Diagnostic performance of thioflavin T in one-step RT-PCR for detection of a viral RNA

Supplementary Information

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Supplementary Table. SI*. Previously reported GQ sequence detected with ThT with sequence name, nucleotide content, melting temperature and the GC content. The GQ sequences are ordered according to the length.*

GQ name	GQ sequence	Length	Tm	GC (%)	Ref
GQ-1	GGGTTTTGGGTTTTGGGTTTTGGGTTTT	28	65.6	43	[3]
GQ-2	TTGGGTAGGGCGGGTTGGGTT	21	65.0	62	[13]
GQ-3	ATGGGAAGGGAGGGATGGGT	20	60.9	60	[14]
PW17	GGGTAGGGCGGGTTGGG	17	60.5	76	[15, 16]
22AG	AGGGTTAGGGTTAGGGTTAGGG	22	59.5	55	[6-11]

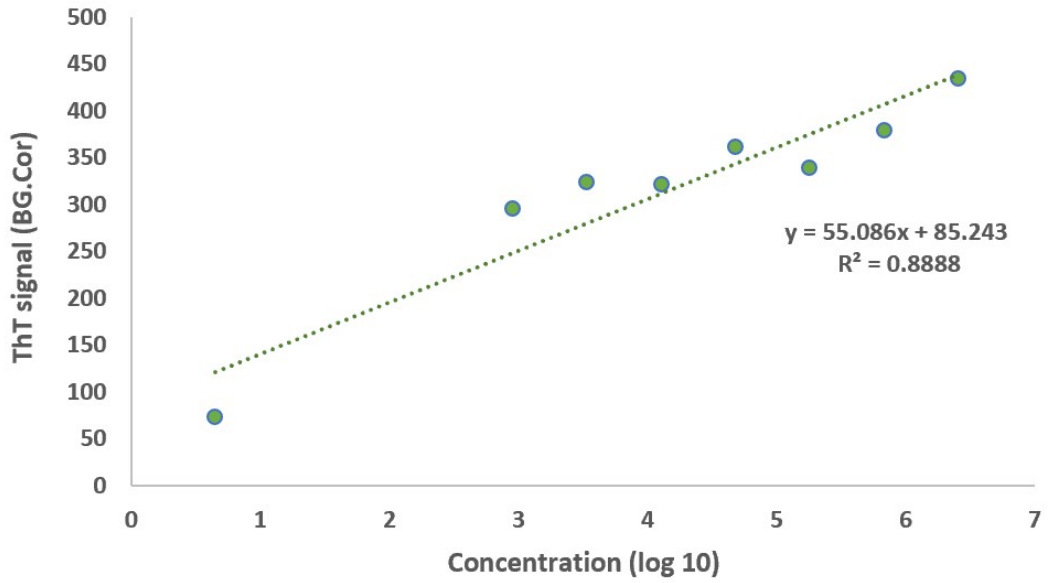
TBA-29	GTCCGTGGTAGGGCAGGTTGGGGTGACT	29	72.7	64	[1, 2]
AGRO 100	GGTGGTGGTGGTTGTGGTGGTGGTGG	26	71.4	65	[5]
ATP aptamer	ACCTGGGGGAGTATTGCG GAGGAAGGT	27	71.8	65	[4]
c-Myc	TGAGGGTGGGTAGGGTGGGTAA	22	63.3	59	[12]

Supplementary Table. SII. *The amplicon sequence of the ScienCell™ SARS-CoV-2 RT-PCR kit. The ScienCell™ primer set amplified the 71-length nucleotide sequence of the SARS-CoV-2 N gene. The GC content of this amplicon is 52% (it is less than 60%), which is a good candidate region to be amplified with the TQsyn RT-PCR method.*

Target Gene	Amplicon sequence	Amplicon size	Target gene position	GC content
N	**GACCCCAAAATCAGCG AAAT**GCACCCCGCATT ACGTTTGGTGGACCCT **CAGATTCAACTGGCAG TAACCAG**	71	28287 - 28358	52.11%

Supplementary Table. SIII. *The fluorescent signal of negative viral RNA samples and the calculated average of them. The TQsyn RT-PCR reactions were done for eleven RNA-negative samples (samples from individuals who were negative for SARS-CoV-2). The calculated average of these samples (1643.45) is considered as the background (B.G.) and is subtracted from the fluorescent signal of any other sample in the report (test or controls).*

Negative sample ID	ThT Fluorescent signal
Neg. 1	1741
Neg. 2	1436
Neg. 3	1503
Neg. 4	1659
Neg. 5	1476
Neg. 6	1599
Neg. 7	1632
Neg. 8	1734
Neg. 9	1734
Neg. 10	1795
Neg. 11	1769
Average background	1643.45
Standard deviation	125.61



Supplementary Figure. SI. *Calibration curve illustrating the relationship between Thioflavin T (ThT) fluorescence signal and log-transformed analyte concentration. Data points represent measured fluorescence intensities at known concentrations, while the fitted linear regression line demonstrates the correlation. The regression equation and coefficient of determination (R^2) are shown on the graph and were used to estimate the Limit of Detection (LOD) and Limit of Quantitation (LOQ) according to the method described by Armbruster & Pry [18] and CLSI EP17-A2 (2012)[1].*

[1] https://clsi.org/shop/standards/ep17/

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