Electronic Supplementary Material (ESI) for Lab on a Chip.

Supplementary Material

Ultra-Rapid Real-Time Microfluidic RT- PCR Instrument for Nucleic Acid Analysis

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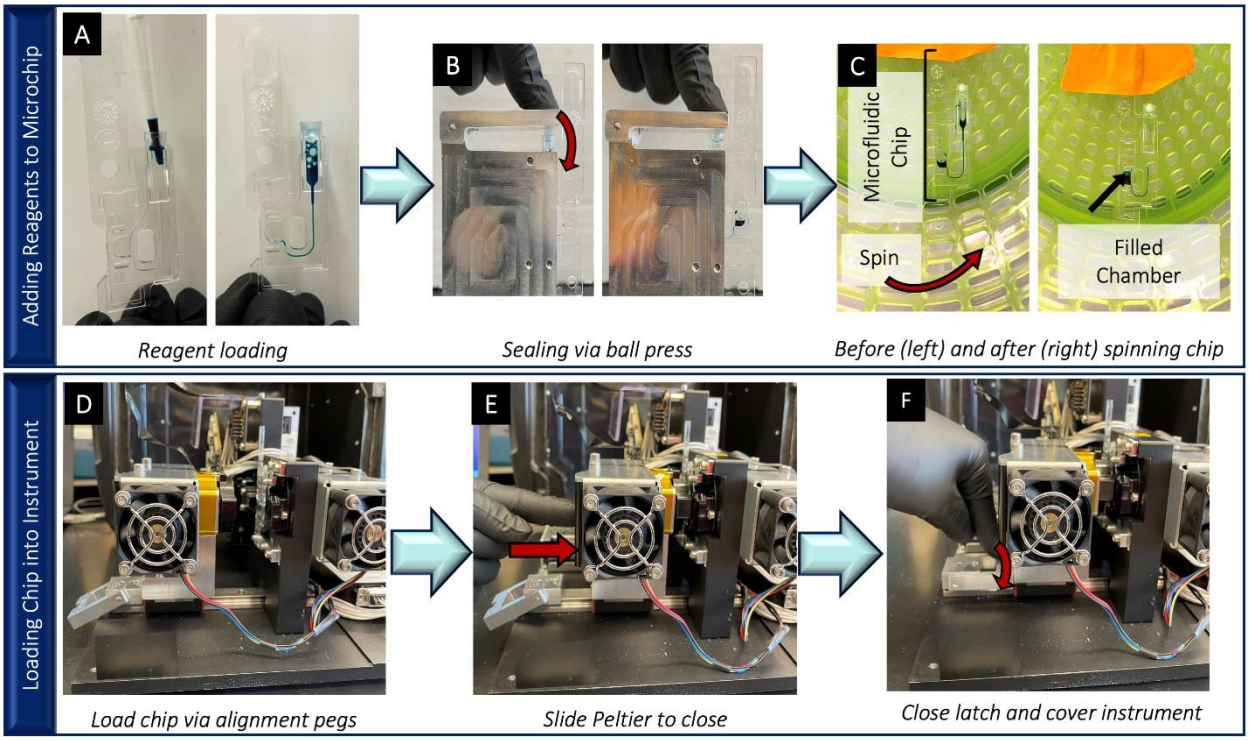

Figure S1. Workflow depicting loading reagents into the microfluidic chip and placing the prepared chip into the mechatronic system. Chip preparation includes **A**) pipetting in pre-mixed reagents (20 ul shown), **B**) sealing the chip with a polymeric ball via a ball press, and **C**) rotationally-driving fluid from the sample inlet to the amplification chamber using a salad spinner. Once ready, the microfluidic chip is **D**) placed along side the right Peltier following the alignment pegs, **E**) clamped between Peltiers following sliding of the left Peltier, and **F**) the Peltiers are locked in place as the latch is closed.

		Initial			PCR Cycles						Extension		
Experiment	Condition	Target Temp (°C)	Ramp Rate (°C/s)	Dwell (s)	Target Temp (°C)	Ramp Rate (°C/s)	Dwell (s)	Target Temp (°C)	Ramp Rate (°C/s)	Dwell (s)	Target Temp (°C)	Ramp Rate (°C/s)	Dwell (s)
Temperature optimization	Plus 1°C	45	8	30 s	96	8	30 s	61	8	30 s	38	8	0 s
	At Temp	45	8	30 s	95	8	30 s	60	8	30 s	38	8	0 s
	Minus 1°C	45	8	30 s	94	8	30 s	59	8	30 s	38	8	0 s
	Water	45	8	30 s	95	8	30 s	60	8	30 s	38	8	0 s
Thermal Cycling Evaluation	Fast Ramp Rates	45	8	1 s	95	12	1 s	60	10	1 s	38	8	0 s
	Standard Ramp Rates	45	8	1 s	95	4	1 s	60	4	1 s	38	8	0 s

Table S1. Amplification conditions input to the ultra-rapid system configuration file. Target temperature, ramp rate, and dwell time settings implemented for various experiments during optimization of the system temperature settings and assessment of the Peltiers. Shaded boxes indicate the conditions that were changed during an experiment: for temperature optimization, the input target temperature was changed while the temperature inside the microamplification chamber was measured; evaluation of the Peltiers was conducted by testing different ramp rates.

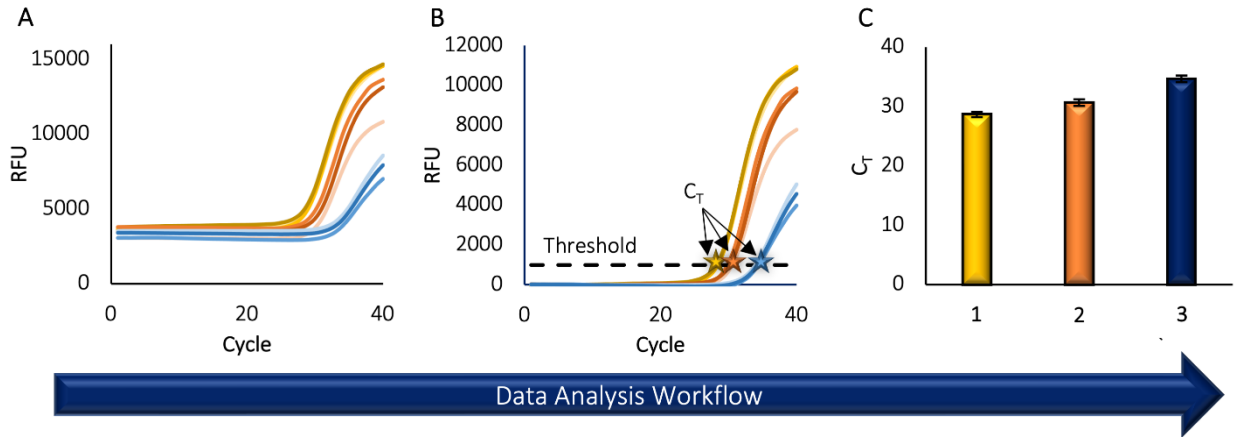

Figure S2. Illustration of the data analysis process on both the ultra-rapid and commercial instruments. **A**) Raw data from the ultra-rapid PCR system. **B**) Baseline-subtracted amplification plots generated by averaging the RFUs from cycles 3-15 and subtracting that average from all points on the corresponding amplification curve. **C**) Bar graph of C_T values generated based on where the amplification curves cross the threshold, which is set at 3 times the standard deviations of the grand mean baseline (i.e., the average of all averages calculated between cycles 3-15 on each amplification curve).

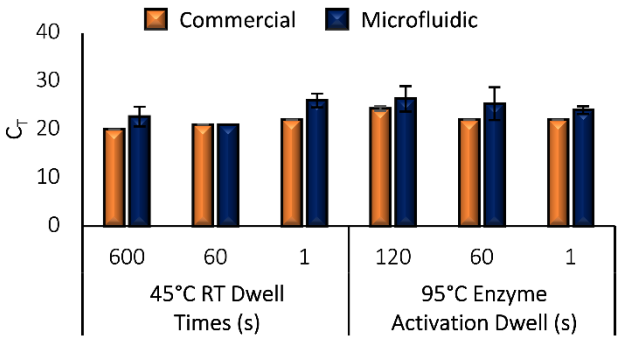

Figure S3. RNA amplification dwell time optimization. Manufacturer recommended times for the reverse transcription (RT) step and the initial 95 ^{o}C enzyme activation step were reduced and amplified on both the ultra-rapid system and the QuantStudio-5.