Electronic supplementary information for

Multiple On-line Active Valves Based Centrifugal Microfluidics for Dynamic Solid-Phase Enrichment and Purification of Viral Nucleic Acid

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This PDF file includes:

Other Supplementary Material for this manuscript includes the following:

Figure S1

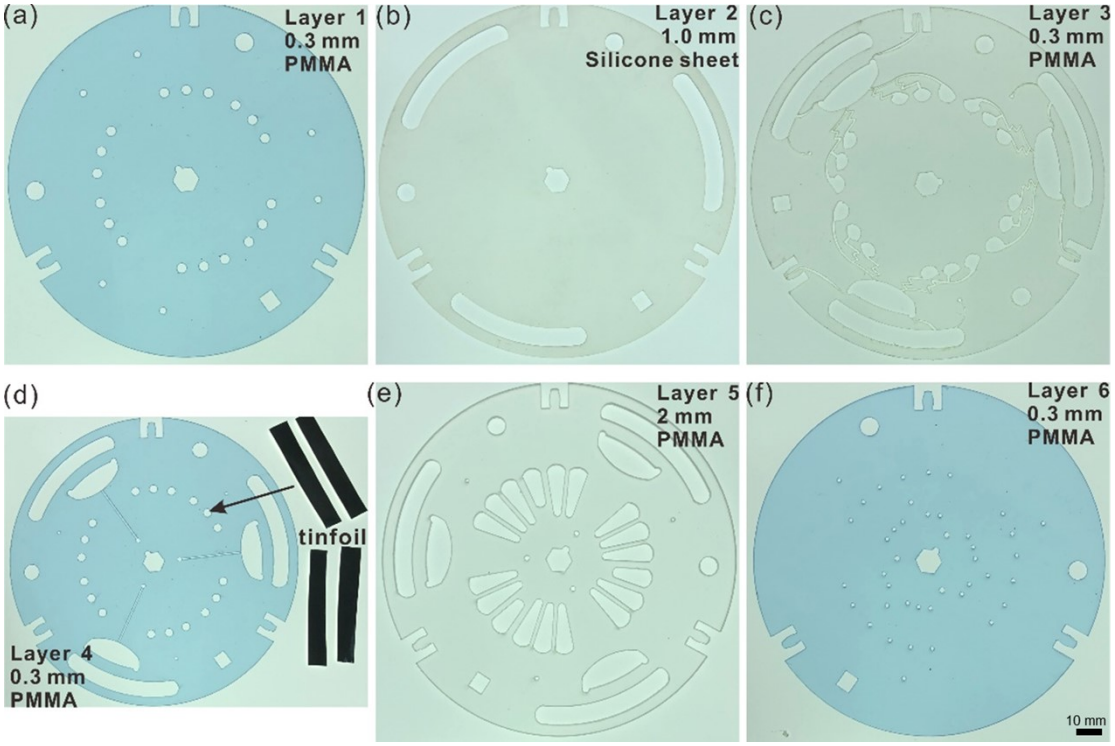

Figure S1: Physical representations of the chip layers: (a) Bottom cover layer - 0.3 mm PMMA; (b) Elastic silicone layer - 1 mm; (c) Drainage layer - 0.3 mm PMMA; (d) Tin foil layer - 0.3 mm PMMA; (e) Liquid storage or reagent layer - 2 mm PMMA; (f) Top cover layer - 0.3 mm PMMA.

Figure S2

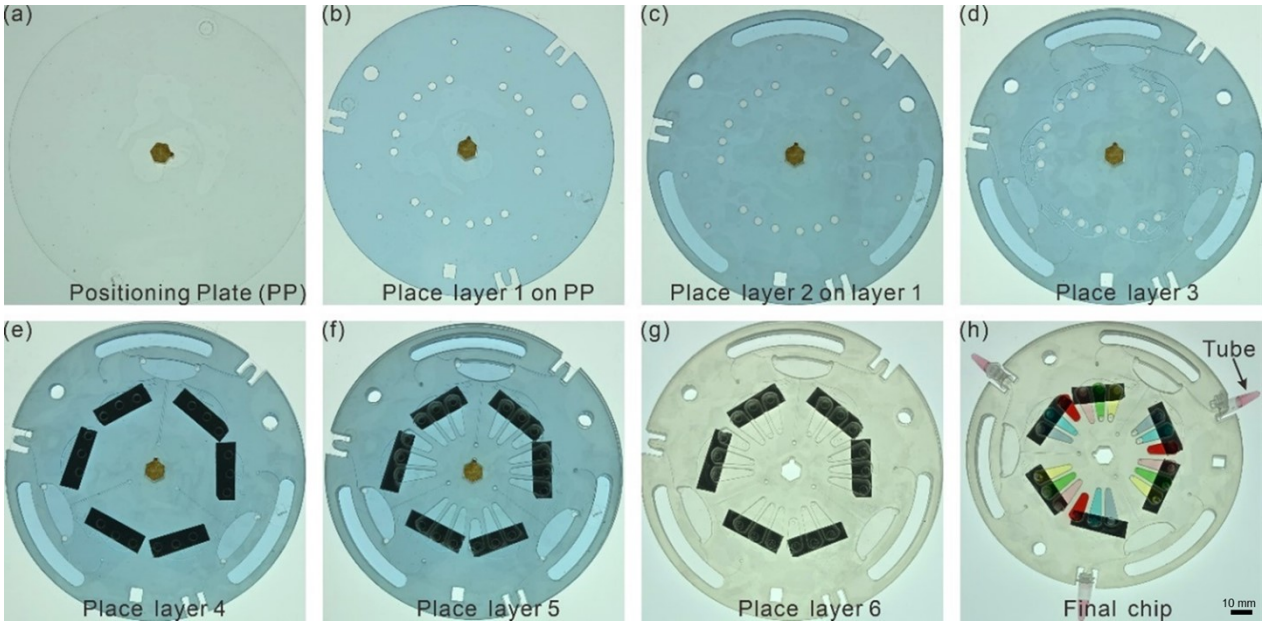

Figure S2: Assembly of the chip: (a) Positioning disc; (b) Assembly of the bottom layer - first layer; (c) Assembly of the second layer; (d) Assembly of the third layer; (e) Assembly of the fourth layer, paying attention to first place the tin foil strip at the corresponding position on the back of this layer; (f) Assembly of the fifth layer; (g) Assembly of the sixth layer, completing the main structure of the chip; (h) Assembly of the centrifuge tube used for collecting purified nucleic acid.

Figure S3

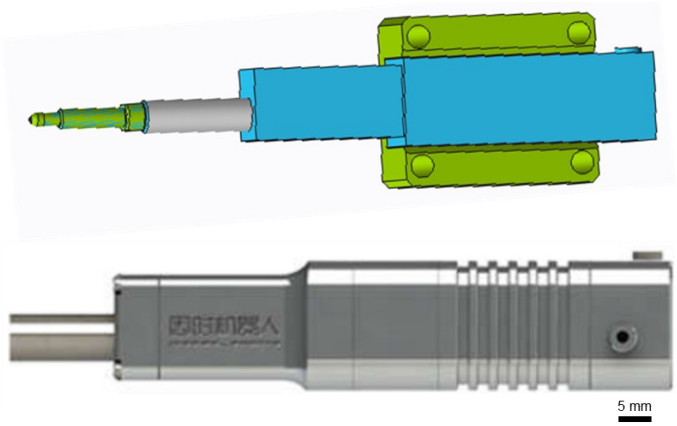

Figure S3: Schematic diagram of the puncture valve motor (top) and its physical representation (bottom, purchased from Beijing Yinshi Robotics Company, total travel distance of 10 mm, using TTL serial communication).

Figure S4

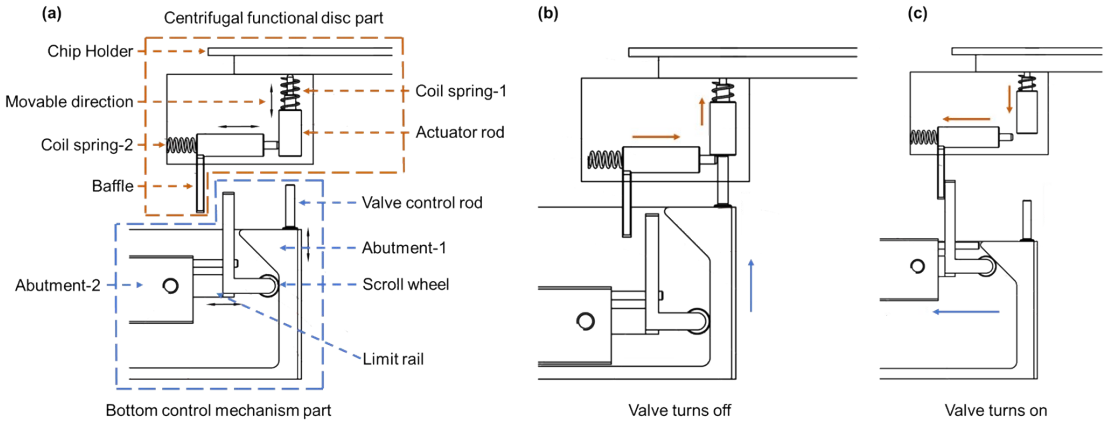

Figure S4: The mechanical structure and principle diagram of the returnable valves. (a). Plan view of the key parts of the centrifugal function disk and the bottom mechanical control structure; (b). Valve turns off: coil spring-1 would be moved upward and coil spring-2 would be moved to the right due to the need of the automatic reset of the coil, and when the mechanical control structure is left, the actuator rod would be fixed above due to coil spring-2; (c). Valve turns on: coil spring-2 would be moved to the left and coil spring-1 would be moved down due to the need of the automatic reset of the coil, ultimately causing the actuator rod to be fixed below.

Figure S5

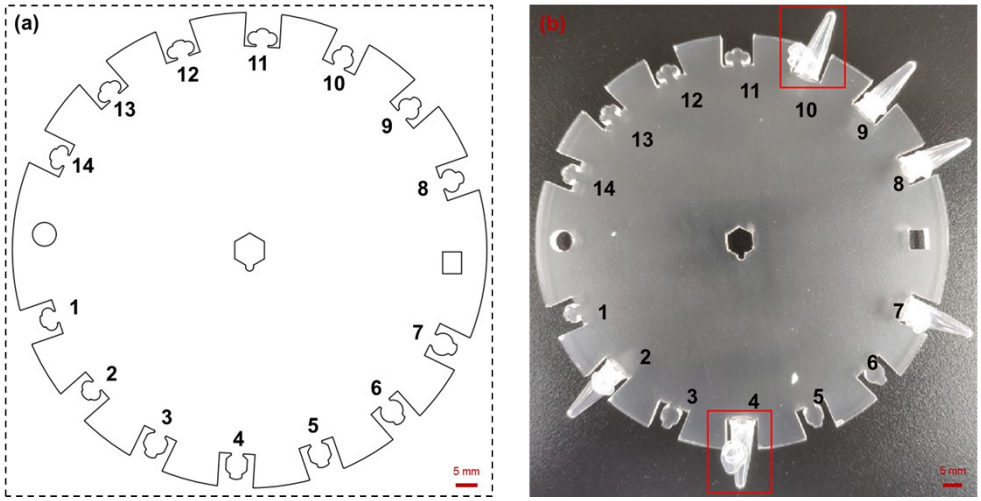

Figure S5: Centrifugal tube clamp dimension selection. (a) 14 centrifuge tube clamp designs. (b) Physical image of centrifuge tube fixation effect after centrifugation at 1500 RPM.

Figure S6

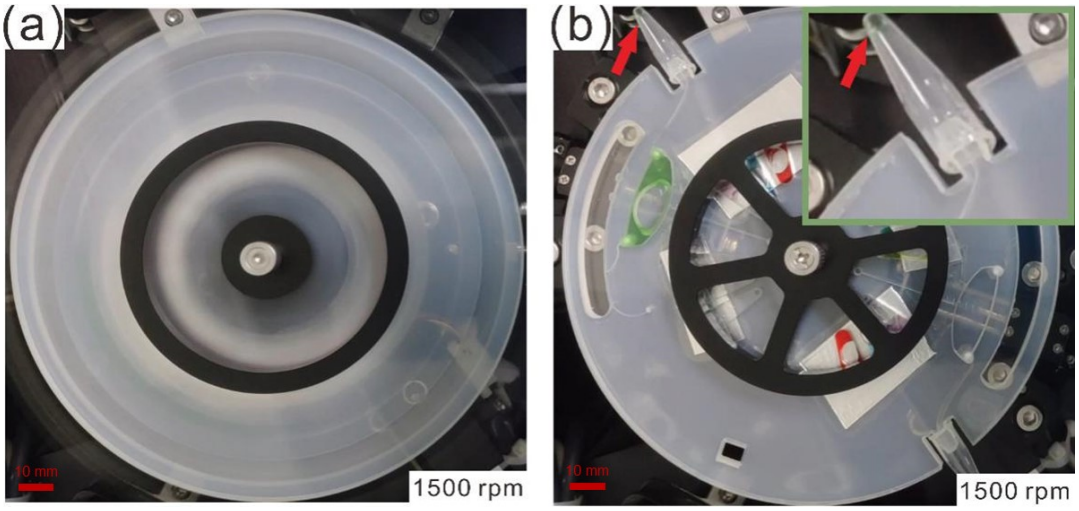

Figure S6: Performance evaluation of the reversible valve: (a) During centrifugation at 1500 rpm; (b) Physical image after centrifugation at 1500 rpm, liquid appears inside the centrifuge tube at the location indicated by the red arrow.

Figure S7

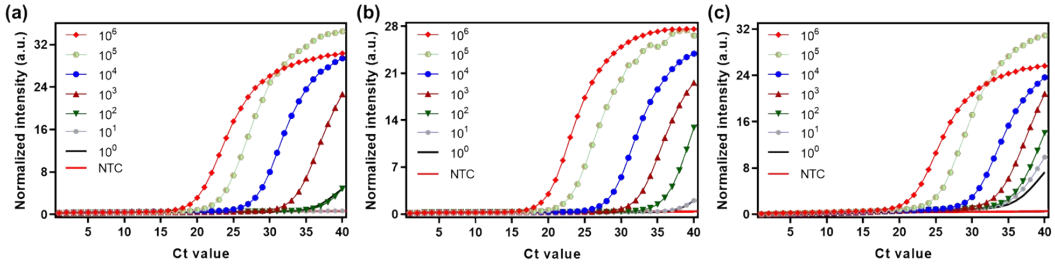

Figure S7: Concentration amplification plot of three target genes in the standard samples: (a) orf1ab gene; (b) N gene; (c) E gene.

Figure S8

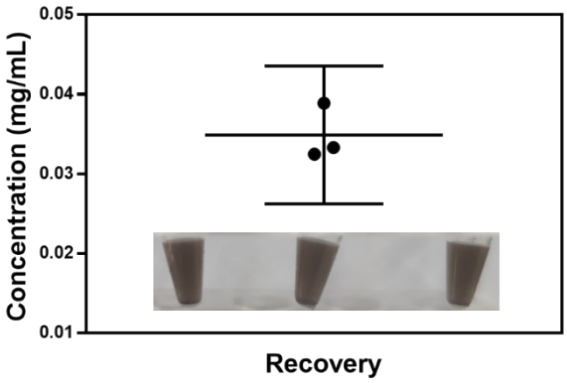

Figure S8: Evaluation of magnetic bead loss rate (from left to right: before extraction, after one round of magnetic actuation and removal of the supernatant, after two rounds of magnetic actuation and removal of the supernatant).

Table S1

Table S1 Detailed manual operational procedures

Step	Operation	Duration
1	Adding sample	1 min
2	adding the lysis buffer	1 min
3	Shock mixing the magnetic beads with the sample and reagents	2 min
4	Incubation at room temperature	10 min
5	The centrifugal tube is placed on the magnetic rack and draws the liquid	6 min
6	Adding wash 1 buffer and mixing	2 min
7	The centrifugal tube is placed on the magnetic rack and draws the liquid	6 min
8	Adding wash 2 buffer and mixing	2 min
9	The centrifugal tube is placed on the magnetic rack and draws the liquid	6 min
10	Adding wash 2 buffer and mixing	2 min
11	The centrifugal tube is placed on the magnetic rack and draws the liquid	6 min
12	Drying of magnetic beads	10 min
13	Adding elution buffer and mixing	3 min
14	Incubation at 65℃	5 min
15	The centrifugal tube is placed on the magnetic rack and draws elution buffer	6 min
16	Overall time	68 min

Table S2

Table S2 Target gene sequences and corresponding primer sequences

SARS-Cov-2 gene	Target gene sequences (5’-3’)	Primer sequences (5’-3’)
N	CCAAAAGGCTTCTACGCAGAAGGGAGCAGAGGC GGCAGTCAAGCCTCTTCTCGTTCCTCATCACGTA GTCGCAACAGTTCAAGAAATTCAACTCCAGGCA GCAGTAGGGGAACTTCTCCTGCTAGAATGGCTG GCAATGGCGGTGATGCTGCTCTTGCTTTGCTGCT	F: GGGGAACTTCTCCT GCTAGAAT; B:

	GCTTGACAGATTGAACCAGCTTGAGAGCAAAAT GTCTGGTAAAGGCCAACAACAACAAGGCCAAAC TGTCACTAAGAAATCTGCTGCTGAGGCTTCTAAG AAGCCTCGGCAAAAACGT ATCGTGTTGTCTGTACTGCCGTTGCCACATAGAT CATCCAAATCCTAAAGGATTTTGTGACTTAAAAG GTAAGTATGTACAAATACCTACAACTTGTGCTAA	CAGACATTTTGCTC TCAAGCTG
Orf1ab	TGACCCTGTGGGTTTTACACTTAAAAACACAGTC TGTACCGTCTGCGGTATGTGGAAAGGTTATGGCT GTAGTTGTGATCAACTCCGCGAACCCATGCTTCA GTCAGCTGATGCACAATCGTTTTTAAACGGGTTT GCGGTGTAAGTGCAGCCCGTCTTACACCGTGCGG CACAGGCACTAGTACTGATGTCGTATACAGGGCT TTTGACATCTACAATGATAAAGTAGCTGGTTTTG CTAAATTCCTAAAAACTAATTGTTGT	F: CCCTGTGGGTTTTA CACTTAA; B: ACGATTGTGCATCA GCTGA
E	ATGTACTCATTCGTTTCGGAAGAGACAGGTACGT TAATAGTTAATAGCGTACTTCTTTTTCTTGCTTTC GTGGTATTCTTGCTAGTTACACTAGCCATCCTTA CTGCGCTTCGATTGTGTGCGTACTGCTGCAATAT TGTTAACGTGAGTCTTGTAAAACCTTCTTTTTAC GTTTACTCTCGTGTTAAAAATCTGAATTCTTCTA GAGTTCCTGATCTTCTGGTCTAA	F: ACAGGTACGTTAAT AGTTAATAGCGT; B: ATATTGCAGCAGTA CGCACACA

Table S3

Table S3 Performance evaluation of functional trays

Modules	Puncture valve		Reversible valve		Mixture	
Conditions	Distance (mm)	Results	Speed (rpm)	Results	Angle (°)	Results
1	0.1	Unpierced	500	Storage	5	No
2	0.5	Unpierced	800	Storage	10	No
3	1	Unpierced	1000	Storage	20	No
4	1.5	Pierced	1300	Storage	30	Yes
5	2	Silicon rupture	1500	Leakage	40	Yes

Table S4

Table S4 Detailed operational procedures

Step	Operation	RV-1	RV-2	Rotation speed (rpm)	Duration
1	Adding sample	off	off	1000	10 s
2	Puncture the lysis reagent chamber and	off	off	1000	15 s

Step	Operation	RV-1	RV-2	Rotation speed (rpm)	Duration
	release the lysis buffer				
3	Puncture the binding reagent chamber and release the binding buffer	off	off	1000	15 s
4	Thoroughly mixing the magnetic beads with the sample and reagents	off	off	Mixing by spinning the centrifuge back and forth with a deflection angle of 30°	10 min
5	Capture of magnetic beads	off	off	0	15 s
6	Transfer waste solution to waste chamber	on	off	3000	10 s
7	De-magnetization	off	off	0	5 s
8	Puncture the wash 1 reagent chamber and release the wash 1 buffer	off	off	1000	15 s
9	Thoroughly mixing the magnetic beads with wash 1 buffer	off	off	Mixing by spinning the centrifuge back and forth with a deflection angle of 30°	15 s
10	Capture of magnetic beads	off	off	0	15 s
11	Transfer waste solution to waste chamber	on	off	3000	10 s
12	De-magnetization	off	off	0	5 s
13	Puncture the wash 2 reagent chamber and release the wash 2 buffer	off	off	1000	15 s
14	Thoroughly mixing the magnetic beads with wash 2 buffer	off	off	Mixing by spinning the centrifuge back and forth with a deflection angle of 30°	15 s
15	Capture of magnetic beads	off	off	0	15 s
16	Transfer waste solution to waste chamber	on	off	3000	10 s

Step	Operation	RV-1	RV-2	Rotation speed (rpm)	Duration
17	De-magnetization	off	off	0	5 s
18	Drying of magnetic beads	off	off	0	10 min
19	Puncture the elution reagent chamber and release the elution buffer	off	off	1000	15 s
20	Thoroughly mixing the magnetic beads with the elution buffer	off	off	Mixing by spinning the centrifuge back and forth with a deflection angle of 30°	5 min
21	Capture of magnetic beads	off	off	0	15 s
22	Transfer nucleic acid solution to tube	off	on	3000	10 s

Table S5

Table S5 Evaluation of nucleic acid enrichment efficiency for pseudo-virus

SARS-Cov-2 gene	Manual		Chip		Chip/Manual (%)
	CT values	Concentrations (copies/μL)	CT values	Concentrations (copies/μL)	
N	27.33	6760.83	24	41686.94	616.60
Orf1ab	26.33	7943.28	22.67	61659.50	776.25
E	26.66	3630.78	21.66	162181.01	4466.84