Electronic supplementary information (ESI) for

Automated sample-to-answer centrifugal microfluidic system for rapid molecular diagnostics of SARS-CoV-2

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Supplementary experimental details

Enclosure chamber

For all experiments involving viral samples, a 3D printed enclosure chamber was placed on the rotating stage to enclose the cartridges and minimize risk of aerosol generation in the event of leakage during the automated operation. The enclosure features a top viewing window sealed using an optically transparent Zeonor substrate (1 mm in thickness). A neoprene rubber gasket (12″ long, 12″ wide, 1/32″ thick; McMaster-Carr, Elmhurst, IL) was trimmed using a Cameo 4 blade cutter (Silhouette, Lindon, UT) and used to seal the enclosure at the top and at the bottom. Thumb screws are used to secure the enclosure on the rotational stage while the cartridge is held in place by the manifold and a spring-based clamping mechanism.

RT-LAMP dry reagents

RT-LAMP master mix for single assay lyophilized RT-LAMP reaction were prepared from custom-ordered glycerol-free liquid reagents (New England Biolabs) and freeze-dried RT-LAMP beads were generated by Evik Diagnostic Innovations (Kanata, ON). Briefly, RT-LAMP beads were freeze-dried each containing 5 µL 10× primer mix, 16 U glycerol free WarmStart Bst 2.0 (New England Biolabs), 22.5 U glycerol free WarmStart RT (New England Biolabs), 0.05 U Antarctic Thermolabile UDG (New England Biolabs), 50 U RNase Inhibitor (New England Biolabs), 1.4 mM dNTPs (Thermo Fisher Scientific), 280 µM dUTP (New England Biolabs), and 10% trehalose (Sigma-Aldrich). For positive control beads, the mix included 4 µL of purified plasmid DNA targets for E and N2 genes (Integrated DNA Technologies). Prior to RT-LAMP reaction, beads were reconstituted with 2× isothermal amplification buffer, 100 µM neutral red (Thermo Fisher Scientific) and nuclease-free water. Finally, each lyophilized RT-LAMP reaction was performed by adding either 4 µL of synthetic SARS-CoV-2 RNA (MT007544.1; Twist Bioscience) or 4 µL of isolated viral RNA from NP swab samples (iSpecimen) for a total volume of 50 µL.

Supplementary tables

Table S1 Characteristics of reservoirs implemented in the fluidic design and corresponding reagents

Reservoir	Number of units	Location on cartridge	Capacity (μL)	Reagent	Reagent volume (μL)
RNA extraction	1	Top side	900	Lysis/binding buffer	400
RT-LAMP reagent storage	5	Bottom side	100 (each)	RT-LAMP master mix	46
RT-LAMP chambers	5	Bottom side	65 (each)		50
Metering chamber	2	Bottom side	4	Eluted RNA	4
Wash	2	Bottom side	850 (each)	Wash buffer 1 or 2	600
Elution buffer	1	Bottom side	100	Elution buffer	30
Waste	1	Bottom side	3000	–	–

Table S2 RNA extraction reagents and volumes used for manual and on-chip RNA isolation

Reagent	Volume per 100 µL sample
Lysis/binding buffer	400 µL
Wash buffer 1	600 µL
Wash buffer 2	600 µL
Elution buffer	30 µL

Table S3 Primers used in the SARS-CoV-2 RT-LAMP assay

Gene	Primer	Sequence (5′ → 3′)	Reference
E	F3	TGGCTACTACCGAAGAGCT	1
	B3	TGCAGCATTGTTAGCAGGAT	
	FIP	TCTGGCCCAGTTCCTAGGTAGTCCAGACGAATTCGTGGTGG	
	BIP	AGACGGCATCATATGGGTTGCACGGGTGCCAATGTGATCT	
	LF	GGACTGAGATCTTTCATTTTACCGT	
	LB	ACTGAGGGAGCCTTGAATACA	
N2	F3	ACCAGGAACTAATCAGACAAG	1
	B3	GACTTGATCTTTGAAATTTGGATCT	
	FIP	TTCCGAAGAACGCTGAAGCGGAACTGATTACAAACATTGGCC	
	BIP	CGCATTGGCATGGAAGTCACAATTTGATGGCACCTGTGTA	
	LF	GGGGGCAAATTGTGCAATTTG	
	LB	CTTCGGGAACGTGGTTGACC	

Table S4 Repeatability of RT-LAMP assays for different SARS-CoV-2 synthetic RNA input concentrations (n = 3) with corresponding RT-qPCR Cq values

	Concentration (c/μL)			
	0.2	1	2	4
E gene				
On-chip RT-LAMP	3/3	3/3	3/3	3/3
Manual RT-LAMP	2/3	3/3	3/3	3/3
RT-qPCR Cq	39.93	37.43	36.35	35.27
N gene				
On-chip RT-LAMP	3/3	3/3	3/3	3/3
Manual RT-LAMP	3/3	3/3	3/3	3/3
RT-qPCR Cq	39.01	36.61	35.58	34.55

Table S5 Summary of RT-LAMP primers used with corresponding targets

Stain/ gene[a]	Primer	Sequence (5′ → 3′)	Reference
NL63/ N gene	F3	TTTGGCTTTAAAGAACTTAGGT	2
	B3	ACCATTCTGAACAAGATCTGA	
	FIP	GGTTGAGAAAGAGGCTTATTAGGTTTTTGATAACCAGTCGAAG TCA	
	BIP	TCGTTGGAAGCGTGTTCCTATGTGATTAAAATCACGAGGAC	
	LF	TCTTAGGAGTGGAAGTACCAGAAG	
	LB	CAGAGAGGAAAATGTTATTCAGTGC	
H1N1/H A gene	F3	AGCAAGAAGTTCAAGCCG	3
	B3	CGTGAACTGGTGTATCTGAA	
	FIP	GGCTCTACTAGTGTCCAGTAATAGT- AAATAGCAATAAGACCCAAAGTG	
	BIP	ATAACATTCGAAGCAACTGGAAATC- TGATAATACCAGATCCAGCATT	
	LF	TCTCCCTTCTTGATCCC	
	LB	TAGTGGTACCGAGATATGCA	
SARS-CoV-2/Rdrp gene	F3	TCACCTTATGGGTTGGGA	4
	B3	CAGTTGTGGCATCTCCTG	
	FIP	CGTTGTATGTTTGCGAGCAAGA-TTTT- GAGCCATGCCTAACATGC	
	BIP	GTGCTCAAGTATTGAGTGAAATGGT-TTTT- ATGAGGTTCCACCTGGTT	
	LF	ACAAGTGAGGCCATAATTCTAAG	
	LB	GTGTGGCGGTTCACTATATGTT	

[a] Obtained from American Type Culture Collection (Manassas, VA).

Supplementary figures

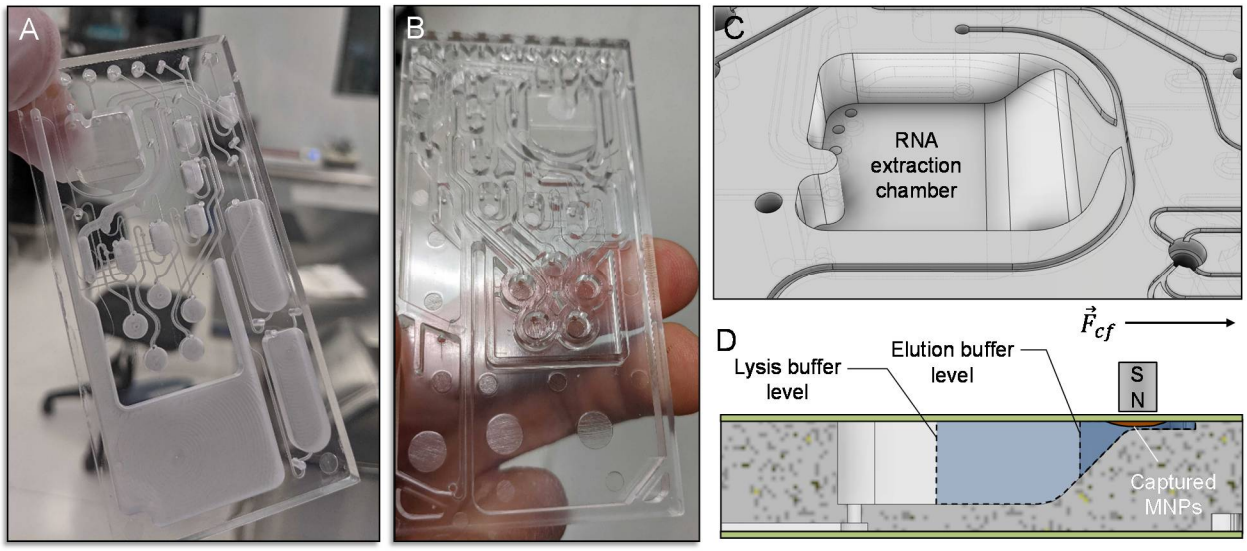

Fig. S1 Microfluidic cartridge. (A) Photograph showing a cartridge variant that was fabricated using CNC machining. (B) Center piece produced by injection molding. (C) 3D design representation of the RNA extraction chamber. (D) Cross-section schematic showing the profile of the RNA extraction chamber, liquid levels for lysis buffer and elution buffer, as well as position of the MNPs after capture with the magnet. The direction of flow is from left to right.

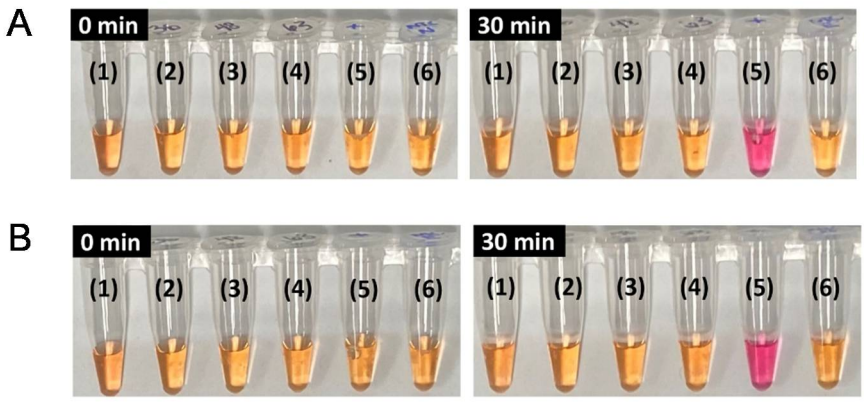

Fig. S2 Images showing the specificity of the RT-LAMP master mix for (A) N gene and (B) E gene primer sets for different nucleic acid templates including (1) genomic DNA from *Mycoplasma pneumoniae* FH strain of Eaton Agent, (2) RNA from influenza A virus (H1N1) strain A, (3) synthetic RNA from MERS-CoV, (4) synthetic RNA from human CoV-NL63, and (5) synthetic RNA from SARS-CoV-2. The remaining sample (6) constitutes NTC.

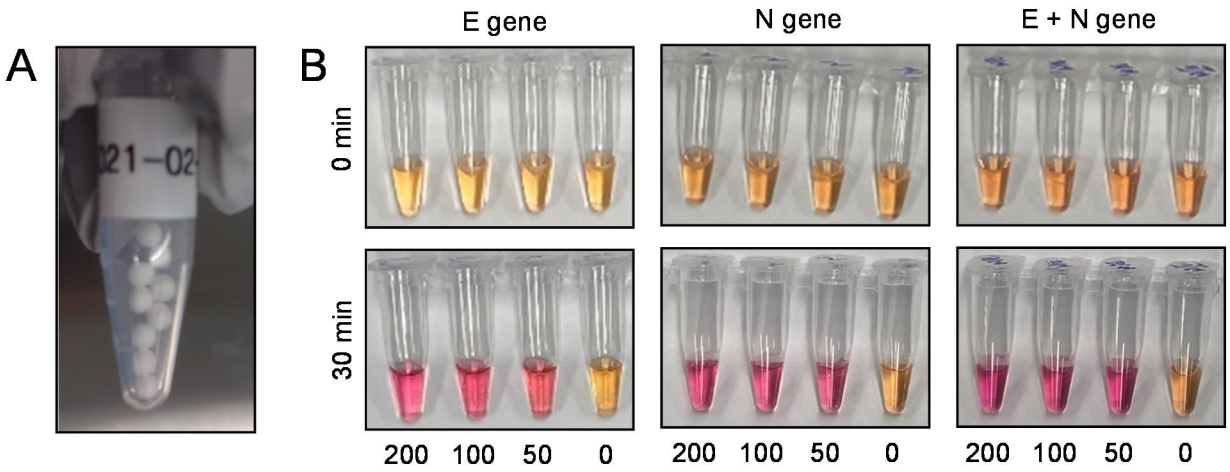

Fig. S3 SARS-CoV-2 RT-LAMP assay performed in tubes using lyophilized reagents. (A) Photograph showing the shape and size of lyophilized beads. (B) Images showing the sensitivity of SARS-CoV-2 RT-LAMP formulation in single assay lyophilized beads containing primers for E or N2 gene alone or in combination detecting 200, 100, and 50 copies of SARS-CoV-2 synthetic RNA. NTC (0 copies) was included as a reference.

Supplementary references

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