Table S2 dMIQE table

ITEM TO CHECK	PROVIDED	Fig 4A-B	Fig 4C-D	Fig 5A-B	Fig 6A	Fig 6B
1. SPECIMEN						
Detailed description of specimen type and numbers	Y	No biological specimen. 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 reactions. - Control reactions contained no EURM-019 with the six bile salt concentrations (included none; standard NTC) in triplicate reactions.//18 reactions.	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 reactions. - Control reactions contained no EURM-019 with the six bile salt concentrations (included none; standard NTC) in triplicate reactions.//18 reactions.	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 reactions. - Control reactions containing no EURM-019 were assessed in parallel. //3 reactions.	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 6 and Fig 7: - Three difference concentrations assessed in triplicate reactions on three separate days: 1000, 100 and 10 copies/uL in the reaction. //36 reactions. - Control reactions containing no EURM-019 were assessed in parallel. //9 reactions.	No biological specimen. Control RNA material provided as multiple non-overlapping IVTs to cover the full (>99.9%) SARS-CoV-2 viral genome. Details of the exact control material and supplier can be obtained by contacting the corresponding author. This approach is taken by the authors as we do not believe that this issue is unique to this supplier.
Sampling procedure (including time to storage)	Y	N/A		Inhibitors (urine samples) were provided by the donors into a 50 mL falcon tube on the day of the experiment.	N/A	N/A
Sample aliquotation, storage conditions and duration	Y	N/A		Fresh urine samples were stored on cooling blocks and directly taken into reaction.	N/A	See next section.
2. NUCLEIC ACID EXTRACTION						
Description of extraction method including amount of sample processed	N	N/A		N/A	N/A	N/A
Volume of solvent used to elute/resuspend extract	N	N/A		N/A	N/A	N/A
Number of extraction replicates	N	N/A		N/A	N/A	N/A
Extraction blanks included?	N	N/A		N/A	N/A	N/A
3. NUCLEIC ACID ASSESSMENT AND STORAGE						
Method to evaluate quality of nucleic acids	N	Not provided by manufacturer.				UV spec details from product insert as ~17 pg per IVT.
Method to evaluate quantity of nucleic acids (including molecular weight and calculations	N	Single unit of EURM-019 provided by the manufacturer as 10^8 copies/uL as determined by UV spectrometry. No further quantification performed prior to RT-dPCR.				Details from product insert. Equal mass concentration of each IVT used.
Storage conditions: temperature, concentration, duration, buffer, aliquots	Y	Details of the stock aliquots here if performed. Stocks were stored in the dark at -20 °C ± 5 °C upon reception and prior to use. 10^5 dilutions were aliquoted and stored as stocks at -20C.				Original stocks were stored in 5-10 uL aliquots and stored at -80 °C.
Clear description of dilution steps used to prepare working DNA solution	Y	For every experiment new working dilutions were diluted from a 10^5copies/µl stock, diluted in NADB. Working concentrations were used for each experimental setup and working conc were directly trashed after the testing is done. The only storage condition was at -20C for the stock. Working concentrations were diluted freshly on the day of experiment and kept on an ice block.				Samples at 1E6 c/µL according to the manufacturer were volumetrically diluted in carrier (1 ng/µL Jurkat Total RNA. Thermo Fisher Scientific; AM7858) to a final concentration of 1E4 c/µL. 1 in 100 dilutions were made from original stock, aliquoted in 25 µL or 40 µL and stored at -80 °C until the analysis.
4. NUCLEIC ACID MODIFICATION						
Template modification (digestion, sonication, pre-amplification, bisulphite etc.)	Y	None				None

Details of repurification following modification if performed	Y	None				None
5. REVERSE TRANSCRIPTION						
cDNA priming method and concentration	Y	Reverse primer				Specific reverse primer (900 nM) in 1-Step RT-ddPCR Advanced Kit for Probes Bio-Rad (Cat#: 186-4021).
One or two step protocol (include reaction details for two step)	Y	One-step protocol using Eppendorf endpoint PCR cycler (C1000): 50 °C for 30 min (reverse transcription), 58 °C for 10 min (inactivation of reverse transcriptase and infinte hold at 12 °C.				One-step protocol (details below).
Amount of RNA added per reaction	Y	1000 copies/µl per reaction	1000 copies/µl per reaction	1000 copies/µl per reaction	1000 + 100 + 10 copies/µl per reaction	5E4 copies per reaction.
Detailed reaction components and conditions	Y	Per reaction duplicate (RT-dPCR and RT-qPCR), 28 µl of a 1x reaction was setup on ice and incubated in a 96 well plate. Reaction volume components: - QIAcuity one-step viral RT-dPCR mastermix (4X):7ul - N1 assay (20X):1.4ul (primer and probe) - N2 assay (20X):1.4ul (primer and probe) -bile: 2,24ul (0.8ug/ul), 1.68ul (0.6ug/ul), 1,12ul (0.4ug/ul), 0.56ul (0.2ug/ul), 0.28ul (0.1ug/ul) - Nuclease-free water: made up to 28 uL.	Per reaction duplicate (RT-dPCR and RT-qPCR), 28 µl of a 1x reaction was setup on ice and incubated in a 96 well plate. Reaction volume components: - QIAcuity one-step viral RT-dPCR mastermix (4X):7ul - N1 assay (20X):1.4ul (primer and probe) - N2 assay (20X):1.4ul (primer and probe) - Nuclease-free water: made up to 28 uL.	Per reaction duplicate (RT-dPCR and RT-qPCR), 28 µl of a 1x reaction was setup on ice and incubated in a 96 well plate. Reaction volume components: - QIAcuity one-step viral RT-dPCR mastermix (4X):7ul - N1 assay (20X):1.4ul (primer and probe) - N2 assay (20X):1.4ul (primer and probe) - Urine: 10% - 2.8 uL, 20: - 5.6 uL, 40% - 11.2 uL - Nuclease-free water: made up to 28 uL.	Per reaction duplicate (RT-dPCR and RT-qPCR), 28 µl of a 1x reaction was setup on ice and incubated in a 96 well plate. Reaction volume components: - QIAcuity one-step viral RT-dPCR mastermix (4X):7ul - N1 assay (20X):1.4ul (primer and probe) - N2 assay (20X):1.4ul (primer and probe) - Nuclease-free water: made up to 28 uL.	22 µL reactions (10% excess) were prepared containing 5.5 µL of One-Step RT-ddPCR Advanced Kit for Probes, 1.1 µL of DTT, 1.1 µL of 20X primer/probe mix, 2.2 µL of reverse transcriptase, 6.6 µL of nuclease-free water and 5.5 µL of RNA template. Control reactions were set up as no template controls (NTCs) containing water in place of DNA template and negative controls containing carrier molecules only or RNA storage solution (RSS). Cycling conditions: Reverse transcription at 47.5 °C for 60 min. Enzyme activation at 95 °C for 10 min, 40 cycles of 94 °C for 30 s, and 55 °C for CDC N2 and 60°C for Sarbecco E for 1 min, followed by enzyme inactivation at 98 °C for 10 min and a 4 °C hold for at least one hour. The ramp rate for each step was set to 2 °C/s except the final cool to 4 °C that was 1 °C/second.
Estimated copies measured with and without addition of RT*	N	Not performed as part of the manufacturer's QC.				Neither CDC N2 nor Sarbecco E copies were measured without the addition of reverse transcriptase.
Manufacturer of reagents used with catalogue and lot numbers	Y	Manufacturer QIAGEN HILDEN, Cat No. 1123145.				One-Step RT-ddPCR Supermix; Bio-Rad, Cat#: 186-4021; Lot #: 64338972. Reverse Transcriptase; Bio-Rad, Cat#: 186-4021; Lot #: 64346506. DTT; Bio-Rad, Cat#: 186-4021; Lot #: 64342343. RNase/DNase-free water; Ambion, Cat#: AM9939; Lot #: 109083.
Storage of cDNA: temperature, concentration, duration, buffer and aliquots	Y	Plates were stored on ice block before aliquoting into qPCR tubes or dPCR nanoplate.				N/A
6. dPCR OLIGONUCLEOTIDES DESIGN AND TARGET INFORMATION						
Sequence accession number or official gene symbol	Y	https://www.cdc.gov/coronavirus/2019-ncov/lab/rt-pcr-detection-instructions.html				CDC N2 assay (as described for Figs 4-5) Sarbeco E assay: Corman V. M. et al. (2020) Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro Surveill. 25:2000045
Method (software) used for design and *in silico* verification	Y	https://www.cdc.gov/coronavirus/2019-ncov/lab/rt-pcr-detection-instructions.html				CDC N2 assay (as described for Figs 4-5) Sarbeco E assay: Gans J. D. and Wolinsky M. (2008) Improved assay-dependent searching of nucleic acid sequence databases. Nucleic Acid Res. 36:e74
Location of amplicon	Y	2019-nCoV_N1: 28287-28358 of reference sequence MN908947.3 2019-nCoV_N2: 29164-29230 of reference sequence MN908947.3				CDC N2 assay (as described for Figs 4-5) Sarbeco E assay: 26269-26381 of reference sequence MN908947.3
Amplicon length	Y	2019-nCoV_N1: 72 nt 2019-nCoV_N2: 67 nt				CDC N2 assay (as described for Figs 4-5) Sarbeco E assay: 113 nt

Primer and probe sequences (or amplicon context sequence)**	Y	2019-nCoV_N1: N1_F: 5’-GACCCCAAAATCAGCGAAAT-3’ N1_R: 5’-TCTGGTTACTGCCAGTTGAATCTG-3’ N1_P: 5'-FAM-ACCCCGCATTACGTTTGGTGGACC-BHQ1-3’ 2019-nCoV_N2: N2_F: 5’-TTACAAACATTGGCCGCAAA-3’ N2 R: 5’-GCGCGACATTCCGAAGAA-3’ N2 P: 5’-TexasRed-ACAATTTGCCCCCAGCGCTTCAG-BHQ1-3’			CDC N2: 2019-nCoV_N2-F: 5'-TTACAAACATTGGCCGCAAA-3' 2019-nCoV_N2-R: 5'-GCGCGACATTCCGAAGAA-3' 2019-nCoV_N2-P_DQ: 5'-FAM-ACAATTTGCCCCCAGCGCTTCAG-BHQNova1-3' Sarbecco E: E_Sarbecco_F1: 5'-ACAGGTACGTTAATAGTTAATAGCGT-3' E_Sarbecco_R2: 5'-ATATTGCAGCAGTACGCACACA-3' E_Sarbecco_P1_DQ: 5'-FAM-ACACTAGCCATCCTTACTGCGCTTCG-BHQNova-3'
Location and identity of any modifications	Y	None.			None.
Manufacturer of oligonucleotides	Y	Integrated DNA Technologies (IDT).			LGC Biosearch.
7. dPCR PROTOCOL					
Manufacturer of dPCR instrument and instrument model	Y	QIAcuity Four (QIAGEN) using 8.5k 96-well Nanoplates			QX200 Droplet Digital PCR System (Bio-Rad)
Buffer/kit manufacturer with catalogue and lot number	Y	Manufacturer QIAGEN HILDEN, Cat No. 1123145			One-Step RT-ddPCR Advanced Kit for Probes (Bio-Rad; Catalog No: 186-4021, lot: 64338972)
Primer and probe concentration	Y	Final concentrations of 0.4uM from each primer and 0.2uM from each probe per target. All reactions performed in duplex.			20X primer/probe mixes prepared: 0.9μM each primer and 0.25 μM of probe
Pre-reaction volume and composition (incl. amount of template and if restriction enzyme added)	Y	Each 28 uL reverse transcription reaction was split between qPCR and dPCR. For dPCR, 12 uL was transferred to the QIAcuity 96-well 8.5k Nanoplate (and 12 uL transferred to qPCR tubes).	Bile salts were added to each of the reactions to give the matched final concentrations added to the RT (Fig 6A-B). The 96-well plate was vortexed and pulsed down before the 12 uL transfer to the QIAcuity 96-well 8.5k Nanoplate.	Each 28 uL reverse transcription reaction was split between qPCR and dPCR. For dPCR, 12 uL was transferred to the QIAcuity 96-well 8.5k Nanoplate (and 12 uL transferred to qPCR tubes).	22 μL reactions (10% excess) were prepared containing 5.5 μL of One-Step RT-ddPCR Advanced Kit for Probes, 1.1 μL of DTT, 1.1 μL of 20X primer/probe mix, 2.2 μL of reverse transcriptase, 6.6 μL of nuclease-free water and 5.5 μL of RNA template. Control reactions were set up as no template controls (NTCs) containing water in place of DNA template and negative controls containing carrier molecules only or RNA storage solution (RSS).
Template treatment (initial heating or chemical denaturation)	Y	None.			The templates were heated to 65°C for 5 minutes and incubated on ice for at least 1 minute.
Polymerase identity and concentration, Mg++ and dNTP concentrations***	N	Proprietary information (Taq polymerase and a M-MLV RT derivative are the two enzymes present in the kit).			One-Step RT-ddPCR Advanced Kit for Probes (Bio-Rad; Catalog No: 186-4021, lot: 64338972).
Complete thermocycling parameters	Y	Initial denaturation of 95 °C for 2 min, 40 cycles of 95 °C for 5 sec and 60 °C for 30 sec.			Reverse transcription at 47.5 °C for 60 min. Enzyme activation at 95 °C for 10 min, 40 cycles of 94 °C for 30 s, and 55 °C for CDC N2 and 60°C for Sarbecco E for 1 min, followed by enzyme inactivation at 98 °C for 10 min and a 4 °C hold for at least one hour. The ramp rate for each step was set to 2 °C/s except the final cool to 4 °C that was 1 °C/second.
8. ASSAY VALIDATION					
Details of optimisation performed	Y	N/A			Temperature gradients performed for both assays. Annealing temperature with largest separation between positive and negative parititons was selected.

Analytical specificity (vs. related sequences) and limit of blank (LOB)	N	N/A				N/A
Analytical sensitivity/LoD and how this was evaluated	Y	See Fig 6A.			Evaluation of 10 copies/uL of EURM-019. No further LoD evaluation was performed as beyond scope of these experiments.	N/A
Testing for inhibitors (from biological matrix/extraction)	Y	The aim of these experiments.			N/A	Not performed
9. DATA ANALYSIS						
Description of dPCR experimental design	Y	See reverse transcription section.				Each aliquot from each batch was analysed once with both assays in tripicate RT-dPCR.
Comprehensive details negative and positive of controls (whether applied for QC or for estimation of error)	Y	See reverse transcription section.				Reactions with >10,000 paritions per reaction and correct final peak fluorescence of the negative paritions compared with the NTC reactions were accepted only. No failed reactions were observed.
Partition classification method (thresholding)	Y	Single manual threshold set across all reactions within the experiment. Set based on the software auto threshold of the NTCs in the plate.				Manual thresholds based on multiple assessment of more than three experiments with the same assays.
Examples of positive and negative experimental results (including fluorescence plots in supplemental material)	Y	CDC N1 with 0.6 ug/uL bile salts (3x 1000 copies/uL, 3 NTCs)	CDC N1 with 0.6 ug/uL bile salts (3x 1000 copies/uL, 3 NTCs)	CDC N1 1000 copies/uL with Donor A 20% urine	CDC N1 without inhibitor (3 1000 copies/uL, 3 NTCs)	CDC N2: (3 positive, 3 NTCs; carrier, RSS, water):
	Y	CDC N2 with 0.6 ug/uL bile salts (3x 1000 copies/uL, 3 NTCs)	CDC N2 with 0.6 ug/uL bile salts (3x 1000 copies/uL, 3 NTCs)	CDC N2 1000 copies/uL with Donor A 20% urine	CDC N2 without inhibitor (3 1000 copies/uL, 3 NTCs)	Sarbeco E: (3 positive, 3 NTCs; carrier, RSS, water):
Description of technical replication	Y	Triplicate RT-dPCR replicates				Triplicate RT-dPCR replicates.
Repeatability (intra-experiment variation)	Y	CDC N1: 4.01% CDC N2: 2.87%	CDC N1: 6.39% CDC N2: 6.24%	CDC N1: 9.90% CDC N2: 6.51%	CDC N1: 7.32% CDC N2: 4.98%	CDC N2: 3% Sarbeco E: 7%
Reproducibility (inter-experiment/user/lab etc. variation)	N	N/A - performed in one laboratory only				N/A - performed in one laboratory only
Number of partitions measured (average and standard deviation)	Y	Average: 8164 Standard deviation: 109	Average: 8157 Standard deviation: 70	Average: 8078 Standard deviation: 154	Average: 8120 Standard deviation: 62	Average: 15056 Standard deviation: 4053
Partition volume	Y	0.85 nL (no VPF performed)				0.776 nL based on laboratory assessment with control material.
Copies per partition (λ or equivalent) (average and standard deviation)	Y	CDC N1: 0.149 (SD=0.097) CDC N2: 0.139 (SD=0.102)	CDC N1: 0.236 (SD=0.010) CDC N2: 0.237 (SD=0.009)	CDC N1: 0.151 (SD=0.097) CDC N2: 0.146 (SD=0.095)	CDC N1: 0.096 (SD=0.112) CDC N2: 0.094 (SD=0.110)	Average: 0.5585 Standard deviation: 0.4186

dPCR analysis program (source, version)	Y	QIAcuity Software Suite version 1.2.18			QuantaSoft v1.7.4.0917
Description of normalisation method	Y	The difference in mean copy number concentration of each bile salt concentration and the matched negative control (no bile salts added) was calculated and presented as a percentage where the negative control was called 100% (no inhibition).	For each donor, the difference in mean copy number concentration of each % urine with control samples containing the EURM-019 with no urine was calculated and presented as a percentage where the negative control was called 100% (no inhibition).	N/A	N/A
Statistical methods used for analysis	Y	Two-way ANOVA of copy number concentrations between different bile salt concentration (0, 0.1, 0.2, 0.4, 0.6 and 0.8 ug/uL) and different quantification platform (qPCR and dPCR) for each EURM-019 concentration with a main effects model. Multiple comparison were performed between each bile concentration for the given platform to determine the bile concentration where the copy number concentration was significantly different. Significance was called when $p < 0.05$ and highly significant when $p < 0.001$.	Two-way ANOVA of copy number concentrations between different urine v/v % added for each donor and different quantification platform (qPCR and dPCR) for each EURM-019 concentration with a main effects model. Multiple comparison were performed between each v/v urine for the given platform to determine the effect of different v/v urine where the copy number concentration was significantly different. Significance was called when $p < 0.05$ and highly significant when $p < 0.001$.	Linear regression of the matched RT samples (split from the single 28 uL RT reaction) on Cq values from qPCR and log2 transformed dPCR copy number concentrations.	Two-way ANOVA of copy number concentrations between the two assays and the different units or batches. Significance was called when $p < 0.05$ and highly significant when $p < 0.001$.
Data transparency		Data avaliable on request.			Data avaliable on request.

* Assessing the absence of DNA using a no RT assay (or where RT has been inactivated) is essential when first extracting RNA. Once the sample has been validated as DNA-free, inclusion of a no-RT control is desirable, but no longer essential.

** Disclosure of the primer and probe sequence is highly desirable and strongly encouraged. However, since not all commercial pre-designed assay vendors provide this information when it is not available assay context sequences must be submitted (Bustin et al. Primer sequence disclosure: A clarification of the miqe guidelines. Clin Chem 2011;57:919-21.)

*** Details of reaction components is highly desirable, however not always possible for commercial disclosure reasons. Inclusion of catalogue number is essential where component reagent details are not available.