Table S1. MIQE checklist for authors, reviewers and editors. a

								COMMENTS/WHERE	?				
ITEM TO CHECK EXPERIMENTAL DESIGN	IMPORTANCE	CHECKLIST		Figure 2A	Figure 2B	Figure 2C	Figure 2D	Figure 3A-B	Figure 3C-D	Fig 4A-B	Fig 4C-D	Fig 5A-B	Fig 6A
Definition of experimental and control groups	E	Yes	All information is provided in open access supplementary information Bustin et al., 2021	Experimental group: Accupite reference material spike into two salaw donors (Donor A and Donor M) and two buccal donors (Donor E and Donor M). Control group: Matched saliva and buccal swab donors with no reference material spike.	Experimental group: Accupies reference material spike into matched samples from three donors for saliva and buccal swaks (from of R, F and E with a succord sample from donor M (Muz]). Control group: Matched saliva and buccal swak donors with no reference material spike. Control group: Extraction blanks with water in place of saliva/buccal swab and reference material spike.	Experimental group: Accupies control material spake into matched samples from one donor for saliva and buccal swabs (Donor I). Control group: Matched saliva and buccal swab donors with no control material spike.	Experimental group. Accupter. control material spiked into matched samples from two donors for saliva and buccal swabs (Donor E and Mr) with 1 ug Polyk added before extention. Two conentrations of spike were assessed: 750 copels/ml. Control group: Matched saliva and buccal swab donors with two control spikes of the spikes	Experimental group: Extraction of a serial dilution on INT RNA molecule in three different carriers, Internal extraction control (IEC) spiked in at a constant concentration. Control group: Extraction of the same dilution series with no carrier molecules. Control group: Extraction blanks control group: Extraction blanks controling the IEC but with water in place of NT RNA molecules with and without carrier molecules.	Experimental group: RNA extraction of the nine samples in the Cinocitic panel (see below) with PolyC carrier molecuse. Internal extraction control (IEC) spiked in at a constant concentration. Control group: Extraction of same Quostics panel without PolyC carrier molecules. Control group: Extraction blanks with water in place of Quostics samples with and without PolyC carrier molecules.	Experimental group: Control RNA molecule spiked into RT-qCR reaction in the presence of bile salts at different concentrations. Control group: Control RNA molecule spiked into the RT-qCR reaction without added bile salts.	Experimental group: Control RNA molecule spiked into RT-qCR reaction with bile salts added after the RT (before PCR) at different concentrations. Control group: Control RNA molecule spiked into the RT-qPCR reaction without added bile salts.	Experimental group: Control RNA molecule spiked into RT-qCR reaction with different v/v concentrations of donor urine from three healthy individuals. Control group: Control RNA molecule spiked into the RT-qPCR reaction without added urine.	Experimental group: Control RNA molecule spike into T =PCR at three concentrations. Control group: none.
Number within each group	E	Yes		Ten extractions per donor sample for experimental and control groups: - 20 extractions from saline with spike - 20 extractions from saline with no spike - 20 extractions from saline with no spike - 20 extractions from buccal swab with no spike - 20 extractions from buccal swab with no spike - 20 extractions from buccal swab with no spike - 80 extractions along with triplicate with two different PCK mastermakes along with triplicate materials spike and couples reference material spike in at the comparable concentration in the RT-qPCR - 243 RT-qPCR + NTCS	Ten extractions per donor sample for experimental and control groups: - 40 extractions from silva with spike - 40 extractions from silva with no spike - 40 extractions from silva with spike - 40 extractions from buccal swab with spike - 1 extraction blank = 161 extractions Each extraction was amplified in single RT-gPCR reactions Each extraction was amplified in single RT-gPCR reactions	Twenty extractions per donor sample for experimental and control groups: - 20 extractions from salina with spike - 20 extractions from salina with no groups: - 30 extractions from buccal swab with spike - 20 extractions from buccal swab with no spike - 30 extractions from buccal swab with no spike - 80 extractions Each extraction was amplified in single RT-grCR reactions - 80 RT-grCR reactions - 80 RT-grCR reactions	Three extractions for each sample in the experimental and control groups: -1.2 extractions from saliva with spike and extractions from saliva with spike and extractions from saliva with spike and PolyA -1.2 extractions from buccal swab with spike and PolyA -1.2 extractions from buccal swab with spike but not PolyA -1.2 extractions from buccal swab with spike but not PolyA -8.4 extraction Each extraction was amplified in single RT-qPCR exactions. -4.8 ETT-qPCR exactions.	Three extractions for each sample in the experiment and control groups: - 60 extractions from a five-point serial of the properties of th	Single extraction for each sample in the experiment and control groups: -18 extractions from the rimes samples in the Chonditic gravel, each extracted in the Chonditic gravel, each extracted: - 6 Blank extractions containing the IEC but with Waster instead of Chonditic sample; three with and three without carrier. = 24 extractions Each extraction was amplified with triplicate RT-qPCR reactions. = 72 RT-qPCR + NTCs	Quantification of EURM-019 (IRC) in the presence of bile salts added directly to the RT-6PCK reaction to give a final concentration of 0, 0, 0, 2, 0, 6, 6 and 1 a	No biological specimen. Quantification of EURM-019 (JRC) in the presence of Dile salts added directly to the effort restriction (jlet PT van Herite (jlet PT van Herite) (jlet PT	Three urine samples from healthy human donors. Quantification of EURM-019 (MC) in the presence of different via via concentrations of the urine at 0%, represented with other fine and other concentrations of the urine at 0%, read donor unit of was evaluated with the three EURM-019 concentrations in triplicate reactions: = \$ 6RT = QCR + NTCs - Control reactions containing no EURM-019 were assessed in parallel = \$ RT = QCR + NTCs	No biological specimen. Quantification of EURN-019 (IRC) that is a single stabilised in vitro transcripted (IVT) synthesis can give stander SNN. The NT molecule contains the target regions for amplification of N1 and N2 (as well as others) based on the reference sequence MN908947.3. Data present is from the same control samples containing no added inhibitors (bile or united) used in rigit and rigit of the same control samples containing no added inhibitors (bile or united) used in rigit and rigit of the same control samples containing no added inhibitors specially stated to the same control samples containing no end of the same control samples containing no experience occurrentations separate days: 1000, 100 and 100 copies/ul. In the reaction 256 RT=QFCR + NTCS - Control reactions containing no EURM-019 were assessed in parallel = 9 RT-QFCR + NTCS
Assay carried out by core	D	Yes		Investigator's lab: EKvH, MK, AB, HH				Investigator's lab: EKvH, MK, AB, HH		Investigator's lab: SB, OK, AM			
lab or investigator's lab? Acknowledgement of	D	Yes		Yes				Yes		Yes			
authors' contributions SAMPLE													
Description	E	Yes		sample was obtained each time. <u>Buccal swabs:</u> The swab (Isohelix Swab Pack (SK-25-x100 contributed a sample to more than one example to more than one example to more than one example to the sample to more than one example to the sample to) was stroked over the inner cheek at least ten pperiment, a fresh sample was obtained each tii 05-0126) is designed as whole-process control (15/Wuhan/Wir/G4/2019 genome (see below).						EURN-019 control material (IRC); spiked in at 10,100 and 1000 copies/uL in the RT-qPCR reaction.	
Volume/mass of sample processed	D	Yes		Samples were diluted 1.1 (v/l) with X PBS (Roth Rotil* Fair PBS pH7.4 1112.1) containing 1 nM DTT (Roth DTT 6908). The reference material was spiked into the diluted saliva to give final concentration of 750 copies/mL saliva. Each extraction was performed on 100 u.t of the spiked diluted saliva. Buccal swabs: Each swab was vortexed in 3 mL UTM (Copan Universal Tansport Media (UTM, 330C, 3ml Tube in bulk). The reference material was spiked into the UTM to give a final concentration of 750 copies/mL saliva. Each extraction was performed on 100 u.t of the spiked UTM. Each extraction was performed on 100 u.t of the spiked UTM. 350 c/ml. (saliva. Each extraction was performed on 100 u.t of the spiked UTM. 350 c/ml. (saliva. Each extraction was performed on 100 u.t of the spiked UTM. 350 c/ml. (saliva. Each extraction was performed on 100 u.t of the spiked UTM. 350 c/ml. (saliva. Each extraction was performed on 100 u.t of the spiked UTM. 350 c/ml. (saliva. Each extraction was performed on 100 u.t of the spiked UTM. 350 c/ml. (saliva. Each extraction was performed on 100 u.t of the spiked UTM. 350 c/ml. (saliva. Each extraction was performed on 100 u.t of the spiked UTM. 350 c/ml. (saliva. Each extraction was performed on 100 u.t of the spiked UTM. 350 c/ml. (saliva. Each extraction was performed on 100 u.t of the spiked UTM. 350 c/ml. (saliva. Each extraction was performed on 100 u.t of the spiked UTM. 350 c/ml. (saliva. Each extraction was performed on 100 u.t of the spiked UTM. 350 c/ml. (saliva. Each extraction was performed on 100 u.t of the spiked UTM. 350 c/ml. (saliva. Each extraction was performed on 100 u.t of the spiked UTM. 350 c/ml. (saliva. Each extraction was performed on 100 u.t of the spiked UTM. 350 c/ml. (saliva. Each extraction was performed on 100 u.t of the spiked UTM. 350 c/ml. (saliva. Each extraction was performed on 100 u.t of the spiked UTM. 350 c/ml. (saliva. Each extraction was performed on 100 u.t of the spiked UTM. 350 c/ml. (saliva. Each extraction was performed on 100 u.t of the sp			Each extraction was performed on 100 ut. of the four different spiked samples (saliva & 750 c/mt, saliva & 3000 c/mt, buccal swab & 750 c/mt and buccal swab & 3000 c/mt), containing either 1 ug Polyk carrier molecules or no carrier	The hophistised IVT RNA template was resuspended in 200 u.f. of Template preparation buffer to give a stock concentration of 1 x 10E5 copies/µL. A fresh serial dilutions series was prepared in nuclease. Few water with and without carrier as 100 000 (stock), 10 000, 1000, 100 and 50 copies/uL.	200 Jul Jilguot was supplied by Primerdesign/Novayr. The panel of samples went through one freeze-thaw cycle from allouding by Primerdesign/Novayr for shipping to the investigation 1b. The samples were thawed once in the investigators lab to add to the extraction reaction only.	N/A		inhibitors (urine samples) were provided by the clones into 50 ml. falcon tube on the day of the experiment.	N/A
Microdissection or	E	Yes		N/A				N/A		N/A			
macrodissection Processing procedure	Е	Yes		Described in above section.				Lyophilised IVT RNA template was stored at -20°C prior to rehydration in Template preparation buffer.	N/A as provided as a panel of ready-to- use samples.	N/A		Fresh urine samples were stored on cooling blocks and directly taken into reaction.	N/A
If frozen - how and how quickly?	E	Yes		N/A as used immediately.			_	Stock samples were provided as dried RNA that were rehydrated just prior to analysis with the remnants discarded (not stored).	Received as frozen liquid 200 µL aliquot (see details above). Stored frozen until and thawed just prior to the extraction procedure.	N/A			
If fixed - with what, how quickly?	E	Yes		N/A as used immediately.				N/A as used immediately	N/A as used immediately	N/A			
Sample storage conditions and duration (especially for FFPE ^b samples)	E	Yes		N/A as used immediately.				Dilutions were not stored; excess was used as the direct spike control and the remainder was discarded after the extraction protocol.	Stored in the original aliquot. Remaining sample was discarded after extractions.	See Table S2.			
NUCLEIC ACID EXTRACTION													

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Procedure and/or instrumentation												
instrumentation	E	Yes	Extraction was carried out on KingFisher F	Flex platform (ThermoFisher) with sbeadex vira	RNA purification kit (LGC Biosearch Technologie	es) as follows:	Extraction was carried out on KingFisher	Extraction was carried out on KingFisher	N/A			
instrumentation			60°C for 11 min (with 1 min slow mixing a	ind 10 min fast mixing).	med by addition of 20 μl Proteinase K Solution (2		Flex platform (ThermoFisher) with sbeadex viral RNA purification kit (LGC Biosearch Technologies).	Flex platform (ThermoFisher) with sbeadex viral RNA purification kit (LGC Biosearch Technologies).				
			mixing. Three washing steps were carried out: a)	400 μl Wash buffer BN1, b) 400 μl Wash buffe	particle suspension EDTA) was added to each res TN1 and c) 400 µl Wash buffer TN2 each for 5 m		1 μL of IVT RNA was added to 100 μL of water with or without 1 μg of respective	90 μL of sample was added to the 100 uL lysis buffer SB. All other buffer				
			RNA was recovered eith a 10 min heated Eluate was stored on ice until added to the	elution at 60°C in 100 μl Elution buffer AMP u	nder fast mixing.		carrier. 280 μl of a premix containing 100 μl	volumes and the automated protocol was performed according to the				
			Eluate was stoled on ice until added to ti	ie Kryrck.			lysis buffer SB, 160 uL binding buffer SB	manufacturer's recommendations as				
							and 20 uL sbeadex particle solution +	described in Fig 3A with the exception				
							EDTA per reaction was added, followed by washing procedure according to	of the lysis step that was performed for 3 min heated. The RNA was eluted in				
							manufacturer's protocol as described	100 μL elution buffer AMP.				
							for Fig2A-D. No heated lysis step on KingFisher platform was performed. The					
							RNA was eluted in 100 µL Elution buffer AMP.					
Name of kit and details of any modifications	Ē.	Yes	As described above.	As described above.	Original protocol: as described above with 11 min lysis, 4 min washing and 5	As described above with addition of 1 ug PolyA carrier (Sigma Aldrich P/N	As described above.	As described above.	N/A			
					min elution. 39 min protocol: as described above	10108626001) per extraction if required.						
					with 3 min lysis step							
					27 min protocol: as described above with 3 min lysis and 1 min washing steps							
					24 min protocol: as described for above							
					with 3 min lysis, 1 min washing and 5 min elution steps.							
Source of additional	D	Yes	As described above in the relevant section	ns.			The volume given is the volume added	The volume given is the volume added	Bile salts (SIGMA) added at the final	Bile salts (SIGMA) added at the final	Donor urine, see details in sample	N/A
reagents used							to lysis buffer prior to each extraction; number in brakets is the total amount	to lysis buffer prior to each extraction; number in brakets is the total amount	concentration in the RT-qPCR as indicated.	concentration in the RT-qPCR as indicated.	section above.	
							added to each extraction:	added to each extraction:	mucateu.	indicated.		
							- genesig Easy RNA Internal extraction control (IEC)(Primerdesign): 20 uL (stock	- Internal extraction Control				
							resuspended in 1000 µL; unknown final	(Primerdesign): as before. - PolyC (Sigma Aldrich P/N4903): as				
							concentration)	before.				
							- PolyA (Sigma Aldrich P/N 10108626001): 2 uL (1 ug per extraction)					
							- PolyC (Sigma Aldrich P/N 4903): 2 uL (1					
							ug per extraction) - Salmon sperm gDNA (Invitrogen P/N					
							15632011V): 2 uL (1µg per extraction,					
							supplied by Novacyt).					
							Additional information on the carrier					
							molecules:					
							 PolyA and PolyC were delivered as lyophylised product and resuspended in 					
							nuclease-free water to 10mg/mL that					
							was stored in single use aliquots at - 80°C. The single use working stocks					
							were diluted 1:20 to give 0.5 mg/mL and					
Details of DNase or RNAse		Yes	Not performed.				remnants were discarded (not stored). Not performed.		Not performed.			
treatment	-											
(DNA or RNA)	E	Yes	Not performed.				Not performed.		Not performed.			
Nucleic acid quantification	E	Yes	Not performed; 5 uL of elutant added to I	RT-qPCR for all experiments.			Not performed.		Not performed.			
Instrument and method	E		N/A				N/A		N/A			
Purity (A260/A280)	D		N/A				N/A		N/A			
Yield RNA integrity	D E	Yes	N/A Not performed.				N/A Not performed.		N/A Not performed.			
RIN/RQI or Cq of 3' and 5'	E						N/A		N/A			
transcripts Electrophorosis trases			N/A									
Electrophoresis traces	D		N/A N/A				N/A		N/A			
Inhibition testing (Cq	D E	Yes	N/A				The IEC acts as a performance indicator: in	nhibition testing is performed by comparison	N/A Aim of the expeirment - experimental gro	up.		N/A
Electrophoresis traces Inhibition testing (Cq dilutions, spike or other)	E E	Yes	N/A N/A				N/A The IEC acts as a performance indicator; in of the extracted IEC Cq with the Cq obtain spike into the RT-qPCR.	nhibition testing is performed by comparison ned from a matched concentration direct	N/A Aim of the experiment - experimental gro	ир.		N/A
Inhibition testing (Cq dilutions, spike or other)	E E		N/A N/A Not performed.	As described for Ein 20 with Maximum v	only		The IEC acts as a performance indicator; in of the extracted IEC Cq with the Cq obtain spike into the RT-qPCR.	ned from a matched concentration direct	Aim of the expeirment - experimental gro		and 12 ut analysed by dDFP	N/A
Inhibition testing (Cq dilutions, spike or other)	E E	Yes	N/A N/A	As described for Fig 3A with Mastermix 1	only.		The IEC acts as a performance indicator; in of the extracted IEC Cq with the Cq obtain spike into the RT-qPCR. One-step RT-qPCR was performed using to PCR COVID-19 (CE IVD) (Primerdesign PN:	ned from a matched concentration direct the Coronavirus COVID-19 genesig* Real-Time 2-Path-COVID-19-CE, manual: 2-Path-COVID-	Aim of the expeirment - experimental gro	up. ared for the RT with 12 uL analysed by qPCR a	ind 12 uL analysed by dPCR.	N/A
Inhibition testing (Cq dilutions, spike or other)	E E		N/A N/A Not performed. One-Step RT-PCR was performed for all experiments.	As described for Fig 3A with Mastermix 1	only.		The IEC acts as a performance indicator; in of the extracted IEC Cq with the Cq obtain spike into the RT-qPCR. One-step RT-qPCR was performed using ti PCR COVID-19 (CE IVD) (Primerdesign PN: 19-CE-IPU Suse 3.0). This sasay contains it sasay contains to	ned from a matched concentration direct he Coronavirus COVID-19 genesig* Real-Time 2-Path-COVID-19-CE, manual: 2-Path-COVID- the primer pair and FAM probe for the SARS-	Aim of the expeirment - experimental gro		ind 12 uL analysed by dPCR.	N/A
Inhibition testing (Cq dilutions, spike or other)	E E		N/A NO1 performed. One-Step RT-PCR was performed for all experiments. Mastermit 1: GoT ang* Probe 1-Step RT-gPCR (Pormega)	As described for Fig 3A with Mastermix 1	only.		The IEC acts as a performance indicator; in of the extracted IEC Cq with the Cq obtain spike into the RT-qPCR. One-step RT-qPCR was performed using ti PCR COVID-19 (CE IVD) (Primerdesign PN: 19-CE-IPU Suse 3.0). This sasay contains it sasay contains to	ned from a matched concentration direct the Coronavirus COVID-19 genesig* Real-Time 2-Path-COVID-19-CE, manual: 2-Path-COVID-	Aim of the expeirment - experimental gro		nd 12 ut analysed by dPCR.	N/A
Inhibition testing (Cq dilutions, spike or other)	E E		N/A N/A Not performed. One-Step RT-PCR was performed for all experiments. Mastermix 1: GoTag* Probe 1-Step RT-gPCR (Promega) Mastermix 2: GuTag* Probe 1-Step RT-gPCR (Promega)	As described for Fig 3A with Masternix. 3	only.		The IEC acts as a performance indicator, in of the extracted IEC Cq with the Cq obtain spike into the RT-qPCR. One-step RT-qPCR was performed using it PCR COVID-19 (CE IVD) (Primerdesign PN: 19-CE-IFU Issue 3.0). This assay contains to CoV-2 target and a primer pair and VIC/IH	ned from a matched concentration direct he Coronavirus COVID-19 genesig* Real-Time 2-Path-COVID-19-CE, manual: 2-Path-COVID- the primer pair and FAM probe for the SARS-	Aim of the expeirment - experimental gro		ind 12 uL analysed by dPCR.	N/A
Inhibition testing (Cq dilutions, spike or other)	E E		N/A NO1 performed. One-Step RT-PCR was performed for all experiments. Mastermit 1: GoT ang* Probe 1-Step RT-gPCR (Pormega)	As described for Fig 3A with Mastermix 1	only.		The IEC acts as a performance indicator, in of the extracted IEC Cq with the Cq obtain spike into the RT-qPCR. One-step RT-qPCR was performed using it PCR COVID-19 (CE IVD) (Primerdesign PN: 19-CE-IFU Issue 3.0). This assay contains to CoV-2 target and a primer pair and VIC/IH	ned from a matched concentration direct he Coronavirus COVID-19 genesig* Real-Time 2-Path-COVID-19-CE, manual: 2-Path-COVID- the primer pair and FAM probe for the SARS-	Aim of the expeirment - experimental gro		nd 12 uL analysed by dPCR.	N/A
Inhibition testing (Cq dilutions, spike or other)	E E		N/A NOT performed. One-Stap RT-PCR was performed for all experiments. Mastermis 1: GoT an ⁴² Probe 1-Step RT-qPCR (Promorga) Mastermis 2: Lucigen RapitoFire Mastermis 2: Lucigen RapitoFire Reverse Transcriptase.	As described for Fig 3A with Mastermix 3	only.		The IEC acts as a performance indicator, in of the extracted IEC Cq with the Cq obtain spike into the RT-qPCR. One-step RT-qPCR was performed using it PCR COVID-19 (CE IVD) (Primerdesign PN: 19-CE-IFU Issue 3.0). This assay contains to CoV-2 target and a primer pair and VIC/IH	ned from a matched concentration direct he Coronavirus COVID-19 genesig* Real-Time 2-Path-COVID-19-CE, manual: 2-Path-COVID- the primer pair and FAM probe for the SARS-	Aim of the expeirment - experimental gro		nd 12 u.L analysed by dPCR.	N/A
Inhibition testing (Cq dilutions, spike or other)	E E		N/A NOx performed. One-Step RT-PCR was performed for all experiments. Mastermix 1. GoT ag* Probe 1-Step RT-gPCR Promorgial Mastermix 2: Lodges RapiDrifre Mastermix supplemented with Episcript Reverse Transcriptase. RT -QPCR assay: 2019-nov LOCG-qualified Probe and Primer Kits (RTT-qualified Probe and Primer Kits (RTT-qualified Probe and Primer Kits (RTT-qualified Probe and Primer Kits (RTT-	As described for Fig 3A with Mastermix 1	only.		The IEC acts as a performance indicator, in of the extracted IEC Cq with the Cq obtain spike into the RT-qPCR. One-step RT-qPCR was performed using it PCR COVID-19 (CE IVD) (Primerdesign PN: 19-CE-IFU Issue 3.0). This assay contains to CoV-2 target and a primer pair and VIC/IH	ned from a matched concentration direct he Coronavirus COVID-19 genesig* Real-Time 2-Path-COVID-19-CE, manual: 2-Path-COVID- the primer pair and FAM probe for the SARS-	Aim of the expeirment - experimental gro		ind 12 ut analysed by dPCR.	N/A
Inhibition testing (Cq dilutions, spike or other)	E E		N/A Not performed. Not performed. One-Step RT-PCR was performed for all experiments. Mastermia: I: GoTaq* Probe 1-Step RT-qPCR (Promeja) Mastermia 2: Lucigen Rapito#ire Mastermia 2: Lucigen Rapito#ire Mastermia 2: Lucigen Rapito#ire Reverse Transcriptase. RT-qPCR saxe_2019-in-CoV CDC-qualified Probe and Primer KIts (RTT- NCOV-P9-1: DOI) containing FAM-	As described for Fig 3A with Mastermix 1	only.		The IEC acts as a performance indicator, in of the extracted IEC Cq with the Cq obtain spike into the RT-qPCR. One-step RT-qPCR was performed using it PCR COVID-19 (CE IVD) (Primerdesign PN: 19-CE-IFU Issue 3.0). This assay contains to CoV-2 target and a primer pair and VIC/IH	ned from a matched concentration direct he Coronavirus COVID-19 genesig* Real-Time 2-Path-COVID-19-CE, manual: 2-Path-COVID- the primer pair and FAM probe for the SARS-	Aim of the expeirment - experimental gro		ind 12 ut analysed by dPCR.	N/A
Inhibition testing (Cq dilutions, spike or other)	D E		N/A NOT performed. One-Step RT-PCR was performed for all experiments. Mastermix 1: GoTaq* Probe 1-Step RT-qPCR (Promeps) Mastermix 2: Lucigen Rapiloriire Mastermix 2: Lucigen Rapiloriire Mastermix 2: Despendented with Episcript Revene Transcriptase. RT qPCR savy 2019—En-CV CDC- qualified Probe and Primer Vita (VIT- NCD-VPP-1 1: DOI) Containing FAM- labeled probes for targets N1, N2 and human Rikase Pwas used. N2 was the	As described for Fig 3A with Mastermix 1	only.		The IEC acts as a performance indicator, in of the extracted IEC Cq with the Cq obtain spike into the RT-qPCR. One-step RT-qPCR was performed using it PCR COVID-19 (CE IVD) (Primerdesign PN: 19-CE-IFU Issue 3.0). This assay contains to CoV-2 target and a primer pair and VIC/IH	ned from a matched concentration direct he Coronavirus COVID-19 genesig* Real-Time 2-Path-COVID-19-CE, manual: 2-Path-COVID- the primer pair and FAM probe for the SARS-	Aim of the expeirment - experimental gro		nd 12 ut. analysed by dPCR.	N/A
Inhibition testing (Cq dilutions, spike or other)	D E		N/A NO1 performed. One-Step RT-PCR was performed for all experiments. Mastermia: 1, GoT aq** Probe 1-Step RT-gPCR Promongal Austermia: 2, Locigon RapiDrafire Mastermia: 2, Locigon RapiDrafire Mastermia: 2, Locigon RapiDrafire Mastermia: 2, Locigon RapiDrafire Mastermia: 2, Locigon RapiDrafire Comparison of the Com	As described for Fig 3A with Mastermix 1	only.		The IEC acts as a performance indicator, in of the extracted IEC Cq with the Cq obtain spike into the RT-qPCR. One-step RT-qPCR was performed using it PCR COVID-19 (CE IVD) (Primerdesign PN: 19-CE-IFU Issue 3.0). This assay contains to CoV-2 target and a primer pair and VIC/IH	ned from a matched concentration direct he Coronavirus COVID-19 genesig* Real-Time 2-Path-COVID-19-CE, manual: 2-Path-COVID- the primer pair and FAM probe for the SARS-	Aim of the expeirment - experimental gro		nd 12 ut analysed by dPCR.	N/A
Inhibition testing (Cq dilutions, spike or other)	D E		N/A N/A NOS performed. One-Step RT-PCR was performed for all experiments. Matterns 1: GoTaq* Probe 1-Step RT-qPCR (Promeja) qPCR (Promeja) Reverse Transcriptase. RT-qPCR Rassay: 2019-nCoV CDC-qualified Probe and Primer KIS; (KIT-NCOV-PS-1) COIQ containing FAM-labeled probes for targets N1, N2 and human Rhitse Prevauced. N2 was the Mannan Rhitse Prevauced. N2 was the SASSY reconstituted as a 15 ufld stock containing 10 mol of each primer should be contained to the same property of	As described for Fig 3A with Masternix 1	only.		The IEC acts as a performance indicator, in of the extracted IEC Cq with the Cq obtain spike into the RT-qPCR. One-step RT-qPCR was performed using it PCR COVID-19 (CE IVD) (Primerdesign PN: 19-CE-IFU Issue 3.0). This assay contains to CoV-2 target and a primer pair and VIC/IH	ned from a matched concentration direct he Coronavirus COVID-19 genesig* Real-Time 2-Path-COVID-19-CE, manual: 2-Path-COVID- the primer pair and FAM probe for the SARS-	Aim of the expeirment - experimental gro		ind 12 uL analysed by dPCR.	N/A
Inhibition testing (Cq dilutions, spike or other) REVERSE TRANSCRIPTION Complete reaction conditions		Yes	N/A NOT performed. One-Step RT-PCR was performed for all experiments. Mastermix 1: GoTaq* Probe 1-Step RT-qPCR (Promega) Mastermix 2: Lucigen RapitoFrire Mastermix 2: Lucigen RapitoFrire Reverse Transcriptase. RT qPCR assex_203=FcaV CDC-qualified Probe and Primer Vol. (VDC-qualified Probe Vol. vol. qualified Probe and Primer Vol. (VDC-qualified Probe Vol. vol. qualified Probe National Probability Company (VDC-qualified Probe Probability Company) Assay reconstituted as a 15 Unit Vol. vol. qualified VDC-qualified	As described for Fig 3A with Masternix 1	only.		The IEC acts as a performance indicator; in of the extracted IEC cq with the Cq obtain spike into the RT-qPCR. One-step RT-qPCR was performed using the PCR COVID-19 (IEC IVD) (Primerdesign PT. 32-CE-IFU ISSUE) and 30.) This assay containst CoV-2 target and a primer pair and VIC/Nt duplex reaction.	hed from a matched concentration direct the Coronavirus COVID-19 genesias "Real-Time. Zepath COVID-39-CE, manual: Zepath COVID- per primer pair and Hady pools for the SARS- SEX probe for the IEC target to be used in a	Alm of the experiment - experimental gro		nd 12 ut analysed by dPCR.	N/A
Inhibition testing (Cq dilutions, spike or other)	D E		N/A N/A NOS performed. One-Step RT-PCR was performed for all experiments. Matterns 1: GoTaq* Probe 1-Step RT-qPCR (Promeja) qPCR (Promeja) Reverse Transcriptase. RT-qPCR Rassay: 2019-nCoV CDC-qualified Probe and Primer KIS; (KIT-NCOV-PS-1) COIQ containing FAM-labeled probes for targets N1, N2 and human Rhitse Prevauced. N2 was the Mannan Rhitse Prevauced. N2 was the SASSY reconstituted as a 15 ufld stock containing 10 mol of each primer should be contained to the same property of		only.		The IEC acts as a performance indicator, in of the extracted IEC Cq with the Cq obtain spike into the RT-qPCR. One-step RT-qPCR was performed using it PCR COVID-19 (CE IVD) (Primerdesign PN: 19-CE-IFU Issue 3.0). This assay contains to CoV-2 target and a primer pair and VIC/IH	ned from a matched concentration direct he Coronavirus COVID-19 genesig* Real-Time 2-Path-COVID-19-CE, manual: 2-Path-COVID- the primer pair and FAM probe for the SARS-	Aim of the expeirment - experimental gro		nd 12 ut analysed by dPCR.	N/A
Inhibition testing (Cq dilutions, spike or other) BRYESSE MANSCRIPTION Complete reaction Conditions Amount of RNA and		Yes	N/A NOT performed. Not performed. One-Step RT-PCR was performed for all experiments. Mastermia: 1:GoT ang* Probe 1-Step RT-gPCR [Promega) Mastermia: Supplemented with Episcript Reverse Transcriptase. RT-gPCR assay: 2019-erOv CCC. qualified Probe and Primer KIS [KIT-NCV-PP1-1000] containing FAM-labeled probe for targets NI, NZ and human Rikise P was used. NZ was the only target used for these experiments. Assay reconstituted as a 15-M stock. 2.5 med of profe die eich primer and 2.5 med of profe die eich primer and 2.5 med of profe die eich primer and 5.5 med of primer and 5.5 us of eleuant from a single extraction. 5 ut of eleuant from a single extraction.	JM primers/probe stock for the NZ assay.	only.		The IEC acts as a performance indicator; in of the extracted IEC or with the Cq obtain spike into the RT-qPCR. One-step RT-qPCR was performed using IPCR COVID-19 (EX PA) This assignment of CQC 2000-19 (EX PA) Thi	hed from a matched concentration direct the Coronavirus COVID-19 generigi* Real-Trine- RP-RPH-COVID-19-CE. manuel: P-RPH-COVID- Herpitrier pair and RPM probe for the APM probe prote has a For each teaction: 8 µ elwant from the extraction was added to 20 µ treaction. MTC reactions contained 8 µt water in	Alm of the experiment - experimental gro		nd 12 u.L analysed by dPCR.	N/A
Inhibition testing (Cq dilutions, spike or other) REVERSE MANUSCRIPTION Complete reaction conditions Amount of RNA and		Yes	N/A NOT performed. One-Step RT-PCR was performed for all experiments. Mastermia: 1:GoT ang* Probe 1-Step RT-gPCR (Promega) Mastermia: Supplemented with Episcript Reverse Transcriptase. RT-gPCR assay: 2019-nCoV CDr. qualified robos and Primer Kits (RT-NCV-PP)-1:000] containing FAM-labeled probes for tragest NI, NZ and human Rikise P was used. NZ was the only target used for these experiments. Assay reconstituted as a 15-bit stock. 2.5 med of prime deech primer and 2.5 med of prime deech primer and 5.5 med of primer and 5.5 med of primer and 5.5 med of primer and 5.5 up of electric forms a single-extraction 15 u.d of mastermix containing 1.5 u.1.5 t. NTC reactions contained 5 µl nuclease-free	JM primers/probe stock for the N2 assay. e water in place of the extraction eluant.			The IEC acts as a performance indicator; in of the extracted IEC ca with the Cq obtain spike into the RT-PCR of the Cq obtain spike into the RT-PCR was performed using the CR COVID-19 (IE VID) (Primeredisgin PN 19-CE-IPU ISSUE 3.0). This assay contains to CoV-2 target and a primer pair and VIC/HI duplex reaction. For each reaction: By it elaunt from the extraction was added to 29 but reaction. Positive controls were performed with 8 us of a 1:100 dullation of each five-point	hed from a matched concentration direct the Coronavirus CDVID-19 genesig* Real-Time P-Path-COVID-19-EC manual: P-Path-COVID- he primer pair and IT-MA probe for the SASS- CA probe for the IEC target to be used in a For each reaction: 8 µt eluant from the extraction was added to 20 µt reaction.	Alm of the experiment - experimental gro		ind 12 ut. analysed by dPCR.	N/A
Inhibition testing (Cq dilutions, spike or other) REVERSE MANUSCRIPTION Complete reaction conditions Amount of RNA and		Yes	N/A NOT performed. One-Step RT-PCR was performed for all experiments. Mastermix 1: GoT ang* Probe 1-Step RT-gPCR (Promega) Mastermix 2: GoT ang* Probe 1-Step RT-gPCR (Promega) Mastermix 2: Lucigen RapitoFire Mastermix 2: Lucigen RapitoFire Reverse Transcriptase. RT-gPCR assay: 2019-nCV CDC-qualified probe and Primer Kits (RTT-NCV-PP3-1000) containing FAM-labeled probes of trargets NJ, NP2 and human RNase P was used. NP2 was the norly target used for trargets AI, NP2 and human RNase P was used. NP2 was the norly target used for trargets and 12 so man of a probe. For each reaction, 5 ut of elaunt from a single extraction 15 ut of materiax containing 15 ut 15 ut NTC reactions contained 5 µl nuclease-fre PCR positive controls and additional sing controls and additional services are controls and additional services.	JM primers/probe stock for the N2 assay. The water in place of the extraction eluant. ≕in controls, 5 µl of Seracure AccuPles SARS-C	only. 2012 Reference Material (5505-0126) was added of species of the control of		The IEC acts as a performance indicator; in of the extracted IEC ca with the Cq obtat spike into the RT-PCR. One-step RT-PCR was performed using the CR occurrence of the CR occurrence occur	hed from a matched concentration direct the Coronavirus COVID-19 generigi* Real-Trine- RP-RPH-COVID-19-CE. manuel: P-RPH-COVID- Herpitrier pair and RPM probe for the APM probe prote has a For each teaction: 8 µ elwant from the extraction was added to 20 µ treaction. MTC reactions contained 8 µt water in	Alm of the experiment - experimental gro		ind 12 ut analysed by dPCR.	N/A
Inhibition testing (Cq dilutions, spike or other) REVERSE MANUSCRIPTION Complete reaction conditions Amount of RNA and		Yes	N/A NOT performed. One-Step RT-PCR was performed for all experiments. Mastermik 1: GoTaq* Probe 1-Step RT-qPCR (Promega) Mastermik 2: Lucigen RapiDriFre Matermix 3: Lucigen RapiDriFre Matermix 1000 containing FAM-labeled probes for targets N1, N2 and human Ribase Pwas used. N2 was the only target used for these experiments. Assay reconstituted as a 15 Ush Va was the only target used for these experiments. Assay reconstituted as a 15 Ush Containing 10 mnol of each primer and 2.5 mnol of probe. For each reaction, 5 Us of elastermix containing 1.5 U. 1.5 L NTC reactions contained 5 Jul nuclease-fire PCR positive controls and additional spike	JM primers/probe stock for the N2 assay. The water in place of the extraction eluant. ≕in controls, 5 µl of Seracure AccuPles SARS-C	ov2 Reference Material (0505-0126) was added d		The IEC acts as a performance indicator; in of the extracted IEC or with the Cq obtain spike into the RT-qPCR. One-step RT-qPCR was performed using it PCR COVID-19 (EE ND) (invinencelsign PTM). The CPTM IS and Spike into the RT-qPCR and Spike into the RT-qPCR and Spike into the RT-qPCR into Company. For each reaction: 8 jut eluant from the extraction was added to 20 jut reaction. Positive controls were performed with 8 u. of a 1:100 dilution of each five-point serial dilution added directly to the RT-qPCR to company with the extractions if	hed from a matched concentration direct the Coronavirus COVID-19 generigi* Real-Trine- RP-RPH-COVID-19-CE. manuel: P-RPH-COVID- Herpitrier pair and RAPI probe for the API price pair and RAPI probe for the API price pair and RAPI probe for the API price pair and in a second of the API probe for the ST probe for the IEC target to be used in a price pair and the API probe for the IEC target to be used in a price pair and the API probe for the IEC target to be used in a price pair price pair and the API	Alm of the experiment - experimental gro		ind 12 ut analysed by dPCR.	N/A
Inhibition testing (Cq dilutions, spike or other) REVERSE MANUSCRIPTION Complete reaction conditions Amount of RNA and		Yes	N/A NOT performed. One-Step RT-PCR was performed for all experiments. Mastermix 1: GoT ang* Probe 1-Step RT-gPCR (Promega) Mastermix 2: GoT ang* Probe 1-Step RT-gPCR (Promega) Mastermix 2: Lucigen RapitoFire Mastermix 2: Lucigen RapitoFire Reverse Transcriptase. RT-gPCR assay: 2019-nCV CDC-qualified probe and Primer Kits (RTT-NCV-PP3-1000) containing FAM-labeled probes of trargets NJ, NP2 and human RNase P was used. NP2 was the norly target used for trargets AI, NP2 and human RNase P was used. NP2 was the norly target used for trargets and 12 so man of a probe. For each reaction, 5 ut of elaunt from a single extraction 15 ut of materiax containing 15 ut 15 ut NTC reactions contained 5 µl nuclease-fre PCR positive controls and additional sing controls and additional services are controls and additional services.	JM primers/probe stock for the N2 assay. The water in place of the extraction eluant. ≕in controls, 5 µl of Seracure AccuPles SARS-C	ov2 Reference Material (0505-0126) was added d		The IEC acts as a performance indicator; in of the extracted IEC cq with the Cq obtain spike into the RT-qPCR. One-step RT-qPCR was performed using it PCR COVID-19 (EE IVD) (immercelian) TM. 19-CE-IFU ISSUE and 30, This assay contains it COV-2 target and a primer pair and VIC/NI dupler reaction. For each reaction: 8 µL eluant from the extraction was added to 20 µL reaction. 8 µL eluant from the extraction was added to 20 µL reaction. 8 µL eluant from the extraction was added to 20 µL reaction. 10 µL reaction: 10 µL reaction: 11 µL reaction: 12 µL reaction: 13 µL reaction: 14 µL reaction: 15 µL reaction: 16 µL reaction: 17 µL reaction: 18 µL eluant from the extraction was added a	hed from a matched concentration direct the Coronavirus COVID-19 generigi* Real-Trine- RP-RPH-COVID-19-CE. manuel: P-RPH-COVID- Herpitrier pair and RAPI probe for the API price pair and RAPI probe for the API price pair and RAPI probe for the API price pair and in a second of the API probe for the ST probe for the IEC target to be used in a price pair and the API probe for the IEC target to be used in a price pair and the API probe for the IEC target to be used in a price pair price pair and the API	Alm of the experiment - experimental gro		ind 12 ut. analysed by dPCR.	N/A
Inhibition testing (Cq dilutions, spike or other) BRYESSE MANSCRIPTION Complete reaction Conditions Amount of RNA and		Yes	N/A NOT performed. One-Step RT-PCR was performed for all experiments. Mastermix 1: GoT ang* Probe 1-Step RT-gPCR (Promega) Mastermix 2: GoT ang* Probe 1-Step RT-gPCR (Promega) Mastermix 2: Lucigen RapitoFire Mastermix 2: Lucigen RapitoFire Reverse Transcriptase. RT-gPCR assay: 2019-nCV CDC-qualified probe and Primer Kits (RTT-NCV-PP3-1000) containing FAM-labeled probes of trargets NJ, NP2 and human RNase P was used. NP2 was the norly target used for trargets AI, NP2 and human RNase P was used. NP2 was the norly target used for trargets and 12 so man of a probe. For each reaction, 5 ut of elaunt from a single extraction 15 ut of materiax containing 15 ut 15 ut NTC reactions contained 5 µl nuclease-fre PCR positive controls and additional sing controls and additional services are controls and additional services.	JM primers/probe stock for the N2 assay. The water in place of the extraction eluant. ≕in controls, 5 µl of Seracure AccuPles SARS-C	ov2 Reference Material (0505-0126) was added d		The IEC acts as a performance indicator; in of the extracted IEC or with the Cq obtain spike into the RT-qPCR. One-step RT-qPCR was performed using it PCR COVID-19 (EE ND) (invinencelsign PTM). The CPTM IS and Spike into the RT-qPCR and Spike into the RT-qPCR and Spike into the RT-qPCR into Company. For each reaction: 8 jut eluant from the extraction was added to 20 jut reaction. Positive controls were performed with 8 u. of a 1:100 dilution of each five-point serial dilution added directly to the RT-qPCR to company with the extractions if	hed from a matched concentration direct the Coronavirus COVID-19 generigi* Real-Trine- RP-RPH-COVID-19-CE. manuel: P-RPH-COVID- Herpitrier pair and RAPI probe for the API price pair and RAPI probe for the API price pair and RAPI probe for the API price pair and in a second of the API probe for the ST probe for the IEC target to be used in a price pair and the API probe for the IEC target to be used in a price pair and the API probe for the IEC target to be used in a price pair price pair and the API	Alm of the experiment - experimental gro		ind 12 ut. analysed by dPCR.	N/A
Inhibition testing (Co didutions, spike or other) REVERSE TANASCRIPTION Complete reaction conditions Amount of RNA and		Yes	N/A NOT performed. One-Step RT-PCR was performed for all experiments. Mastermix 1: GoT ang* Probe 1-Step RT-gPCR (Promega) Mastermix 2: GoT ang* Probe 1-Step RT-gPCR (Promega) Mastermix 2: Lucigen RapitoFire Mastermix 2: Lucigen RapitoFire Reverse Transcriptase. RT-gPCR assay: 2019-nCV CDC-qualified probe and Primer Kits (RTT-NCV-PP3-1000) containing FAM-labeled probes of trargets NJ, NP2 and human RNase P was used. NP2 was the norly target used for trargets AI, NP2 and human RNase P was used. NP2 was the norly target used for trargets and 12 so man of a probe. For each reaction, 5 ut of elaunt from a single extraction 15 ut of materiax containing 15 ut 15 ut NTC reactions contained 5 µl nuclease-fre PCR positive controls and additional sing controls and additional services are controls and additional services.	IM primers/probe stock for the N2 assay. The water in place of the extraction eluant. The controls, 5 µl of Seracure Accupies SARS-Centration of 3.75 copies/RT-qPCR and/or 15 co	ov2 Reference Material (0505-0126) was added d		The IEC acts as a performance indicator; in of the extracted IEC ca with the Cq obtain spike into the RT-PCR was performed using the CR or the CR	he Groma matched concentration direct the Coronavirus COVID-19 generals* Real-Trine. Zeath COVID-19 Generals* Real-Trine. Zeath COVID-19 CE, manual: Zeath-COVID- per princip pair and Affil probe for the SASS- X probe for the IEC target to be used in a For each reaction: 8 µL eluant from the extraction was added to 20 µL reaction. NTC reactions contained 8 µL water in place of eluant.	Alm of the experiment - experimental gro		nd 12 ut. analysed by dPCR.	N/A

Reverse transcriptase					Page 3 c		
and concentration	E	Yes	Mastermix 1. GoScript** TR Mix for 1- Step RT-QPC Stocks (f. Q4 µl per reaction). Mastermax 2. EpiScript** Rikase +- Reverse Transcriptase, 200 U/µl (0.5 µl per reaction).	Qasig [™] One-Step 2x RT-qPCR Master Mix lyophilised, resuspended in 325µI Qasig [™] resuspension buffer.	See Table S2.		
Temperature and time	E	Yes	Mastermix 1: 15 min at 45 °C. Mastermix 2: 15 min at 42 °C. As described for Fig 3A with Mastermix 1 only.	55 ℃ for 10 min	See Table S2.		
Manufacturer of reagents and catalogue numbers	D	Yes	Mastermix 1: Promaga: GoTaq* Probe 1: Step RT-qCR: System, A612: Mastermix 1: Quigen: RapilDrire qCR SX Master Mix Cpf, Ind., 30050-1, with EpiScript RNase H- Reverse Transcriptase, ERT12925K.	genetig* Real-Time PCR COVID-19 (CE), One step RT-PCR (Primerdesign PN Z-Path- COVID-19-CE-).	See Table S2.		
Cqs with and without RT	Dc	Yes	Not performed as one-step mastermix.	It is not possible to separate RT from PCR as it is provided as a mastermix so this was not performed.	Not performed as one-step mastermix.		
Storage conditions of cDNA	D	Yes	Not performed as one-step mastermix.	Not performed as one-step mastermix.	Not performed as one-step mastermix.		
qPCR TARGET INFORMATION							
If multiplex, efficiency and LOD of each assay.	Ł	Yes	No multiplex performed. The used assay is the 2019-nCoV CDC-qualified Probe and Primer Kits for SARS-CoV-2 that targets SARS Cov2 Nucleocapsid gene N1 and N2. N2 was the only target used for experiments.		See Table S2 (CDC N1 and CDC N2 in duplex reactions).		
Sequence accession number	E	Yes	Accession number NC_045512 was submitted first in December 2020. Since no viral extractions were publically available, in vitro transcribed RNA (N gene; GenBank acces MN908947.2) was used for validation of LOD.	in: SARS-CoV-2 target assay: manufacturer refers to GISAID EpiCoV database, GISAID EpiCoV database itself refers to hCoV-13/Wuhan/WIV04/2019 (WIV04, EPI_ISL_402124) as the official reference genome.	See Table S2.		
Location of amplicon	D	Yes	Nucleocapsid phosphoprotein (N), NC_045512v2: 29163-29230.	SARS-CoV-2 target: Orf1 ab gene, see CE-IVD certified product	See Table S2.		
Amplicon length	E	Yes	According to Accession Number January 2020/NC_0.445512_2: 67bp (UCSC Browser, In-silico PCR tool)	IEC target: unknown Unknown (CE-IVD certified product); not supplied by manufacturer.	See Table S2.		
In silico specificity screen (BLAST, etc)	E	Yes	According to FDA, CDC.000-00018, Revision: 08, EUA Instruction for use: CDC.2019 nCoV Real-Time RT-PCR Diagnostic Panel were evaluated against 31,623 sequences avoid Global Initiative on Sharing All Influenza Data (GSDA). Interpol. yold was gained a property of the CDT-PCR Diagnostic Panel. Moreover, in silico analysis were performed using BLASTn (ncb), for details please refer to CDC-008-00019 EUA instruction for use).	Time RT- database (see above) is daily reviewed and an in silico analysis is performed on weekly basis to confirm validity of the target region, cross reactivity was reported for two sequences of Bat coronavirus and Pangolin Coronavirus (see current Z-Path-COVID-19- CE-IFU Issue 5.00: p.23, 27f)	See Table S2.		
Pseudogenes,	D D		N/A	N/A	N/A as viral target.		
Sequence alignment Secondary structure	D		N/A N/A	Current Z-Path-Covid-CE-IFU Issue 5.00 : p27 f Unknown (CE-IVD certified product); not supplied by manufacturer.	See Table S2. See Table S2.		
analysis of amplicon Location of each primer by exon or intron (if applicable)	E		N/A as viral target.	N/A as viral target.	N/A as viral target.		
What splice variants are	E		N/A as viral target.	N/A as viral target.	N/A as viral target.		
qPCR OLIGONUCLEOTIDES				Unknown (CE-IVD certified product): not supplied by manufacturer.	See Table S2 (CDC N1 and N2 targets).		
Primer sequences	E	Yes	N2 FW 5' TTACAAACATTGGCCGCAAA 3' N2 RV 5' GCGCGACATTCCGAAGAA 3'	, , , , , , , , , , , , , , , , , , , ,			
RTPrimerDB Identification Number	D		N/A	N/A	See Table S2.		
Probe sequences	D ^d	Yes	N2 Probe 5' FAM-ACAATTTGCCCCAGGGGTTCAG-BHQ-1 3'	Unknown (CE-IVD certified product); not supplied by manufacturer. SARS CoV2 Target Probe FAM labeled. Internal Extraction Control WC/FURS labeled.	See Table S2.		
Probe sequences Location and identity of any modifications	D ^d	Yes	N2 Probe 5' FAM-ACAATTIGCCCCAGCGCTTCAG-BHQ-1 3' None.	SARS CoV2 Target Probe FAM labeled.	See Table S2. See Table S2.		
Location and identity of any modifications	D ^d E			SARS CoV2 Target Probe FAM labeled. Internal Extraction Control VIC/HEX labeled.			
Location and identity of any modifications		Yes	None. Biosearch Technologies	SARS COV2 Target Probe FAM labeled. Internal Extraction Control VIC/HEX labeled. None. Primerdesign Ltd	See Table S2. See Table S2.		
Location and identity of any modifications Manufacturer of oligonucleotides Purification method GPCR PROTOCOL		Yes Yes Yes	None. Biosearch Technologies Primer AX HPLC, Probe Dual HPLC.	SARS COV2 Target Probe FAM labeled. Internal Extraction Control VIC/HEX labeled. None. Primerdesign Ltd Unknown (CE-IVO certified product); not supplied by manufacturer.	See Table S2. See Table S2. See Table S2.		
Location and identity of any modifications Manufacturer of oligonucleotides Purification method (BPCR PROTOCO) Complete reaction conditions	D D	Yes Yes Yes Yes	None. Biosearch Technologies Primer AX HPLC, Probe Dual HPLC. One-step as described in reverse transcription section.	SARS COV2 Target probe FAM labeled. Internal Estraction Control VIC/HEX labeled. None. Primerdesign Ltd Unknown (CE-IVD certified product); not supplied by manufacturer. One-step as described in reverse transcription section. See CE-IVD certified product manual with further details below.	See Table 52. See Table 52. See Table 52. See Table 52. Each 28 ut reverse transcription reaction was split between qPCR and dPCR - see Table 52 for details. For qPCR, 12 ut was transferred to 0.1 mt tubes for the Rotor-Gene Q (and 12 ut transferred to QIAcuity 96-well 8.5k Nanoplate wells.		
Location and identity of any modifications Manufacturer of oligonucleotides Purification method IPCR RRITOCOL Complete reaction conditions Reaction volume and		Yes Yes Yes	None. Biosearch Technologies Primer AX HPLC, Probe Dual HPLC. One-step as described in reverse transcription section. N/A as one-step (see reverse transcription section).	SARS COV2 Target probe FAM labeled. Internal Estraction Control VIC/HEX labeled. None. Primerdesign Ltd Unknown (CE-VTO certified product); not supplied by manufacturer. One-step as described in reverse transcription section. See CE-VTO certified product manual with further details below. N/A as one-step (see reverse transcription section).	See Table S2. See Ta		
Location and identity of any modifications Manufacturer of oligonucleotides Purification method (BPCR PROTOCO) Complete reaction conditions	D D	Yes Yes Yes Yes	None. Biosearch Technologies Primer AX HPLC, Probe Dual HPLC. One-step as described in reverse transcription section.	SARS COV2 Target probe FAM labeled. Internal Extraction Control VIC/HEX labeled. None. Primerdesign Ltd Unknown (CE-VD0 certified product); not supplied by manufacturer. One-step as described in reverse transcription section. See CE-VD0 certified product manual with further details below. N/A as one-step (see reverse transcription section). Unknown (CE-VD0 certified product); not supplied by manufacturer.	See Table 52. See Table 52. See Table 52. See Table 52. Each 28 Lit reverse transcription reaction was split between qPCR and dPCR - see Table 52 for details. For qPCR, 12 Lit was transferred to 0.1 mt tubes for the Rotor-Gene Q (and 12 Lit transfered to Q (Acutly 96-well 8.5k Nanoplate wells.)		
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Location and identity of any modifications. Manufacturer of oiligenucleotides Purification method (PRCR PROTOCO). Complete reaction conditions Reaction volume and amount of cDNA/DNA Primer, (probe), Mg+ and MVTP connectrations Polymerase identity and	D E E	Yes Yes Yes Yes Yes Yes	None. Biosearch Technologies Primer AX HPLC, Probe Dual HPLC. One-step as described in reverse transcription section. N/A as one-step (see reverse transcription section). RT-qPCR assay: 2019-nCoV CDC-qualified Probe and Primer Kits (NT-NCOV-PP1-1000) from Biosearch Technologies. The N2 assay was supplied in a single tube containing hypophilised oligonucleotides of 30 nmol for each primer and 2.5 nmol of the FAM-BHQ1 probe. The assays was recovered that the containing supplied in the containing that the containing hypophilised oligonucleotides of 30 nmol for each primer and 2.5 nmol of the FAM-BHQ1 probe. The assays was recovered that the containing supplied to the containing hypophilised oligonucleotides of 30 nmol for each primer and 2.5 nmol of the FAM-BHQ1 probe. The assays was recovered that the containing supplied to the containing hypophilised oligonucleotides of 30 nmol for each primer and 2.5 nmol of the FAM-BHQ1 probe. The assays was recovered that the containing hypophilised oligonucleotides of 30 nmol for each primer and 2.5 nmol of the FAM-BHQ1 probe. The assays was recovered that the containing hypophilised oligonucleotides of 30 nmol for each primer and 2.5 nmol of the FAM-BHQ1 probe. The assays was recovered that the containing hypophilised oligonucleotides of 30 nmol for each primer and 2.5 nmol of the FAM-BHQ1 probe. The assays was recovered to the containing hypophilised oligonucleotides of 30 nmol for each primer and 2.5 nmol of the FAM-BHQ1 probe.	SARS COV2 Target probe FAM labeled. Internal Extraction Control VIC/HEX labeled. None. Primerdesign Ltd Unknown (CE-ND certified product); not supplied by manufacturer. One-step as described in reverse transcription section. See CE-ND certified product manual with further details below. N/A as one-step (see reverse transcription section). Unknown (CE-ND certified product); not supplied by manufacturer.	See Table S2. See Table S2. See Table S2. Each 28 ut. reverse transcription reaction was split between qPCR and dPCR - see Table S2 for details. For qPCR, 12 ut. was transferred to 0.1 m/L tubes for the Rotor-Gene Q (and 12 ut. transferred to Qxturily 96-well 8.5% Nanophite wells. N/A as one-step (see reverse transcription section). See Table S2.		
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Location and identity of any modifications. Manufacturer of oligonucleotides Purification method (PRCR PROTOCO). Complete reaction conditions. Reaction volume and amount of cDNA/DNA Primer, (probe), Mg+ amount of cDNA/DNA Primer, (prob), Mg+ amount of constitution of the buffer obstitution obstitution of the buffer obstitution obst	D D E E D D E E	Yes	None. Biosearch Technologies Primer AX HPLC, Probe Dual HPLC. One-step as described in reverse transcription section. N/A as one-step (see reverse transcription section). RT-qPCR assays; 2015-nCoV CDC-qualified Probe and Primer Kits (RT-NCOV-PP1-1000) from Biosearch Technologies. The NZ assay was supplied in a seight bub containing lyophilised oligonucleotides of 10 nmol for each primer and 2.5 nmol of the FAM-BHQ1 probe. The assays was record with nuclease-free water to give a working cotic. of 15 u.M. One-step as described in reverse transcription section. One-step as described in reverse transcription section. Not provided by manufacturer. None. MicroAMP Optical 96-well Reaction plate, Applied Biosystems, NB010560, MicroAmP Optical 96-well Reaction plate, Applied Biosystems, NB010560, MicroAmP Optical 96-well Reaction plate plate Biosystems, NB010560, MicroAmP Optical 96-well Reaction plate, Applied Biosystems, NB010560, MicroAmP Optical 96-well Reactions; Initial denaburation 95 °C for 2 min 45 cycles of denaburation 49 °C for 15 sec with annealing/elongation at 60 °C for 60 sec. Manual AB17500 Real Time PCR system (Applied Biosystems)	SARS COV2 Target probe FAM labeled. Internal Extraction Control VIC/HEX labeled. None. Primerdesign Ltd Unknown (CE-VD certified product), not supplied by manufacturer. One-step as described in reverse transcription section. See CE-VD certified product manual with further details below. N/A as one-step (see reverse transcription section). Unknown (CE-VD certified product); not supplied by manufacturer. Unknown (CE-VD certified product); not supplied by manufacturer. Unknown (CE-VD certified product); not supplied by manufacturer. Coronavirus COVID-19 genesig* Real-Time PCR assay. Primerdesign Ltd (Novacyt Group). Unknown, CE-VD certified product, information not supplied by the manufacturer, but delivered hyphilised and must be reconstituted in resuspension buffer for 10x final Unknown (CE-VD certified product, information not supplied by the manufacturer, but delivered hyphilised and must be reconstituted in resuspension buffer for 10x final Unknown (CE-VD certified product, inot supplied by manufacturer. MicroAMP Optical 8-wall Reaction pale, applied Biosystems, 80010550, MicroAmp Optical 8-wall Reaction pale, applied Biosystems. Reverse Transcription (55°C for 2 min see above) PCR cycling conditions (all reactions): Initial denaturation 95°C for 2 min 45 cycles of denaturation 95°C for 3 min 45 cycles of denaturation 95°C for 10 sec and annealing/elongation at 60°C for 60 sec.	See Table 52. See Table 52. See Table 52. Each 28 full reverse transcription reaction was split between qPCR and dPCR - see Table 52 for details. For qPCR, 12 vi. was transferred to 0.1 mil. tubes for the Rotor-Gene Q (and 12 vi. transferred to Qlxcuty 96-well 6.5k Nanopitte wells. N/A as one-step (see reverse transcription section). See Table 52. See Table 52. See Table 52. Not provided by manufacturer. None. 0.1 mil. tubes for Rotor-Gene Q. Reverse transcription (as described in Table 51). PCR cycling: initial denaturation of 95 °C for 2 min 40 cycles of 95 °C for 5 see and 60 °C for 30 sec. Manual Rotor-Gene Q.		

Table S1. MIQE checklist											
Standard curves with slope and y-intercept	E		Not performed as only two concentr	ations of reference material spike used.			Direct IVT spike: y = -3.55x + 32.51 No carrier: y = -3.647x + 32.5 PolyA: y = -3.694x + 32.94 PolyC: y = -3.613x + 32.53 ssgDNA: y = -3.409x + 33.01	No carrier: y = -3.641 + 46.16 PolyC: y = -3.38x + 44.73	See Fig 6A.	CDC N1: y = -3.39x + 33.29 CDC N2: y = -3.23x + 32.10	
PCR efficiency calculated from slope	E		≥ 95%				Direct IVT spike: 91% No carrier: 88% PolyA: 97% PolyC: 89% ssgDNA: 96%	No carrier: 88% PolyC: 98%	See Fig GA.	CDC N1: 97% CDC N2: 104%	
Confidence interval for PCR efficiency or standard error	D		Not performed as only two concentr	ations of reference material spike used.			Direct IVT spike: 88-95% No carrier: 82-95% PolyA: 84-90% PolyC: 85-94% ssgDNA: 95-98%	No carrier: 86-91% PolyC: 95-100%	See Fig 6A.	CDC N1: 91-103% CDC N2: 98-109%	
r2 of standard curve	E		Not performed as only two concentr	ations of reference material spike used.			Direct IVT spike: 0.9979 No carrier: 0.9915 PolyA: 0.9980 PolyC: 0.9963 ssgDNA: 0.9998	No carrier: 0.9907 PolyC: 0.9928	See Fig 6A.	CDC N1: 0.9259 CDC N2: 0.9509	
Linear dynamic range	E		Not performed as only two concentr	ations of reference material spike used.			Below 50 copies per extraction (equivalent to 5 copies/RT-qPCR)	No carrier: down to 500 copies/mL PolyC: down to 50 copies/mL	See Fig GA.	Down to 10 copies/uL in RT-qPCR is equivalent of 120 copies/reaction as the lowest concentration tested.	
Cq variation at lower limit	E	Yes	SD of Cq values: <0.73	SD of Cq values: <0.88	SD of Cq values: <0.96	SD of Cq values: <0.34	Current Z-Path-Covid-CE-IFU Issue 5.00 : p21f	No carrier: 1.05% PolyC: 1.01%	See Fig 6A.	CDC N1: 1.79% CDC N2: 2.57%	
Confidence intervals	D						Current Z-Path-Covid-CE-IFU Issue 5.00 : p24f	Current Z-Path-Covid-CE-IFU Issue 5.00 :	See Fig 6A.	Not performed.	
throughout range Evidence for limit of detection	E			The Accupies reference material was spiked into the pre-extracted samples at 750 copies/mL. Assuming 100% extraction efficiency, this would result in 3-4 copies/RT-qPCR reaction For 219 out of 20 spiked extractions (either from multiple donors for same spiked amount SARS CoV2 copies or for single donor) with 750 copies/mL, the N2 assay was detectable with a Cqc 41.				o24f Limit of detection defined as the concentration at which all three replicates of the RT-qPCR are detected: No carrier: down to 500 copies/mL	See Fig 6A.	Not performed, but 120 copies/reaction was measured 100% of the time.	
If multiplex, efficiency and	E		No multiplex experiments performed	L.			Unknown (CE-IVD certified product); not	PolyC: down to 50 copies/mL upplied by manufacturer.	See Fig 6A.	Details above.	
LOD of each assay. DATA ANALYSIS									qPCR data collected with Rotor-Gene Q2.3.1 (Build 49) software. Data analysis performed with Prism v9.0.0 (GraphPad) as descr		
qPCR analysis program (source, version)	E	Yes	qPCR data collected with 7500 Softw Data analysis performed with Prism ROX reference dve .	are v2.0.6 (Applied Biosystems) 9.0.0 (GraphPad)			qPCR data collected with 7500 Software v.2.0.6 (Applied Biosystems) QPCR data collected with Rotor-Gene Q2.3.1 (Build 49) software. Data analysis performed with Prism v9.0.0 (GraphPa Data analysis performed with Prism v9.0.0 (GraphPa No passive reference, normalized buseline. Cq threshold set manually on the normalized fluorescence axis (N1: 0.018 and N2: 0.044).			ibed in Table SZ.	
determination Outlier identification and	E	Yes	N/A as evaluation of different protor	ol variations so all data was included in the analy	ses.		No outliers identified.		N/A as evaluation of different protocol variations so all data was included in the analyses.		
disposition Results of NTCs	E		All amplification curves (MM1) - no	mplification in NTCs	All amplification curves (saliva and bucci	al) - no amplification in NTCs	No amplification.		CDC N1: 0.2 ug/ut bile salts (1000, 100, CDC N1: 0.8 ug/ut bile salts (1000, 100, CDC N1 Donor C 40% example 10 & 0 copies/ut in rxn) 10 & 0 copies/ut in rxn)	CDC N1 (NO INHIBITORS), 1000, 100, 10 and 0 copies/uL in reaction	
			0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	u s s u u s s u u u u u cycle	1 0.02756	and the second s			CDC N2: 0.2 ug/ut bile salts (1000, 100, 100 to N2: 0.8 ug/ut bile salts (1000, 100, 100 to COC N2 Donor C 40% example 10 & 0 copies/ut in non) CDC N2: 0.8 ug/ut bile salts (1000, 100, 100 to N2 Donor C 40% example 10 & 0 copies/ut in non)	CDC N2 (NO INHIBITORS), 1000, 100, 10 and 0 caples/out in reaction	
Justification of number and choice of reference genes	E	Yes	No reference gene used.				No reference gene, but performance asse extraction control.	ssed based on amplification of the internal	No reference gene as comparison with control group.		
Description of normalisation method	E	Yes	Normalised to baseline, normalised				Normalised to baseline.		Normalised to baseline.		
Number and concordance of biological replicates	D	Yes	Ten extractions per donor sample fo experimental and control groups (80 extractions in total).		20 extraction replicates per donor sample for experiment and control groups (80 extractions in total).	3 extraction replicates per donor sample for experiment and control groups (48 extractions in total).	Three extraction replicates for each dilution point and carrier condition. Each of the triplicate extractions was prepared from a single dilution series.	One extraction replicate per panel sample due to limited volume (200 uL provided).	See Table S2.		
Number and stage (RT or qPCR) of technical replicates	E	Yes	Triplicate RT-qPCR per extraction wil each mastermix (six RT-qPCR per extraction in total).	h Triplicate RT-qPCR per extraction replicate.	Single RT-qPCR per extraction replicate.	Triplicate RT-qPCR for each extraction. The Accupiex reference material was spiked into the pre-extracted samples at 750 copies/mL or 3000 copies/mL. Assuming 100% extraction efficiency, this would result in 34 copies/RT-qPCR or 15 copies/RT-qPCR, respectively.	Single RT-qPCR per extraction replicate.	Triplicate RT-qPCR per extraction replicate.	See Table 52.		
Repeatability (intra-assay variation)	E	Yes	At 750 copies/mL spike into matrix: At 3000 copies/mL spike into matrix				IEC target: 4.3% with no carrier <1.8% with carrier 5ARS-CoV-2 target: <1% with no carrier <1 with carrier	IEC target: No carrier: <1.8% PolyC: <1.6% SARS-COV-2 target: No carrier: <2.5% PolyC: <1.2%	See Table S2.		
Reproducibility (inter-assay variation, %CV)	D		Not assessed as performed with one	assay for each target and in one laboratory.			Not assessed as performed with one assa	for each target and in one laboratory.	Not assessed as performed with one assay for each target and in one laboratory.		
			Not performed.				Not performed.		Not performed.		

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Tubic 32. Miliqu circulat									
Statistical methods for	E	Yes	Correlation between Cq values obtained	Two-way ANOVA using the main effects	Two-way ANOVA using the main effects	Two-way ANOVA using the main effects	Two-way ANOVA using the main effects	Two-way ANOVA using the main effects	See Table S2.
result significance			by mastermix 1 (x-axis) and mastermix 2	model with donor and matrix as the	model with protocol and matrix as the	model with donor and carrier as the	model with IVT concentration and	model with Qnostics sample and carrier	
			(y-axis).	variables. Significance called when p <	variables. Significance called when p <	variables. One-way ANOVA using spike	carrier as the variables and multiple	as the variables and multiple	
				0.05. P values are given in the figure	0.05. P values are given in the figure	concentration as the variable.	comparison option selected. Linear	comparison option selected. Linear	
			Two-way ANOVA using the main effects	legend.	legend.	Significance called when p < 0.05. P	regression of Cq of IVT dilution series to	regression of Cq of Qnostics panel to	
			model with mastermix and matrix as the			values are given in the figure legend.	obtain reaction efficiency statistics.	obtain reaction efficiency statistics.	
			variables. Significance called when p <				Significance called when p < 0.05. P	Significance called when p < 0.05. P	
			0.05. P values are given in the figure				values are given in the figure legend.	values are given in the figure legend.	
			legend.						
Software (source, version)	E	Yes	7500 Software v2.0.6 (Applied Biosystems	7500 Software v2.0.6 (Applied Biosystems)					Rotor-Gene Q2.3.1 (Build 49).
Cq or raw data submission	D	Yes	Avaliable on request.				Avaliable on request.		Avaliable on request.
using PDMI									

a All essential information (E) must be submitted with the manuscript. Desirable information (D) should be submitted if available. If primers are from RTPrimerDB, information on qPCR target, oligonucleotides, protocols, and validation is available from that source. b FFRF, formalin-fixed, parafilm-embedded; RIN, RNA integrity number, PGL, RNA quality indicator, CSP, gene-specific priming; eNTP, decount-decided triphosphate.

A session the absence of DNA with a non-evener transcription cassary is essential when first extraction RNA Once the sension has been validated as DNA free. inclusion of a no-everse transcription control is desirable but no longer essential.

d Disclosure of the probe sequence is highly desirable and strongly encouraged; however, because not all vendors of commercial predesigned assays provide this information, it cannot be an essential requirement. Use of such assays is discouraged.