

SUPPLEMENTARY INFORMATION: Description of the Lab Developed Test

Table 1: Summary of LDT Extraction Methods

	LDT-1	LDT-2D	LDT-2R	LDT-FUS
Extraction Reagents	MagMAX (Thermo Scientific)	DNA Mini Kit (QIAGEN)	RNeasy Kit (QIAGEN)	Panther Fusion reagents (Hologic)
Extraction Instrument	KingFisher (Thermo Scientific)	QIACube (QIAGEN)	QIACube (QIAGEN)	Panther Fusion (Hologic)
Specimen Volume for Extraction	200 µL	140 µL	200 µL	500 µL loaded (≈150 µL extracted) [†]
Elution Volume from Extraction	110 µL	50 µL	50 µL	50 µL

[†] 500 µL of specimen is mixed with 710 µL of Hologic Lysis Buffer and loaded on to the Panther Fusion instrument.

The on-board extraction process uses 360 µL of this mixture, of which ≈150 µL is derived from the specimen.

Table 2: Summary of LDT RT-PCR Methods

	LDT-1	LDT-2	LDT-FUS
RT-PCR System	CFX96 (BioRad)	Rotor Gene Q (QIAGEN)	Panther Fusion (Hologic)
Template (Eluate) Volume	5 uL	5 uL	5 uL
Master Mix Buffer	Taqman Fast 1-Step Virus Mix (Thermo Scientific)	Taqman Fast 1-Step Virus Mix (Thermo Scientific)	PPR Mix supplemented with 3 mM MgCl ₂ and 100 mM KCl plus Open Access RNA/DNA Enzyme Cartridge (Hologic)

Table 3: Primer and Probe Sequences:

Screening Test Oligonucleotide Name	Oligonucleotide Sequence	Concentration (nM)		
		LDT-1	LDT-2	LDT-FUS
E Forward	5'-ACAGGTACGTTAATAGTTAATAGCGT-3'	400	400	400
E Reverse	5'-ATATTGCAGCAGTACGCACACA-3'	400	400	400
E Probe	5'-FAM-ACACTAGCCATCCTTACTGCGCTTCG-BHQ1-3'	200	200	200
RNaseP Forward	5'-AGATTTGGACCTGCGAGC-3'	100	100	100
RNaseP Reverse	5'-GAGCGGCTGTCTCCACAAGT-3'	100	100	100
RNaseP Probe	5'-HEX-TTCTGACCTGAAGGCTCTGCGCG-BHQ1-3'	100	100	100

Confirmatory Test Oligonucleotide Name	Oligonucleotide Sequence	Concentration (nM)		
		LDT-1	LDT-2	LDT-FUS
N1 Forward	5'-GACCCCAAATCAGCGAAAT-3'	400	400	400
N1 Reverse	5'-TCTGGTTACTGCCAGTTGAATCTG-3'	400	400	400
N1 Probe	5'-FAM-ACCCCGCATTACGTTTGGTGGACC-BHQ1-3'	200	200	200

‡ Oligonucleotide sequences are derived from the previously published Corman et al. (E gene) and US CDC (N1 gene) RT-PCR protocols.