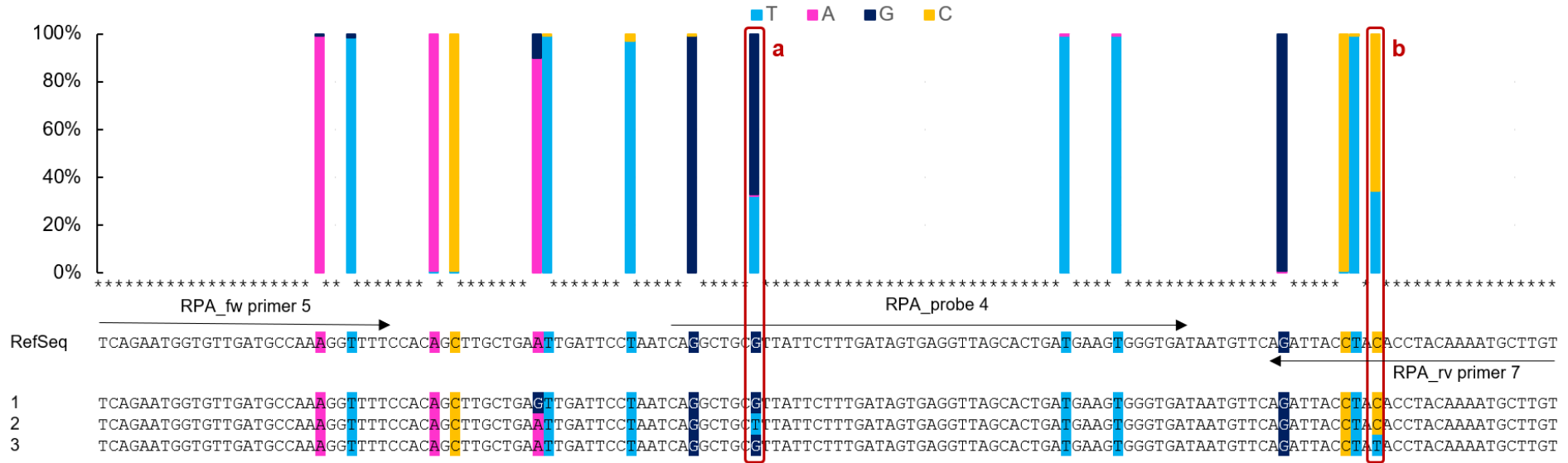


## **Supplemental data**

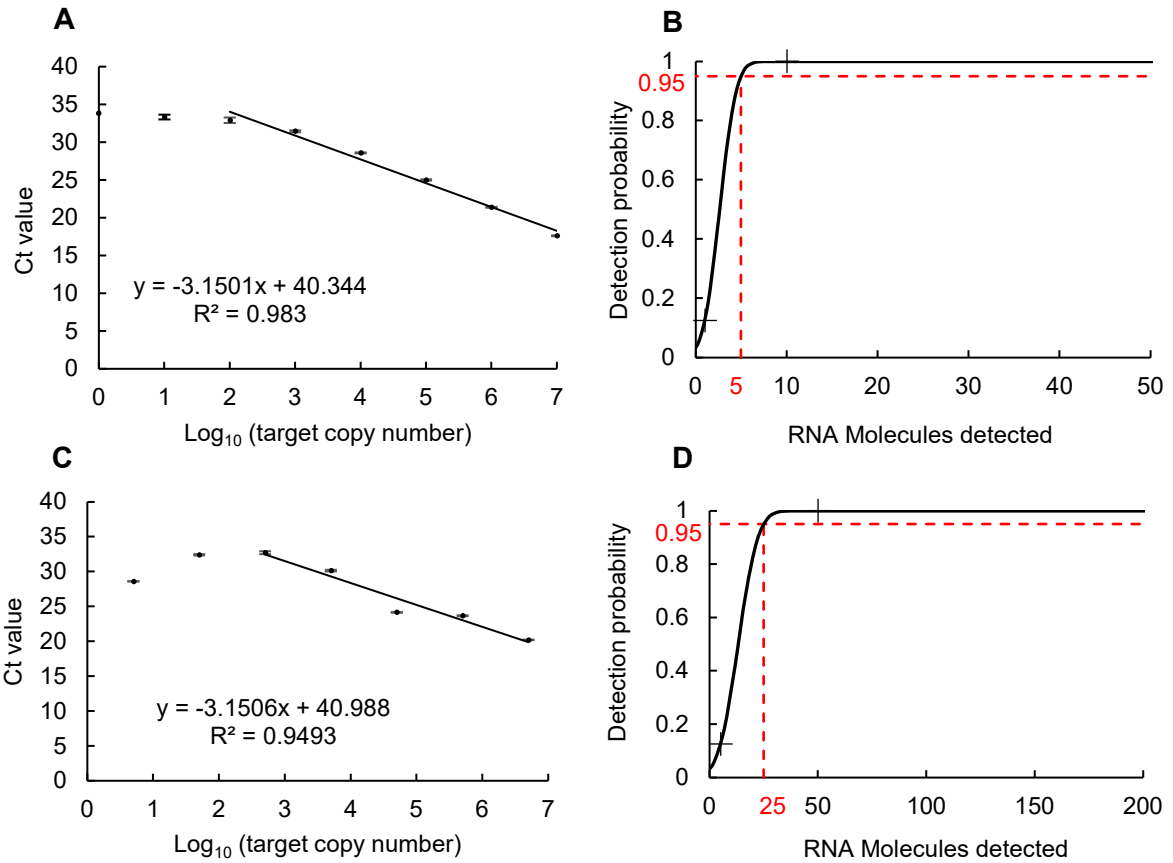
**Supplemental table 1: List of used viral nucleic acids for specificity analysis.** Respiratory virus panel from patient samples (except human coronaviruses) provided by Friedrich Loeffler institute, Germany and Quality Control for Molecular Diagnostics (QCMD), UK). Coronavirus RNA specificity panel from cell culture was provided by European Virus Archive Global, Rotterdam, Netherlands.

<b>Name of sample</b>	<b>Name of virus</b>
Adenoviruses	
ADV 12-01	Adenovirus Type 34
ADV 12-05	Adenovirus Type 1
ADV 12-06	Adenovirus Type 4
Avian respiratory viruses	
ILTV 168/13	Infectious laryngotracheitis virus U76 (Gallid herpesvirus 1)
IBV M41	Avian infectious bronchitis virus strain M41
Coronaviruses	
HCoV-229E	Human coronavirus 229E
HCoV-OC43	Human coronavirus OC43
SARS-CoV HKU39849	Severe acute respiratory syndrome coronavirus HKU39849
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
MERS-CoV	Middle east respiratory syndrome-related coronavirus
Influenza viruses	
INFHT 12-04	Influenza Virus A H1N1 pdm09 (2009 pandemic strain isolated from a patient in 2009)
INFHT 12-05	Influenza Virus A H7N7 (A/Mallard/Netherlands/2/2009)
INFHT 12-06	Influenza Virus A H1N1 H275Y (Oseltamivir resistant A, H1 pre- pandemic strain isolated in The Netherlands in 2011)
INFHT 12-07	Influenza Virus A H3N2 (A/Netherlands/344/2006)
INF RNA 12-01	Influenza Virus B (Victoria)
INF RNA 12-08	Influenza Virus B (Yamagata)
H5N1 Isolate 264	Influenza Virus A H5N1 (patient isolate)
H7N7 Isolate 301	Influenza Virus A H7N7 (patient isolate)
H1N1 Isolate 330	Influenza Virus A H1N1 (patient isolate)
Metapneumoviruses	
MPV 12-02	Human metapneumovirus B2
MPV 12-04	Human metapneumovirus A1
Parainfluenza viruses (Patient isolate)	
Parainfluenza 1	Parainfluenza 1

Parainfluenza 2	Parainfluenza 2
Parainfluenza 10.3	Parainfluenza 3
Parainfluenza 20.4	Parainfluenza 4
Parainfluenza 4a	Parainfluenza 4
<b>Name of sample</b>	<b>Name of virus</b>
Parainfluenza 4b	Parainfluenza 4
Respiratory syncytial virus	
RSV 12-08	Respiratory syncytial virus A
Rhinoviruses	
RV 12-02	Human rhinovirus A 16
RV 12-06	Human rhinovirus A 90
RV 12-11	Human rhinovirus C
RV 12-12	Human rhinovirus B 5



**Supplemental figure 1: Alignment of circulating HCoV-NL63 strains.** Reference sequence (NC005831.2) was aligned to 136 sequences from circulating HCoV-NL63 strains (NCBI genbank search). Results are demonstrated in the bar chart, revealing two important point mutations. a) In 33 % of the sequences there is a thymidine instead of a guanine, which is in position 9 from the 5' end of the probe and exemplarily shown in sequence 2. b) In 34 % of the sequences there is a thymidine instead of a cytosine in position 18 from the 5' end of the reverse primer and exemplarily shown in sequence 3. Both mutations were never found simultaneously within the same strain. Sequence 1 exemplarily shows a point mutation from adenine to guanine (10 % of all sequences) at position 43 from 5' end of the amplicon within an area where no primer nor probe is located. All other mismatches occur rarely and only in individual sequences and do not persist throughout the 136 strains. Sequence 1 KY862074.1 France (Caen) 2004; sequence 2 LC488389.2 Japan (Tokyo) 2018; sequence 3 JQ765570.1 USA (Denver) 2005.



**Supplemental figure 2: Calibration lines and probit regressions of HCoV-NL63 RT-PCR sensitivity assays.**

Calibration line for the detection of A) Synthetic HCoV-NL63 RNA and C) genomic viral HCoV-NL63 RNA from cell culture in human RNA from swab sample material in the RT-PCR assay. Shown are linear correlations of threshold cycles over the decadic logarithm of the RNA target concentration ( $10^7 - 10^0$  RNA molecules/reaction). Probit regression for B) synthetic HCoV-NL63 RNA in PCR-grade water and D) genomic viral HCoV-NL63 RNA from cell culture and human RNA from swab sample material revealing a 95 % detection probability (analytical sensitivity) of 5 RNA copies/reaction and 25 RNA copies/reaction.