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

ITEM TO CHECK	IMPORTANCE	CHECKLIST	COMMENTS/WHERE?										
			Fig 1	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
EXPERIMENTAL DESIGN													
Definition of experimental and control groups	E	Yes	All information is provided in open access supplementary information Bustin et al., 2021	Experimental group: Accuplex reference material spiked into two saliva 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: Accuplex reference material spiked into matched samples from three donors for saliva and buccal swabs (Donor M, F and E with a second sample from donor M (M#2)). Control group: Matched saliva and buccal swab donors with no reference material spike. Control group: Extraction blanks with water in place of saliva/buccal swab and reference material spike.	Experimental group: Accuplex control material spiked into matched samples from one donor for saliva and buccal swabs (Donor F). Control group: Matched saliva and buccal swab donors with no control material spike.	Experimental group: Accuplex control material spiked into matched samples from two donors for saliva and buccal swabs (Donor E and M) with 1 ug PolyA added before extraction. Two conentrations of spike were assessed: 750 copies/mL and 3000 copies/mL. Control group: Matched saliva and buccal swab donors with two concentrations of the control material spike but no addition of PolyA carrier.	Experimental group: Extraction of a serial dilution of an IVT 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 containing the IEC but with water in place of IVT RNA molecules with and without carrier molecules.	Experimental group: RNA extraction of the nine samples in the Qnostics panel (see below) with PolyC carrier molecues. Internal extraction control (IEC) spiked in at a constant concentration. Control group: Extraction of same Qnostics panel without PolyC carrier molecules. Control group: Extraction blanks with water in place of Qnostics samples with and without PolyC carrier molecules.	Experimental group: Control RNA molecule spiked into RT-qPCR reaction in the presence of bile salts at different concentrations. Control group: Control RNA molecule spiked into the RT-qPCR reaction without added bile salts.	Experimental group: Control RNA molecule 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-qPCR 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 spiked into RT-aPCR 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 saliva with spike - 20 extractions from saliva with no spike - 20 extractions from buccal swab with spike - 20 extractions from buccal swab with no spike **= 80 extractions** Each extraction was amplified in triplicate with two different PCR mastermixes along with triplicate reactions with direct addition of the Accuplex reference material spiked 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 saliva with spike - 40 extractions from saliva with no spike - 40 extractions from buccal swab with spike - 40 extractions from buccal swab with no spike - 1 extraction blank **= 161 extractions** Each extraction was amplified in single RT-qPCR reactions **= 161 RT-qPCR + NTCs**	Twenty extractions per donor sample for experimental and control groups: - 20 extractions from saliva with spike - 20 extractions from saliva with no spike - 20 extractsions from buccal swab with spike - 20 extractions from buccal swab with no spike **= 80 extractions** Each extraction was amplified in single RT-qPCR reactions **= 80 RT-qPCR + NTCs**	Three extractions for each sample in the experimental and control groups: - 12 extractions from saliva with spike and PolyA - 12 extractions from saliva with spike but no PolyA - 12 extractions from buccal swab with spike and PolyA - 12 extractions from buccal swab with spike but not PolyA **= 48 extractions** Each extraction was amplified in single RT-qPCR reactions. **= 48 RT-qPCR + NTCs**	Three extractions for each sample in the experiment and control groups: - 60 extractsions from a five-point serial dilution of the IVT RNA molecule: 50, 100, 1 000, 10 000 & 100 000 RNA molecules per extraction with four carrier conditions: PolyA, PolyC, salmon sperm gDNA and no carrier. - 12 Extraction blanks containing the IEC with and without carrier **= 72 extractions** Each extraction was amplified in single RT-qPCR reactions. **= 72 RT-qPCR + NTCs**	Single extractsion for each sample in the experiment and control groups: - 18 extractions from the nine samples in the Qnostics panel, each extracted with PolyC and without carrier - 6 blank extractions containing the IEC but with water instead of Qnostics 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 (JRC) in the presence of bile salts added directly to the RT-dPCR reaction to give a final concentration of 0, 0.1, 0.2, 0.4, 0.6 and 0.8 ug/uL. - Each bile salt concentration evaluated with three different EURM-019 concentrations in triplicate reactions **= 54 RT-qPCR + NTCs** - Control reactions contained no EURM-019 with the six bile salt concentrations (included none; standard NTC) in triplicate reactions **= 18 RT-qPCR + NTCs**	No biological specimen. Quantification of EURM-019 (JRC) in the presence of bile salts added directly to the dPCR reaction (after RT was complete) to give a final concentration of 0, 0.1, 0.2, 0.4, 0.6 and 0.8 ug/uL. - Each bile salt concentration evaluated with three different EURM-019 concentrations in triplicate reactions **= 54 RT-qPCR + NTCs** - Control reactions contained no EURM-019 with the six bile salt concentrations (included none; standard NTC) in triplicate reactions **= 18 RT-qPCR + NTCs**	Three urine samples from healthy human donors. Quantification of EURM-019 (JRC) in the presence of different v/v concentrations of the urine at 0%, 10%, 20% and 40% of the final reaction volume. - Each donor urine % was evaluated with the three EURM-019 concentrations in triplicate reactions **= 36 RT-qPCR + NTCs** - Control reactions containing no EURM-019 were assessed in parallel **= 3 RT-qPCR + NTCs**	No biological specimen. Quantification of EURM-019 (JRC) that is a single stabilised in vitro transcripted (IVT) synthetic single stranded RNA (ssRNA) of 880 nt in buffered solution. The IVT 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 urine) used in Fig 4 and Fig 5: - Three difference concentrations assessed in triplicate reactions on three separate days: 1000, 100 and 10 copies/uL in the reaction **= 36 RT=qPCR + NTCs** - Control reactions containing no EURM-019 were assessed in parallel **= 9 RT-dPCR + NTCs**
Assay carried out by core lab or investigator's lab?	D	Yes		Investigator`s lab: EKvH, MK, AB, HH				Investigator`s lab: EKvH, MK, AB, HH		Investigator's lab: SB, OK, AM			
Acknowledgement of authors' contributions	D	Yes		Yes				Yes		Yes			
SAMPLE													
Description	E	Yes		<u>Saliva samples:</u> Saliva was collected in a 50 ml reaction tube for each donor just prior to use in the experiment. Where a donor has contributed a sample to more than one experiment, a fresh sample was obtained each time. <u>Buccal swabs:</u> The swab (Isohelix Swab Pack (SK-2S-x100) was stroked over the inner cheek at least ten times on each side for each donor just prior to use in the experiment. Where a donor has contributed a sample to more than one experiment, a fresh sample was obtained each time. <u>Control material spike:</u> Seracare Accuplex Reference material (0505-0126) is designed as whole-process control (extraction and qRT-PCR). It contains fragments of SARS-CoV2 Virus packaged in protein coat and covers the whole viral genome. Manufacturers copy number concentration is 5000 copies/mL.				The lyophilised IVT RNA template contains 6 x 10^7 copies of a SARS-CoV-2 RNA sequence (Novocyte). The sequence GenBank number or genomic coordinates are not supplied but the assay targets a region in the Orf1ab region of the hCoV-19/Wuhan/WIV04/2019 genome (see below).	SARS-CoV-2 Q Controls (Qnostics SCV2AQP01); nine samples containing a dilution series of whole pathogen sample in transport medium.	EURM-019 control material (JRC); spiked in at 1000 copies/uL in the RT-qPCR reaction.			EURM-019 control material (JRC); spiked in at 10, 100 and 1000 copies/uL in the RT-qPCR reaction.
Volume/mass of sample processed	D	Yes		<u>Saliva samples:</u> Samples were diluted 1:1 (v/v) with 1X PBS (Roth Roti® 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 uL of the spiked diluted saliva. <u>Buccal swabs:</u> 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 uL of the spiked UTM.			Saliva and buccal swabs were diluted as described for Fig 2A-C. The reference material was spiked into the samples at one of two concentrations to give final concentration of either 750 copies/mL or 3000 copies/mL. Each extraction was performed on 100 uL of the four different spiked samples (saliva & 750 c/mL, saliva & 3000 c/mL, buccal swab & 750 c/mL and buccal swab & 3000 c/mL) containing either 1 ug PolyA carrier molecules or no carrier molecules.	The lyophislised IVT RNA template was resuspended in 200 uL of Template preparation buffer to give a stock concentration of 1 x 10E5 copies/µL. A fresh serial dilution series was prepared in nuclease-free water with and without carrier as 100 000 (stock), 10 000, 1 000, 100 and 50 copies/uL.	200 µl aliquot was supplied by Primerdesign/Novacyt. The panel of samples went through one freeze-thaw cycle from aliquoting by Primerdesign/Novacyt for shipping to the investigator lab. 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 donors into a 50 mL falcon tube on the day of the experiment.	N/A
Microdissection or macrodissection	E	Yes		N/A				N/A		N/A			
Processing procedure	E	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													

Procedure and/or instrumentation	E	Yes		Extraction was carried out on KingFisher Flex platform (ThermoFisher) with sbeadex viral RNA purification kit (LGC Biosearch Technologies) as follows: For each extraction, 100 µl sample was added to 100 µl Lysis Buffer SB. Lysis was performed by addition of 20 µl Proteinase K Solution (20mg/ml) to each sample and incubation at 60°C for 11 min (with 1 min slow mixing and 10 min fast mixing). After lysis, 180 µl of binding mix (containing 160 µl Binding buffer SB and 20 µl sbeadex particle suspension EDTA) was added to each reaction and incubated for 4 min with fast mixing. Three washing steps were carried out: a) 400 µl Wash buffer BN1, b) 400 µl Wash buffer TN1 and c) 400 µl Wash buffer TN2 each for 5 min with fast mixing. RNA was recovered eith a 10 min heated elution at 60°C in 100 µl Elution buffer AMP under fast mixing. Eluate was stored on ice until added to the RT-qPCR.				Extraction was carried out on KingFisher Flex platform (ThermoFisher) with sbeadex viral RNA purification kit (LGC Biosearch Technologies). 1 µL of IVT RNA was added to 100 µL of water with or without 1 µg of respective carrier. 280 µl of a premix containing 100 µl lysis buffer SB, 160 uL binding buffer SB and 20 uL sbeadex particle solution + EDTA per reaction was added, followed by washing procedure according to manufacturer`s protocol as described for Fig2A-D. No heated lysis step on KingFisher platform was performed. The RNA was eluted in 100 µL Elution buffer AMP.	Extraction was carried out on KingFisher Flex platform (ThermoFisher) with sbeadex viral RNA purification kit (LGC Biosearch Technologies). 90 µL of sample was added to the 100 uL lysis buffer SB. All other buffer volumes and the automated protocol was performed according to the manufacturer's recommendations as described in Fig 3A with the exception of the lysis step that was performed for 3 min heated. The RNA was eluted in 100 µL elution buffer AMP.	N/A			
Name of kit and details of any modifications	E	Yes		As described above.	As described above.	Original protocol: as described above with 11 min lysis, 4 min washing and 5 min elution. 39 min protocol: as described above 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.	As described above with addition of 1 ug PolyA carrier (Sigma Aldrich P/N 10108626001) per extraction if required.	As described above.	As described above.	N/A			
Source of additional reagents used	D	Yes		As described above in the relevant sections.				The volume given is the volume added to lysis buffer prior to each extraction; number in brakets is the total amount added to each extraction: - genesig Easy RNA Internal extraction control (IEC)(Primerdesign): 20 µL (stock resuspended in 1000 µL; unknown final concentration) - 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 remnants were discarded (not stored).	The volume given is the volume added to lysis buffer prior to each extraction; number in brakets is the total amount added to each extraction: - Internal extraction Control (Primerdesign): as before. - PolyC (Sigma Aldrich P/N4903): as before.	Bile salts (SIGMA) added at the final concentration in the RT-qPCR as indicated.	Bile salts (SIGMA) added at the final concentration in the RT-qPCR as indicated.	Donor urine, see details in sample section above.	N/A
Details of DNase or RNAse treatment	E	Yes		Not performed.				Not performed.		Not performed.			
Contamination assessment (DNA or RNA)	E	Yes		Not performed.				Not performed.		Not performed.			
Nucleic acid quantification	E	Yes		Not performed; 5 uL of elutant added to 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	D			N/A				N/A		N/A			
RNA integrity	E	Yes		Not performed.				Not performed.		Not performed.			
RIN/RQI or Cq of 3' and 5' transcripts	E			N/A				N/A		N/A			
Electrophoresis traces	D			N/A				N/A		N/A			
Inhibition testing (Cq dilutions, spike or other)	E	Yes		Not performed.				The IEC acts as a performance indicator; inhibition testing is performed by comparison of the extracted IEC Cq with the Cq obtained from a matched concentration direct spike into the RT-qPCR.		Aim of the expeirment - experimental group.			N/A
REVERSE TRANSCRIPTION													
Complete reaction conditions	E	Yes		One-Step RT-PCR was performed for all experiments. Mastermix 1: GoTaq® Probe 1-Step RT-qPCR (Promega) Mastermix 2: Lucigen RapiDxFire Mastermix supplemented with Episcript Reverse Transcriptase. RT-qPCR assay: 2019-nCoV CDC-qualified Probe and Primer Kits (KIT-NCOV-PP1-1000) containing FAM-labeled probes for targets N1, N2 and human RNase P was used. N2 was the only target used for these experiments. Assay reconstituted as a 15 uM stock containing 10 nmol of each primer and 2.5 nmol of probe.	As described for Fig 3A with Mastermix 1 only.			One-step RT-qPCR was performed using the Coronavirus COVID-19 genesig® Real-Time PCR COVID-19 (CE IVD) (Primerdesign PN Z-Path-COVID-19-CE, manual: Z-Path-COVID-19-CE-IFU Issue 3.0). This assay contains the primer pair and FAM probe for the SARS-CoV-2 target and a primer pair and VIC/HEX probe for the IEC target to be used in a duplex reaction.		See Table S2. Briefly, 28 uL reactions prepared for the RT with 12 uL analysed by qPCR and 12 uL analysed by dPCR.			
Amount of RNA and reaction volume	E	Yes		For each reaction, 5 uL of eluant from a single extraction 15 uL of mastermix containing 1.5 uL 15 uM primers/probe stock for the N2 assay. NTC reactions contained 5 µl nuclease-free water in place of the extraction eluant. PCR positive controls and additional spike-in controls, 5 µl of Seracare AccuPlex SARS-CoV2 Reference Material (0505-0126) was added directly to reaction without undergoing extraction procedure to give a final concentration of 3.75 copies/RT-qPCR and/or 15 copies/RT-qPCR (matched with the carry through of the reference material spike into the 100 uL spiked sample).				For each reaction: 8 µL eluant from the extraction was added to 20 µL reaction. Positive controls were performed with 8 uL of a 1:100 dilution of each five-point serial dilution added directly to the RT-qPCR (match the copy numbers per RT-qPCR to compare with the extractions if 100% recovery is obtained). NTC reactions contained 8 µl nuclease-free water in place of eluant.	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.	See Table S2.			
Priming oligonucleotide (if using GSP) and concentration	E	Yes		One-Step RT-PCR so priming performed with reverse primer.				Unknown (CE-IVD certified product) but likely to be primed with the reverse primer.		See Table S2 (reverse primer).			

Reverse transcriptase and concentration	**E**	**Yes**		Mastermix 1: GoScript™ RT Mix for 1-Step RT-qPCR 50x stock (0.4 µl per reaction). Mastermix 2: EpiScript™ RNase H-Reverse Transcriptase, 200 U/µl (0.5 µl per reaction).	As described for Fig 3A with Mastermix 1 only.	Oasig ™ OneStep 2x RT-qPCR Master Mix lyophilised, resuspended in 525µl Oasig™ 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 °C for 10 min	See Table S2.
Manufacturer of reagents and catalogue numbers	D	**Yes**		Mastermix 1: Promega : GoTaq® Probe 1-Step RT-qPCR System, A6121. Mastermix 2: Lucigen : RapiDxFire qPCR 5X Master Mix GF, 1mL, 30050-1, with EpiScript RNase H- Reverse Transcriptase, ERT12925K.	As described for Fig 3A with Mastermix 1 only.	genesig® 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	D[c]	**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.	**E**	**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 the experiments.		Multiplex reactions performed with the IEC assay and SARS-CoV-2 assay. Details in CE-IVD certified product manual (current Z-Path-COVID-19-CE-IFU Issue 5.00: p.20 ff).	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 accession: MN908947.2) was used for validation of LOD.		SARS-CoV-2 target assay: manufacturer refers to GISAID EpiCoV database, GISAID EpiCoV database itself refers to hCoV-19/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 IEC target: unknown	See Table S2.
Amplicon length	**E**	**Yes**		According to Accession Number January 2020/NC_0.445512_2: 67bp (UCSC Browser, In-silico PCR tool)		Unknown (CE-IVD certified product); not supplied by manufacturer.	See Table S2.
In silico specificity screen (BLAST, etc)	**E**	**Yes**		According to FDA, CDC-006-00019, Revision: 06, EUA instruction for use: CDC 2019 nCoV Real-Time RT-PCR Diagnostic Panel were evaluated against 31,623 sequences available in the Global Initiative on Sharing All Influenza Data (GISAID, https://www.gisaid.org) database as of June 20, 2020, to demonstrate the predicted inclusivity of the 2019-nCoV Real-Time RT-PCR Diagnostic Panel. Moreover, in silico analysis were performed using BLASTn (ncbi, for details please refer to CDC-006-00019 EUA instruction for use).		Unknown (CE-IVD certified product); not supplied, but manufacturer state that 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			N/A		N/A	N/A as viral target.
Sequence alignment	D			N/A		Current Z-Path-Covid-CE-IFU Issue 5.00 : p27 f	See Table S2.
Secondary structure analysis of amplicon	D			N/A		Unknown (CE-IVD certified product); not supplied by manufacturer.	See Table S2.
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 targeted?	**E**			N/A as viral target.		N/A as viral target.	N/A as viral target.
qPCR OLIGONUCLEOTIDES							
Primer sequences	**E**	**Yes**		N2 Fw 5’ TTACAAACATTGGCCGCAAA 3’ N2 Rv 5’ GCGCGACATTCCGAAGAA 3’		Unknown (CE-IVD certified product); not supplied by manufacturer.	See Table S2 (CDC N1 and N2 targets).
RTPrimerDB Identification Number	D			N/A		N/A	See Table S2.
Probe sequences	D[d]	**Yes**		N2 Probe 5’ FAM-ACAATTTGCCCCCAGCGCTTCAG-BHQ-1 3’		Unknown (CE-IVD certified product); not supplied by manufacturer. SARS CoV2 Target Probe FAM labeled. Internal Extraction Control VIC/HEX labeled.	See Table S2.
Location and identity of any modifications	**E**	**Yes**		None.		None.	See Table S2.
Manufacturer of oligonucleotides	D	**Yes**		Biosearch Technologies		Primerdesign Ltd	See Table S2.
Purification method	D	**Yes**		Primer AX HPLC, Probe Dual HPLC.		Unknown (CE-IVD certified product); not supplied by manufacturer.	See Table S2.
qPCR PROTOCOL							
Complete reaction conditions	**E**	**Yes**		One-step as described in reverse transcription section.		One-step as described in reverse transcription section. See CE-IVD certified product manual with further details below.	Each 28 uL reverse transcription reaction was split between qPCR and dPCR - see Table S2 for details. For qPCR, 12 uL was transferred to 0.1 mL tubes for the Rotor-Gene Q (and 12 uL transfered to QIAcuity 96-well 8.5k Nanoplate wells.
Reaction volume and amount of cDNA/DNA	**E**	**Yes**		N/A as one-step (see reverse transcription section).		N/A as one-step (see reverse transcription section).	N/A as one-step (see reverse transcription section).
Primer, (probe), Mg++ and dNTP concentrations	**E**	**Yes**		RT-qPCR assay: 2019-nCoV CDC-qualified Probe and Primer Kits (KIT-NCOV-PP1-1000) from Biosearch Technologies. The N2 assay was supplied in a single tube containing lyophilised oligonucleotides of 10 nmol for each primer and 2.5 nmol of the FAM-BHQ1 probe. The assays was reconstituted with nuclease-free water to give a working sotkc of 15 uM.		Unknown (CE-IVD certified product); not supplied by manufacturer.	See Table S2.
Polymerase identity and concentration	**E**	**Yes**		One-step as described in reverse transcription section.		Unknown (CE-IVD certified product); not supplied by manufacturer.	See Table S2.
Buffer/kit identity and manufacturer	**E**	**Yes**		One-step as described in reverse transcription section.		Coronavirus COVID-19 genesig® Real-Time PCR assay. Primerdesign Ltd (Novacyt Group).	See Table S2.
Exact chemical constitution of the buffer	D			Not provided by manufacturer.		Unknown, CE-IVD certified product, information not supplied by the manufacturer, but delivered lyophilised and must be reconstituted in resuspension buffer for 10X final	Not provided by manufacturer.
Additives (SYBR Green I, DMSO, etc.)	**E**	**Yes**		None.		Unknown (CE-IVD certified product); not supplied by manufacturer.	None.
Manufacturer of plates/tubes and catalog number	D	**Yes**		MicroAMP Optical 96-well Reaction plate, Applied Biosystems, N8010560, MicroAmp Optical Adhesive Film, 4311971, Applied Biosystems.		MicroAMP Optical 96-well Reaction plate, Applied Biosystems, N8010560, MicroAmp Optical Adhesive Film, 4311971, Applied Biosystems.	0.1 mL tubes for Rotor- Gene Q.
Complete thermocycling parameters	**E**	**Yes**		Reverse Transcription conditions described in reverse transcription section above (45 °C (Promega) or 42 °C (Lucigen) for 15 min) PCR cycling conditions (all reactions): Initial denaturation: 95 °C for 2 min 45 cycles of denaturation at 95 °C for 15 sec with annealing/elongation at 60 °C for 60 sec.		Reverse Transcription (55 °C for 10 min; see above) PCR cycling conditions (all reactions): Initial denaturation 95 °C for 2 min 45 cycles of denaturation at 95 °C for 10 sec and annealing/elongation at 60 °C for 60 sec.	Reverse transcription (as described in Table S2). PCR cycling: Initial denaturation of 95 °C for 2 min 40 cycles of 95 °C for 5 sec and 60 °C for 30 sec.
Reaction setup (manual/robotic)	D	**Yes**		Manual		Manual	Manual
Manufacturer of qPCR instrument	**E**	**Yes**		ABI7500 Real Time PCR System (Applied Biosystems)		ABI7500 Real Time PCR System (Applied Biosystems)	Rotor-Gene Q
qPCR VALIDATION							
Evidence of optimisation (from gradients)	D			Not performed. Used standard cycling conditions based on original publication of the assay.		Not performed. Used standard cycling conditions based on current Z-Path-Covid-CE-IFU Issue 5.00 : p28	Not performed.
Specificity (gel, sequence, melt, or digest)	**E**			Specificity against other species described in original publication of the assay - specific for SARS-CoV-2.		Unknown (CE-IVD certified product); not supplied by manufacturer.	See Table S2.
For SYBR Green I, Cq of the NTC	**E**			N/A as probe based detection.		N/A as probe based detection.	N/A as probe based detection.

Standard curves with slope and y-intercept	E			Not performed as only two concentrations 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 6A.			CDC N1: 97% CDC N2: 104%
Confidence interval for PCR efficiency or standard error	D			Not performed as only two concentrations 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 concentrations 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 concentrations 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 6A.			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 throughout range	D							Current Z-Path-Covid-CE-IFU Issue 5.00 : p24f	Current Z-Path-Covid-CE-IFU Issue 5.00 : p24f	See Fig 6A.			Not performed.
Evidence for limit of detection	E			The Accuplex 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 ≥19 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 Cq< 41.				All concentrations of IVT, including 5 copies/reaction were detected in all extraction replicates. Limit of detection is defined as lower than this.	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 PolyC: down to 50 copies/mL	See Fig 6A.			Not performed, but 120 copies/reaction was measured 100% of the time.
If multiplex, efficiency and LOD of each assay.	E			No multiplex experiments performed.				Unknown (CE-IVD certified product); not supplied by manufacturer.		See Fig 6A.			Details above.
DATA ANALYSIS													
qPCR analysis program (source, version)	E	Yes		qPCR data collected with 7500 Software v2.0.6 (Applied Biosystems) Data analysis performed with Prism v9.0.0 (GraphPad)				qPCR data collected with 7500 Software v2.0.6 (Applied Biosystems) Data analysis performed with Prism v9.0.0 (GraphPad)		qPCR data collected with Rotor-Gene Q2.3.1 (Build 49) software. Data analysis performed with Prism v9.0.0 (GraphPad) as described in Table S2.			
Cq method determination	E			ROX reference dye .				No passive reference, normalized to baseline.		Cq threshold set manually on the normalized fluorescence axis (N1: 0.018 and N2: 0.044).			
Outlier identification and disposition	E	Yes		N/A as evaluation of different protocol variations so all data was included in the analyses.				No outliers identified.		N/A as evaluation of different protocol variations so all data was included in the analyses.			
Results of NTCs	E			All amplification curves (MM1) - no amplification in NTCs		All amplification curves (saliva and buccal) - no amplification in NTCs		No amplification.		CDC N1: 0.2 ug/uL bile salts (1000, 100, 10 & 0 copies/uL in rxn)	CDC N1: 0.8 ug/uL bile salts (1000, 100, 10 & 0 copies/uL in rxn)	CDC N1 Donor C 40% example	CDC N1 (NO INHIBITORS), 1000, 100, 10 and 0 copies/uL in reaction
										CDC N2: 0.2 ug/uL bile salts (1000, 100, 10 & 0 copies/uL in rxn)	CDC N2: 0.8 ug/uL bile salts (1000, 100, 10 & 0 copies/uL in rxn)	CDC N2 Donor C 40% example	CDC N2 (NO INHIBITORS), 1000, 100, 10 and 0 copies/uL in reaction
Justification of number and choice of reference genes	E	Yes		No reference gene used.				No reference gene, but performance assessed based on amplification of the internal extraction control.		No reference gene as comparison with control group.			
Description of normalisation method	E	Yes		Normalised to baseline, normalised to ROX levels.				Normalised to baseline.		Normalised to baseline.			
Number and concordance of biological replicates	D	Yes		Ten extractions per donor sample for experimental and control groups (80 extractions in total).	10 extraction replicates per donor sample for experiment and control groups (161 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 with each mastermix (six RT-qPCR per extraction in total).	Triplicate RT-qPCR per extraction replicate.	Single RT-qPCR per extraction replicate.	Triplicate RT-qPCR for each extraction. The Accuplex 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 3-4 copies/RT-qPCR or 15 copies/RT-qPCR, respectively.	Single RT-qPCR per extraction replicate.	Triplicate RT-qPCR per extraction replicate.	See Table S2.			
Repeatability (intra-assay variation)	E	Yes		At 750 copies/mL spike into matrix: 1.87% At 3000 copies/mL spike into matrix: 0.32%				IEC target: 4.3% with no carrier <1.8% with carrier SARS-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 assay for each target and in one laboratory.		Not assessed as performed with one assay for each target and in one laboratory.			
Power analysis	D			Not performed.				Not performed.		Not performed.			

Statistical methods for result significance	**E**	**Yes**		Correlation between Cq values obtained by mastermix 1 (x-axis) and mastermix 2 (y-axis). Two-way ANOVA using the main effects model with mastermix and matrix as the variables. Significance called when $p < 0.05$. P values are given in the figure legend.	Two-way ANOVA using the main effects model with donor and matrix as the variables. Significance called when $p < 0.05$. P values are given in the figure legend.	Two-way ANOVA using the main effects model with protocol and matrix as the variables. Significance called when $p < 0.05$. P values are given in the figure legend.	Two-way ANOVA using the main effects model with donor and carrier as the variables. One-way ANOVA using spike concentration as the variable. Significance called when $p < 0.05$. P values are given in the figure legend.	Two-way ANOVA using the main effects model with IVT concentration and carrier as the variables and multiple comparison option selected. Linear regression of Cq of IVT dilution series to obtain reaction efficiency statistics. Significance called when $p < 0.05$. P values are given in the figure legend.	Two-way ANOVA using the main effects model with Qnostics sample and carrier as the variables and multiple comparison option selected. Linear regression of Cq of Qnostics panel to obtain reaction efficiency statistics. Significance called when $p < 0.05$. P values are given in the figure legend.	See Table S2.
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 using RDML	D	**Yes**		Avaliable on request.				Avaliable on request.		Avaliable on request.

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 FFPE, formalin-fixed, paraffin-embedded; RIN, RNA integrity number; RQI, RNA quality indicator; GSP, gene-specific priming; dNTP, deoxynucleoside triphosphate.
c Assessing the absence of DNA with a no–reverse transcription assay is essential when first extracting RNA. Once the sample has been validated as DNA free, inclusion of a no–reverse 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.