625
EDTA	Sigma Aldrich	E5134-5009
TCEP	Sigma Aldrich	C4706
Glycerol	Sigma Aldrich	G6279-1L
LbCas12a	NEB	M0653
N-gene plasmid (2019-nCov-Npostive	IDT	10006625
lateral flow strips	TwistDx	*MILENIA01*
RT-LAMP mastermix	NEB	E1700L
Chimney 96 wells Plates (black)	Greiner Bio	655209
Chimney 384 wells Plates (black)	Greiner Bio	781076
MicroAmp optical adhesive film	Thermo	431197
Superscript III first strand Reverse transcriptase	Thermo Fisher	18080-051
Phusion High-Fidelity PCR kit	Thermo Scientific	MAN0013363

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Table 1.1

application	specific name	sequence
isothermalamplification	N-geneF3	AACACAAGCTTTCGGCAG
isothermalamplification	N-geneB3	GAAATTTGGATCTTTGTCATCC
isothermalamplification	N-geneFIP	TGCGGCCAATGTTTGTAATCAGCCAAGGAAATTTTGGGGAC
isothermalamplification	N-geneBIP	CGCATTGGCATGGAAGTCACTTTGATGGCACCTGTGTAG
isothermalamplification	N-geneLF	TTCCTTGTCTGATTAGTTC
isothermalamplification	N-geneLB	ACCTTCGGGAACGTGGTT
isothermalamplification	RNasePPOP7F3*	TTGATGAGCTGGAGCCA
isothermalamplification	RNasePPOP7B3*	CACCCTCAATGCAGAGTC
isothermalamplification	RNasePPOP7FIP*	GTGTGACCCTGAAGACTCGGTTTTAGCCACTGACTCGGATC
isothermalamplification	RNasePPOP7BIP*	CCTCCGTGATATGGCTCTTCGTTTTTTTCTTACATGGCTCTGGTC
isothermalamplification	RNasePPOP7LF*	ATGTGGATGGCTGAGTTGTT
isothermalamplification	RNasePPOP7LB*	CATGCTGAGTACTGGACCTC
N gene detection	Guide 1	CCCCCAGCGCTTCAGCGTTC
N gene detection	Guide 2	GGGACCAGGAACTAATCAGAC
RNAse P detection	RnasePgRNA(ctrl)	AATTACTTGGGTGTGACCCT
8nt probe DETECTR-HT	PAMreporter-HT	/56FAM/TTATTATT/3IABkFQ
POCT	PAMreporter	/5-FITC/TTATTATT/3Bio/
12nt probe DETECTR-HT	PAMreporter-HT	/56FAM/TTATTATTATTA/3IABkFQ

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Table 1.2

N-gene	10X workstock		
Primer	**Description**	**Volume (µl)**	**Final concentration (µM)**
COVID-19_1	N-gene F3 primer (100 uM)	20	2
COVID-19_2	N-gene B3 primer (100 uM)	20	2
COVID-19_3	N-gene FIP primer (100 uM)	160	16
COVID-19_4	N-gene BIP primer (100 uM)	160	16
COVID-19_5	N-gene LF primer (100 uM)	80	8
COVID-19_6	N-gene LB primer (100 uM)	80	8
	ultrapure water	480	

Rnase P	10X workstock		
Primer	**Description**	**Volume (µl)**	**Final concentration (µM)**
COVID-19_13	Rnase P F3 primer	20	2
COVID-19_14	Rnase P B3 primer	20	2
COVID-19_15	Rnase P FIP primer	160	16
COVID-19_16	Rnase P BIP primer	160	16
COVID-19_17	Rnase P LF primer	80	8
COVID-19_18	Rnase P LB primer	80	8
	ultrapure water	480	

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Table 1.3

Reagent	Volume 1 rnx (uL)
RT-LAMP mastermix (NEB	12.5
10x primer mix (N-gene, POP7 or E-gene)	2.5
Nuclease free water	5
Total Volume	20

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Table 1.4

Cas12 storage buffer	
	final concentration
NaCl	500 mM
sodium acetate	20 mM
EDTA	1 mM
TCEP	1 mM
Glycerol	50% v/v
Ultra pure DNAse and Rnase free water	na
pH 6.0 @ 25°C	

general stock			
	dry weight (pmol)	**Cas12A storage buffer (µl)**	**Final concentration (µM)**
lbcas12a (NEB M0653T)	2000	2000	100

Working solution			
	Volume of general stock (µl)	**Cas12A storage buffer (µl)**	**Final concentration (µM)**
lbcas12a (general stock)	10	1990	0.5

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Table 1.5

RNP formation mix for 1 condition			
	volume in µl		final concentration
Nuclease free water	14	0	na
10X NEBuffer 2.1	2	0	1X
0,5 µM lbCas12a	2	0	50 nM
0,625 µM gRNA (N-gene)	2	0	62,5 nM
Total volume	20	0	

incubate RNP reaction mix for 30 minutes at 37oC

add probe

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Table 1.6

Reverse transcriptase reaction		
RT-Mix 1	Superscript III first strand Reverse transcriptase	Volume/rxn (µl)
	Total RNA	5
	10 µm Primer Fw **N-geneF3** AACACAAGCTTTCGGCAG	0.5
	10 µm Primer Rv **N-geneB3** GAAATTTGGATCTTTGTCATCC	0.5
	10 mM dNTP mix	1
	MQ	3
		10
RT-Mix 2	Superscript III first strand Reverse transcriptase	Volume/rxn (µl)
	10X RT buffer	2
	25mM MgCl2	4
	0.1 M DTT	2
	RNaseOUT (40U/uL)	1
	Superscript III RT (200U/uL)	1
	total	10

1 Combine the components of mix 1

2 Incubate at 65oC for 5 min

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3	place on ice for 1 min
4	prepare cDNA synthesis mixes according mix 2
5	add 10 uL of cDNA synthesis mix to each well and mix
6	incubate for 50 min at 50oC
7	terminate the reaction at 85oC for 5 min
8	spin down briefly
9	add 1 uL Rnase H to each tube and incubate for 20 min at 37C

RT-PCR

RT-PCR Mix	**Phusion High-Fidelity PCR Kit**	Volume/rxn (µl)
	H2O	12.4
	5X Phusion HF Buffer	4
	10 mM dNTPs	0.4
	10 µm Primer Fw **N-geneF3** AACACAAGCTTTCGGCAG	0.5
	10 µm Primer Rv **N-geneB3** GAAATTTGGATCTTTGTCATCC	0.5
	RT-template	2
	HF phusion polymerase	0.2
	Total	20

PCR program

	time in seconds	degrees	
1	30	98	
2	5	98	35 cycly
	15	58	

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	10	72
3	10	72

1	Combine the components of RT-PCR Mix
2	Run PCR according PCR program
3	run fragments on 2% Agarose gel for 30 minutes 130v

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Figure 1

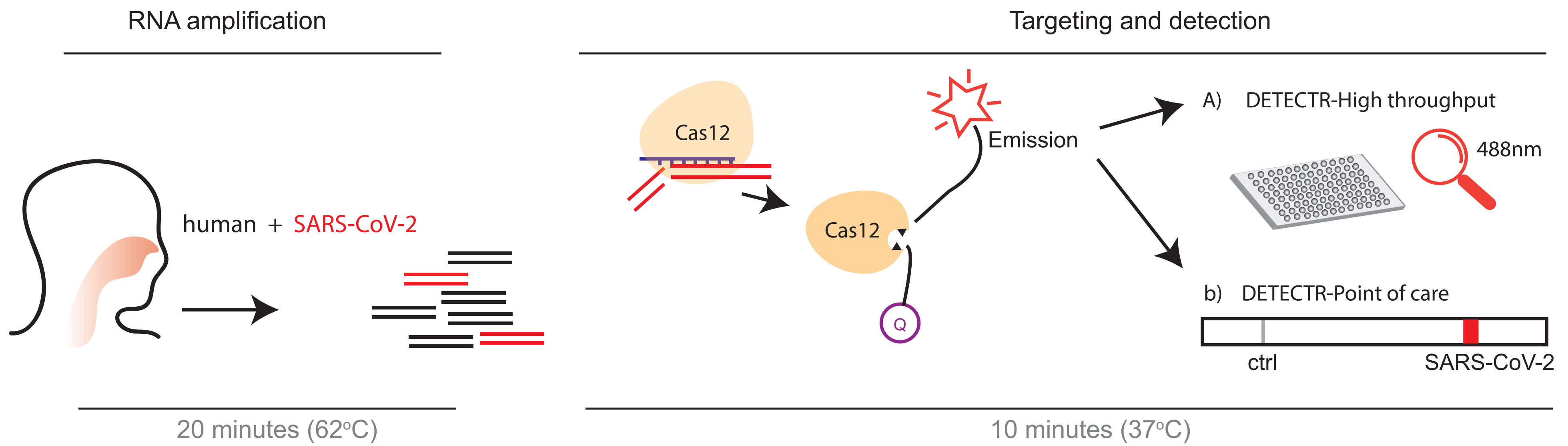
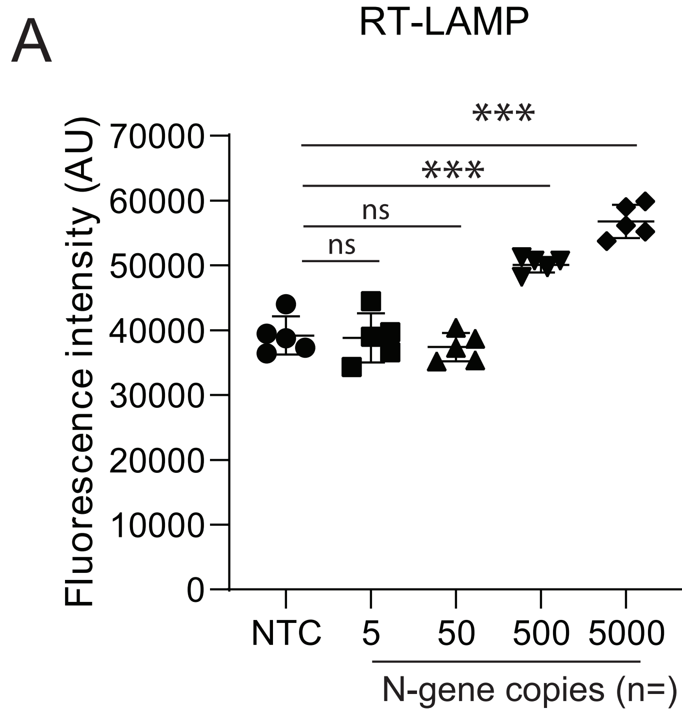
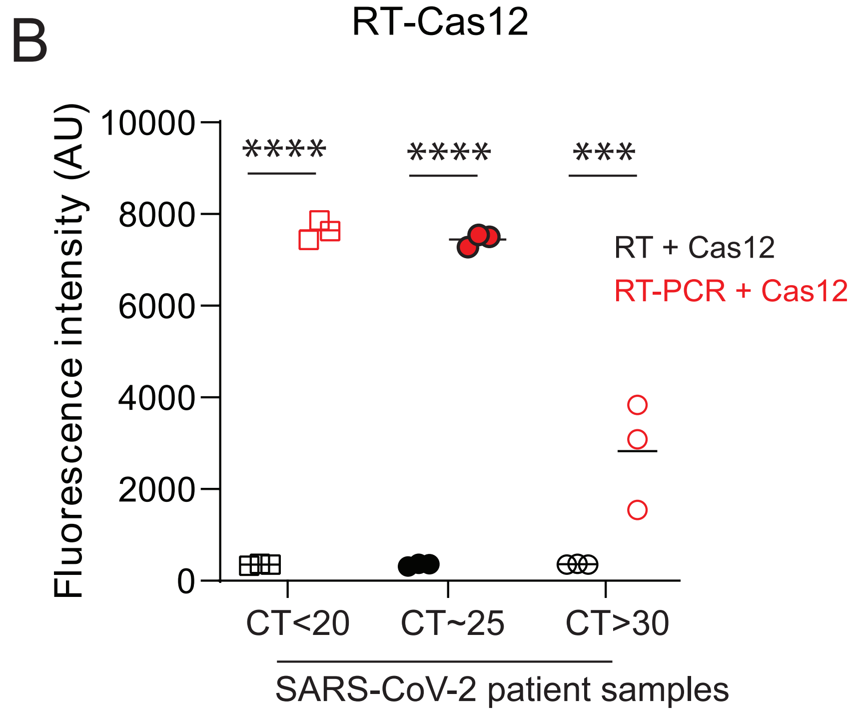
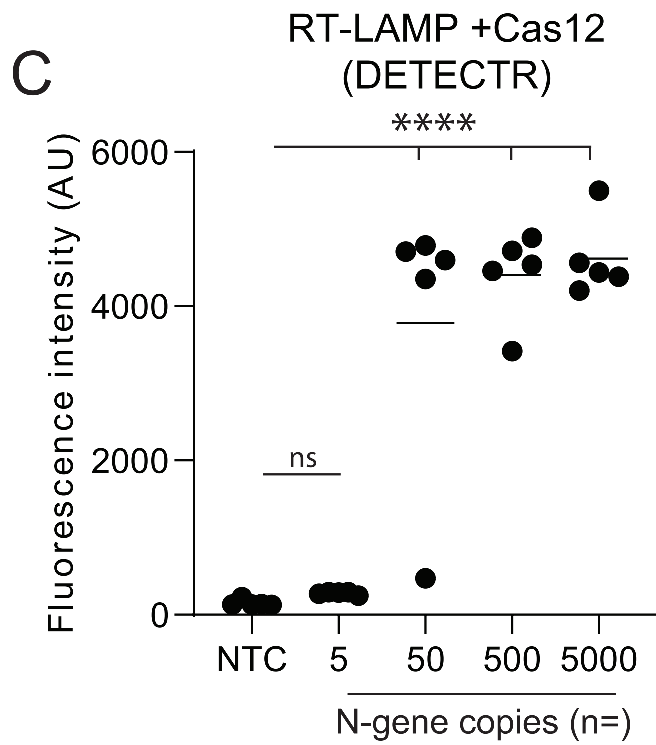


Figure 2







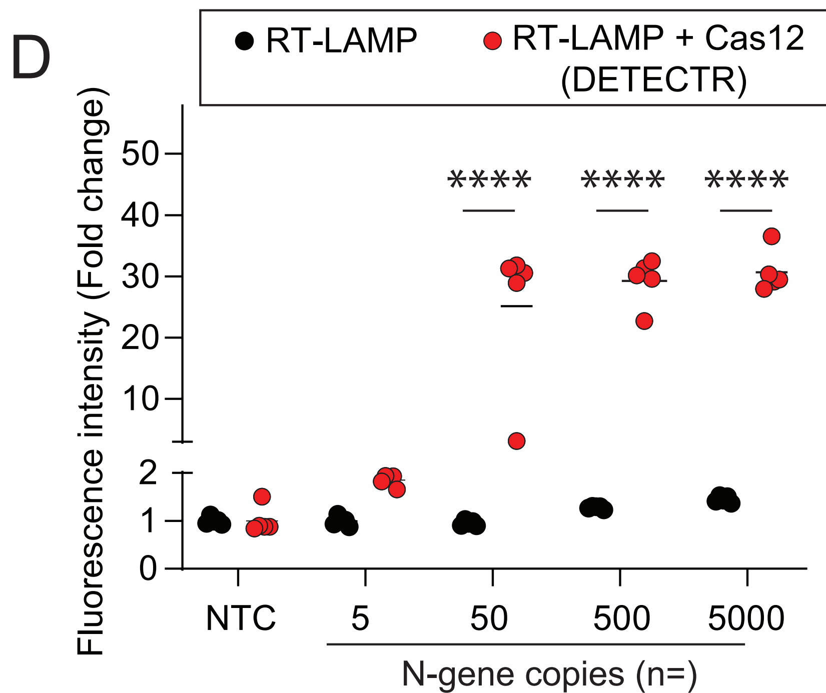
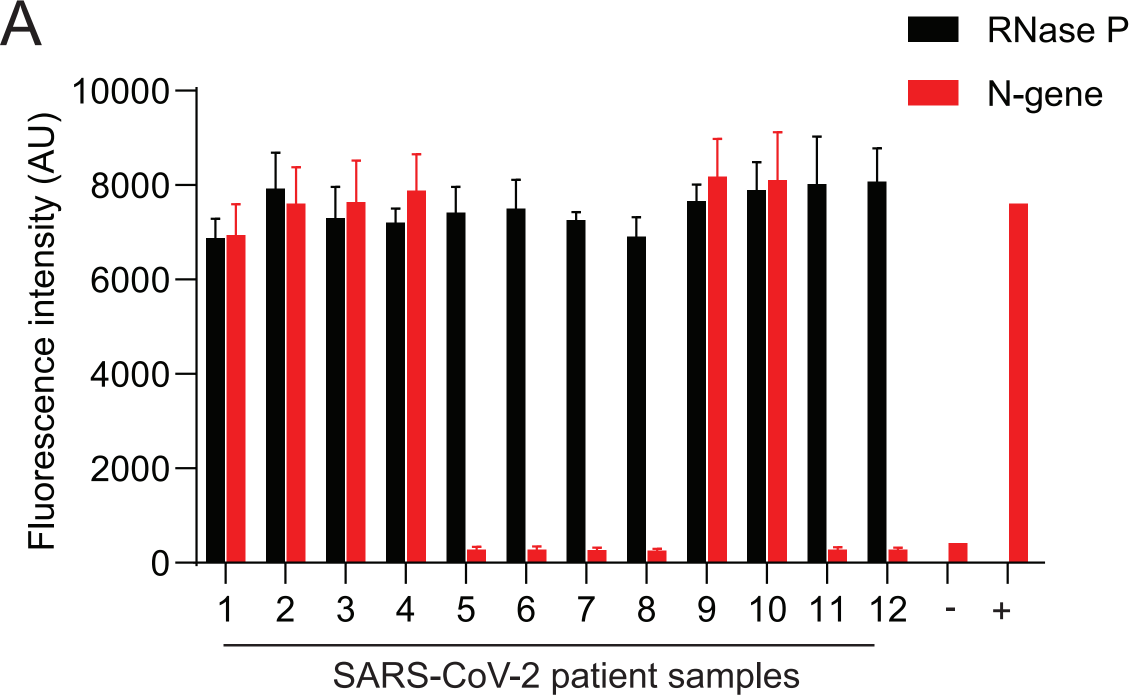


Figure 3



B

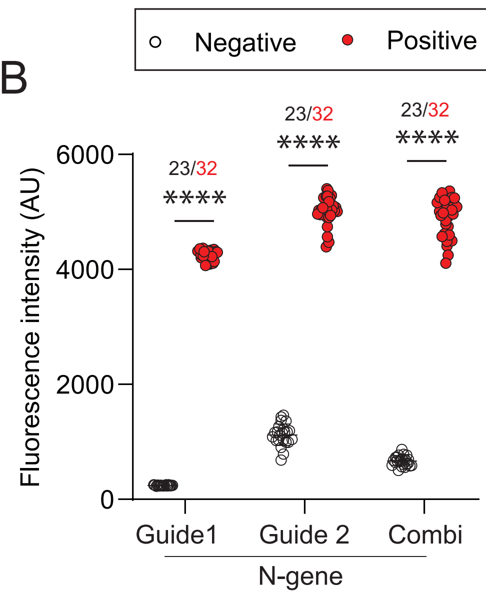
Patient ID	qRT-PCR Status	qRT-PCR Ct-Value	DETECTR score (AU)
1	Positive	23,29	7594
2		27,35	8377
3		23,51	8521
4		25,56	8658
5	Negative	ND	336
6		ND	348
7		ND	321
8		ND	297
9	Ambiguous	NI	8980
10		NI	9119
11		NI	326
12		NI	321

Figure 4

A

Dilution	Patient 1		Patient 2	
	qRT-PCR	DETECTR	qRT-PCR	DETECTR
1:10	+	+	+	+
$1:10^2$	+	+	+	+
$1:10^3$	+	+	+	+
$1:10^4$	+	+	-	+
$1:10^5$	-	+	-	-
$1:10^6$	-	+	-	-
$1:10^7$	-	-	-	-

B



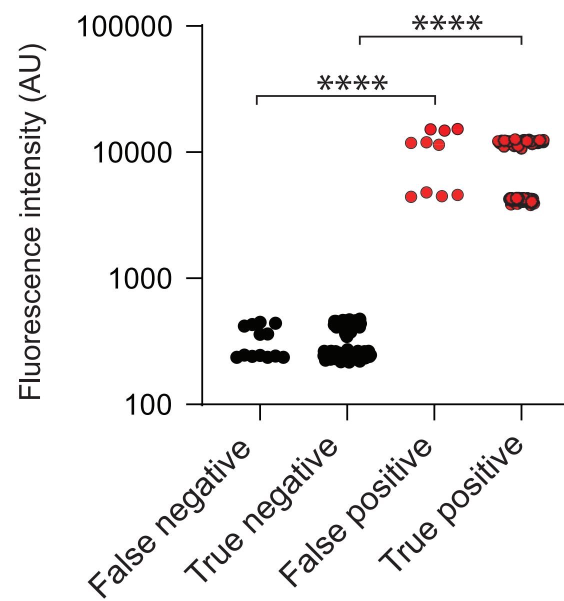
C

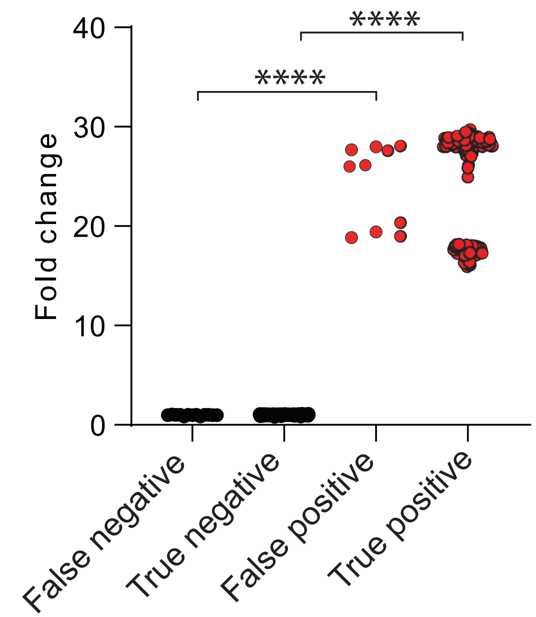
	Average Ct Value	Range Ct Value	DETECTR SARS-CoV-2
CoV-229E	28,8	28,8-28,8	0/1
CoV-HKU1	24,0	17,7-33,7	0/10
CoV-NL63E	30,0	25,6-32,2	0/7
CoV-OC43	31,6	27,9-33,8	0/4

Figure 5

A

		qRT-PCR	
		Negative	Positive
DETECTR	Negative	213	11
	Positive	10	144





B

	Average Cq Value	Range Cq Value	DETECTR result SARS-CoV-2
<15	<15,0	<15,0	1/1
15-20	17,3	15,0-19,4	16/16
20-25	22,4	20,3-23,7	36/37
25-30	27,8	25,0-29,9	61/64
30-32	30,8	30,0-31,9	24/25
>32	33,4	32,1-37,3	6/12

C