The US CDC RT-PCR probes for the Wuhan 2019-nCoV are non-specific for SARS - there is a better specific 500bp option (21852-22427) within the spike-protein

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Letter

The World Health Organization has declared the Wuhan coronavirus [1–3] outbreak a public health emergency on 30th Jan, 2020. An accurate detection of the Wuhan virus is imperative to avoid false positives, since 2019-nCoV [2,3] shares significant homology [4] with other species.

On the 24th of Jan, the CDC has published a list of 3 probes (Fig 1) for detecting the 2019-nCoV. Detection of all three probes would give a positive result, while detection of one or two gives an 'inconclusive result' (and obviously negative when none are found).

All 3 probes will have significant homology in other strains (Table 1 and Fig 2) - specifically SARS (Severe Acute Respiratory Syndrome). A much more specific option is to look within a 500bp genomic fragment in the N-terminal of the spike protein (Accid:NC_045512.2 [2],21852-22427, SI.cdc:nCoVFULLSLICE.fa), which seems to be the most perturbed in this virulent strain [5].

It is also important to have a common repository for all countries to be following a similar protocol. Many papers do not provide what PCR was used to detect coronaviruses [6].

References

1. Perlman S (2020). Another decade, another coronavirus.
2. Zhu N, Zhang D, Wang W, Li X, Yang B, et al. (2020) A novel coronavirus from patients with pneumonia in China, 2019. New England Journal of Medicine .
3. Chan JFW, Yuan S, Kok KH, To KKW, Chu H, et al. (2020) A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. The Lancet .

Table 1: **Non-specificity of the three primers CDC has published** This will flag positive for other coronaviruses of SARS species - an example of BAT-SARS (Accid:DQ412043.1). These differences (SI.cdc:Blastresults.txt) are all well within the mismatch tolerance of the PCR's [7]. On the other, primers within the 500bp region within the spike protein in the Wuhan 2019-nCoV has no homology in other species.

Primer	Wuhan nCoV	Bat-SARS	Identities
F=GACCCCAAAATCAGCGAAAT R=TCTGGTTACTGCCAGTTGAATCTG	28287-28358	28145-28215	64/71(90%)
F=TTACAAACATTGGCCGCAA R=GCGCGACATTCCGAAGAA	29164-29230	29022-29088	59/67(88%)
F=GGGAGCCTTGAATACACCAAAA R= TGTAGCACGATTGCAGCATTTG	28681-28752	28539-28610	68/72(94%)

2019-Novel Coronavirus (2019-nCoV) Real-time rRT-PCR Panel

Primers and Probes

Division of Viral Diseases

2019-Novel Coronavirus (2019-nCoV) Real-time rRT-PCR Panel Primers and Probes				
Name	Description	Oligonucleotide Sequence (5'→3')	Label ¹	Working Conc.
2019-nCoV_N1-F	2019-nCoV_N1 Forward Primer	5'-GAC CCC AAA ATC AGC GAA AT-3'	None	20 µM
2019-nCoV_N1-R	2019-nCoV_N1 Reverse Primer	5'-TCT GGT TAC TGC CAG TTG AAT CTG-3'	None	20 µM
2019-nCoV_N1-P	2019-nCoV_N1 Probe	5'-FAM-ACC CCG CAT TAC GTT TGG TGG ACC-BHQ1-3'	FAM, BHQ-1	5 µM
2019-nCoV_N2-F	2019-nCoV_N2 Forward Primer	5'-TTA CAA ACA TTG GCC GCA AA-3'	None	20 µM
2019-nCoV_N2-R	2019-nCoV_N2 Reverse Primer	5'-GCG CGA CAT TCC GAA GAA-3'	None	20 µM
2019-nCoV_N2-P	2019-nCoV_N2 Probe	5'-FAM-ACA ATT TGC CCC CAG CGC TTC AG-BHQ1-3'	FAM, BHQ-1	5 µM
2019-nCoV_N3-F	2019-nCoV_N3 Forward Primer	5'-GGG AGC CTT GAA TAC ACC AAA A-3'	None	20 µM
2019-nCoV_N3-R	2019-nCoV_N3 Reverse Primer	5'-TGT AGC ACG ATT GCA GCA TTG-3'	None	20 µM
2019-nCoV_N3-P	2019-nCoV_N3 Probe	5'-FAM-AYC ACA TTG GCA CCC GCA ATC CTG-BHQ1-3'	FAM, BHQ-1	5 µM
RP-F	RNAse P Forward Primer	5'-AGA TTT GGA CCT GCG AGC G-3'	None	20 µM
RP-R	RNAse P Reverse Primer	5'-GAG CGG CTG TCT CCA CAA GT-3'	None	20 µM
RP-P	RNAse P Probe	5'-FAM - TTC TGA CCT GAA GGC TCT GCG CG - BHQ-1-3'	FAM, BHQ-1	5 µM

¹TaqMan® probes are labeled at the 5'-end with the reporter molecule 6-carboxyfluorescein (FAM) and with the quencher, Black Hole Quencher 1 (BHQ-1) (Biosearch Technologies, Inc., Novato, CA) at the 3'-end.

Note: Oligonucleotide sequences are subject to future changes as the 2019-Novel Coronavirus evolves.

Figure 1: **RT-PCR test to detect Wuhan 2019-nCoV published by CDC on 24th Jan, 2020** Information provided here-www.cdc.gov/2019-ncov/downloads/rt-pcr-panel-primer-probesPrimerProbes-CDC.

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- Chakraborty S (2020). Ubiquitous genomic fragment in human 2019-ncov viruses in the spike-protein, also encoding a novel 87 aa protein, completely missing in all other coronaviruses. doi: 10.31219/osf.io/6vd54. URL osf.io/6vd54.
- Li Q, Guan X, Wu P, Wang X, Zhou L, et al. (0) Early transmission dynamics in wuhan, china, of novel coronavirus-infected pneumonia. *New England Journal of Medicine* 0: null.
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Figure 2: Multiple sequence alignment of the 3 PCR probes in Wuhan 2019-nCoV and BAT-SARS