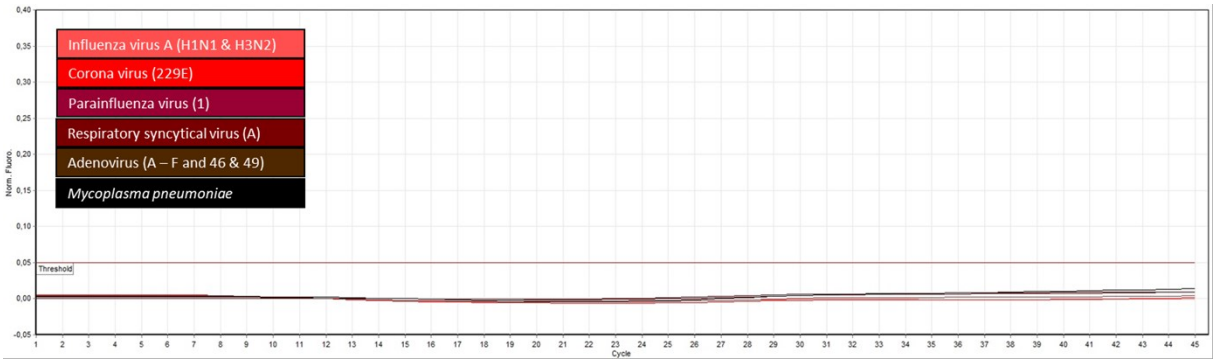
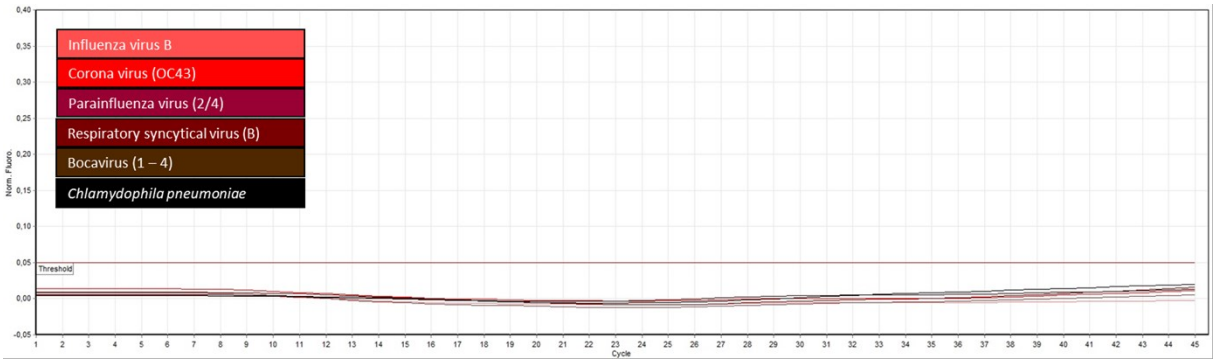
Electronic Supplementary Material (ESI) for Analyst.

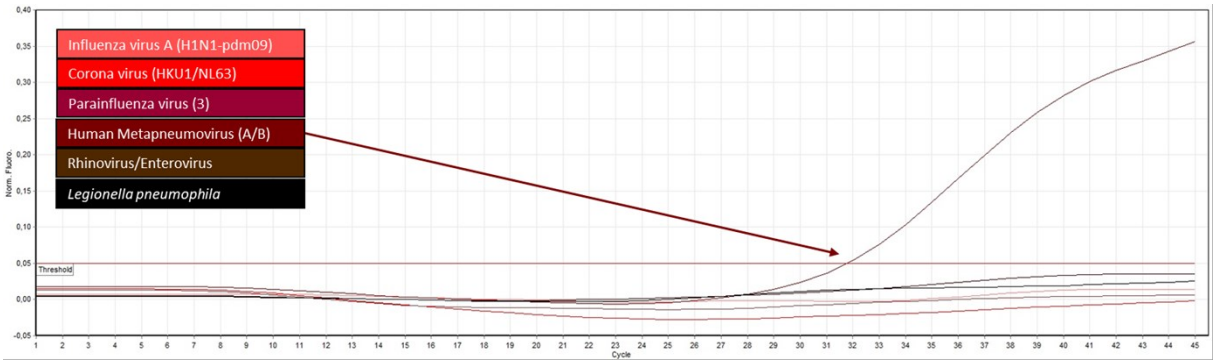
1. RT-PCR results in a single chamber



(a)



(b)



(c)

Figure S1: Standardised real-time PCR curves of all three fluorescence channels from a RespiDisk run analysing sample 10 of the RESPII17S panel (Human MPV Type A1). The figures show the fluorescence signal in the FAM (a), MAX (b) and TexasRed (c) channels for all six RealAccurate® Respiratory Quadruplex qPCR pathogen panels (see table 2 in manuscript). Threshold value was set to 0.05 std. RFUs for all runs. Data normalization and analysis was done using the RotorGeneQ software (QIAGEN, Hilden).

2. Protocol for processing of the RespiDisk

S1 Table. Microfluidic protocol as used to run the sample-to-answer LabDisk for detection of respiratory pathogens (RespiDisk).

Step[a]	#	Action description	Rotation frequency [Hz]	Rotation acceleration [Hz s^{-1}]	Temperature [°C]	Duration[b] [s]
Sample addition	0-1	Add 200 µL sample to the sample inlet using a pipette. Seal with tape[c]	0	N/A	N/A	N/A
	0-2	Spin sample into lysis chamber	20	10		0
Lysis	1-1	Thermal weakening of stick-pack seams			60	180
	1-2	Buffer release from stick-packs	70	10		20
	1-3	Set lysis temperature			22	0
	1-4	Air pressure equilibration in microfluidic network	30	10		30
	1-5	Lysis incubation loop[d]	1 s @ 20 Hz / 1 s @ 25 Hz	10		600
Lysate transfer [1]	2-1	Set rotation	12	10		0
	2-2	Activate overpressure valving			50	0
	2-3	Transfer lysate	25	10		60
Rehydrate magnetic beads	3-1	Bead rehydration	8	10		5
Binding step	4-1	Binding of NAs to beads loop	20 s @ 8 Hz / 1 s @ 15 Hz	10		600
	4-2	Transfer beads using bead	10	5		20

		transfer under rotation [2]	8	5		5
			7	5		5
			6	5		5
			5	5		90
			4	5		90
			3	5		60
Washing step I	5-1	Sediment beads	30	10		20
	5-2	Mixing loop	20 s @ 10 Hz/ 1 s @ 15 Hz	10		42
	5-3	Transfer beads using bead transfer under rotation Repeat step 4-2				
Washing step II	6-1	Sediment beads	30	10		0
	6-2	Mixing loop Repeat step 5-2				
	6-3	Transfer beads using bead transfer under rotation Repeat step 4-2				
Elution step	7-1	Sediment beads	30	10		20
	7-2	Rotation frequency reduction	13	5		10
	7-3	Set elution temperature			50	
	7-4	Elution loop	10 s @ 13 Hz/ 1 s @ 18 Hz	10		240
1st TCRe actuated valving [3]	8-1	Heating for valve actuation			60	10
	8-2	Sediment beads	20	5		2

	8-3	Set valving frequency	9	5		5
	8-4	Cooling down to activate valve			35	
	8-5	Load compression chamber	40	5		70
Centrifugo-pneumatic inward pumping [4]	9-1	Inward pumping	5	8		2
	9-2	Increase temperature to empty compression chamber			60	
	9-3	Air pressure equilibration in microfluidic network	25	5		5
TCR actuated mixing [5,6]	10-1	Cool down system for bubble mixing			40	15
	10-2	Rotation frequency reduction for bubble mixing	6	10		0
	10-3	Heating to initiate bubble mixing			60	
	10-4	Rotation frequency for cooling	25	5		15
	10-5	Loop: Repeat 4× steps 10-1 to 10-4				
2nd TCR actuated valving [3]	11-1	Set rotation frequency for valving	9	5		10
	11-2	Cool down for valving			40	
Aliquoting and transfer into reaction chambers [7]	12-1	Metering	12	0,5		1
	12-2	Metering	16	0,2		1
	12-3	Empty valve	30	5		5
	12-4	Transfer into reaction chambers	45	5		5
	12-5	Transfer into reaction	5	5		5

		chambers				
	12-6	Repeat steps 12-4 & 12-5 3x				
RT-PCR reaction & detection	13-1	Set rotation frequency	25	5		5
	13-2	RT reaction			50	600
	13-3	Set PCR denaturation temperature			95	10
	13-4	Set PCR annealing and extension temperature			60	30
	13-5	Sequential (chamber 1 → 6) detection in FAM, MAX, TexasRed and TYE665 channels				
	13-6	PCR reaction loop: Repeat steps 13-3 to 13-5 45x				

[a]: If no value is stated for a parameter, it remains constant as stated before.

[b]: "Duration" refers to the time a set of parameters is kept constant, before the next protocol step is executed. The time starts, when the given parameters (frequency, acceleration, temperature) are reached.

[c]: Diagnostic tape # 9795R, 3M, USA.

[d]: During loops, the described operations are repeated until the given duration is reached.

[e]: Temperature change rate.

N/A: Parameter not applicable and/or not controlled by the device.

References

[1] Abi-Samra K et al., *Microfluidics and Nanofluidics*, 2011, **11**, 643–652

[2] Hin S et al., *20th International Conference on Solid-State Sensors, Actuators and Microsystems & Eurosensors XXXIII*, 2019, 130–133. (Full paper in preparation).

[3] Keller M et al., *Lab Chip*, 2017, **17**, 864-875.

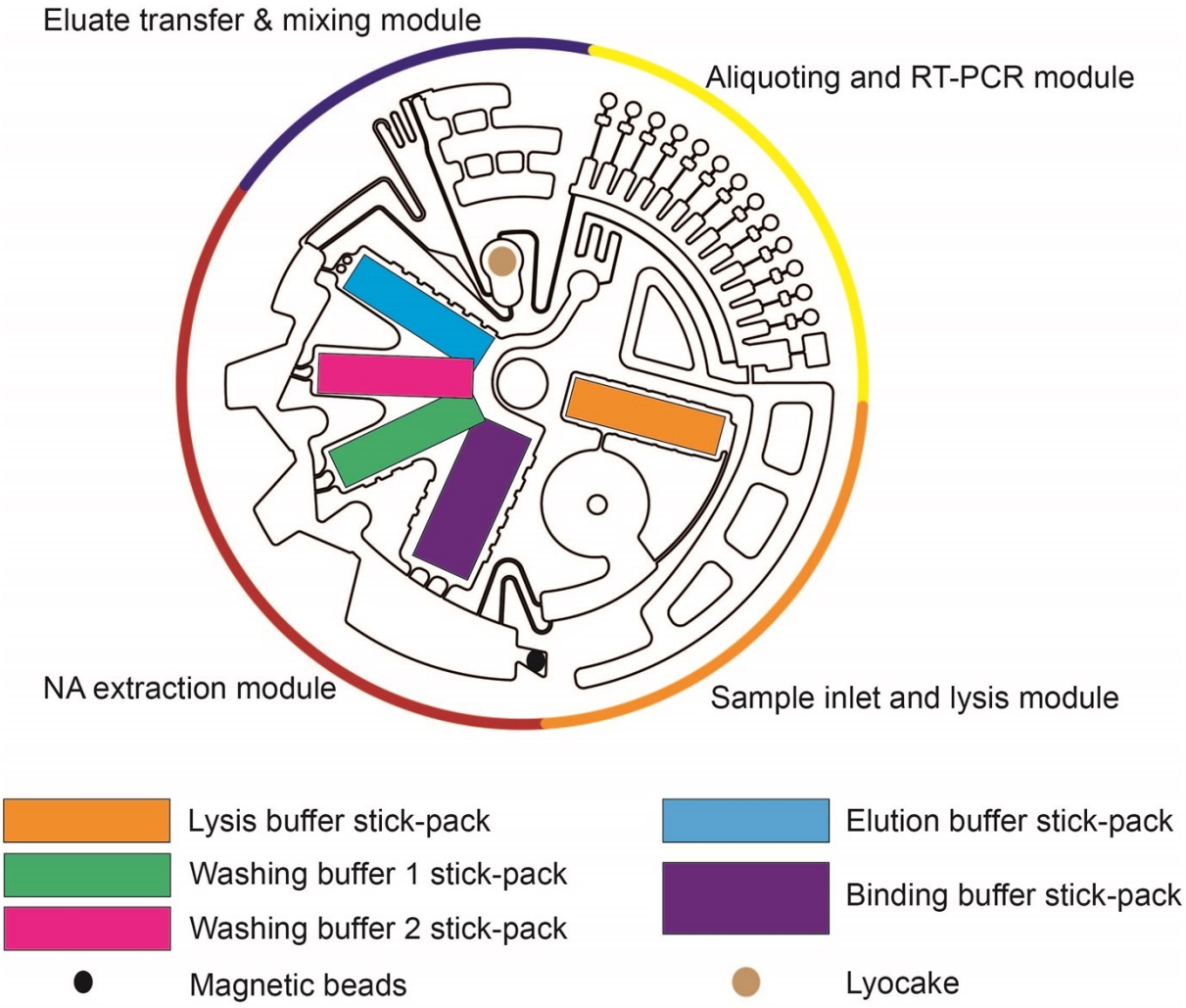
[4] Zehnle S et al., *LabChip*, 2012, **12**, 5142 – 5145

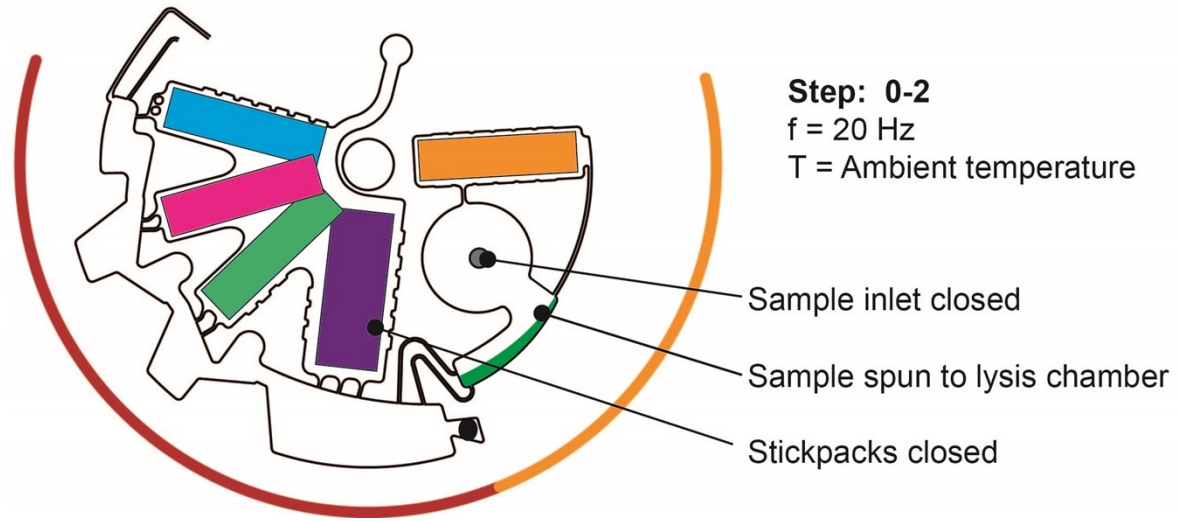
[5] Burger S et al., *Lab Chip*, 2016, **16**, 261 – 268

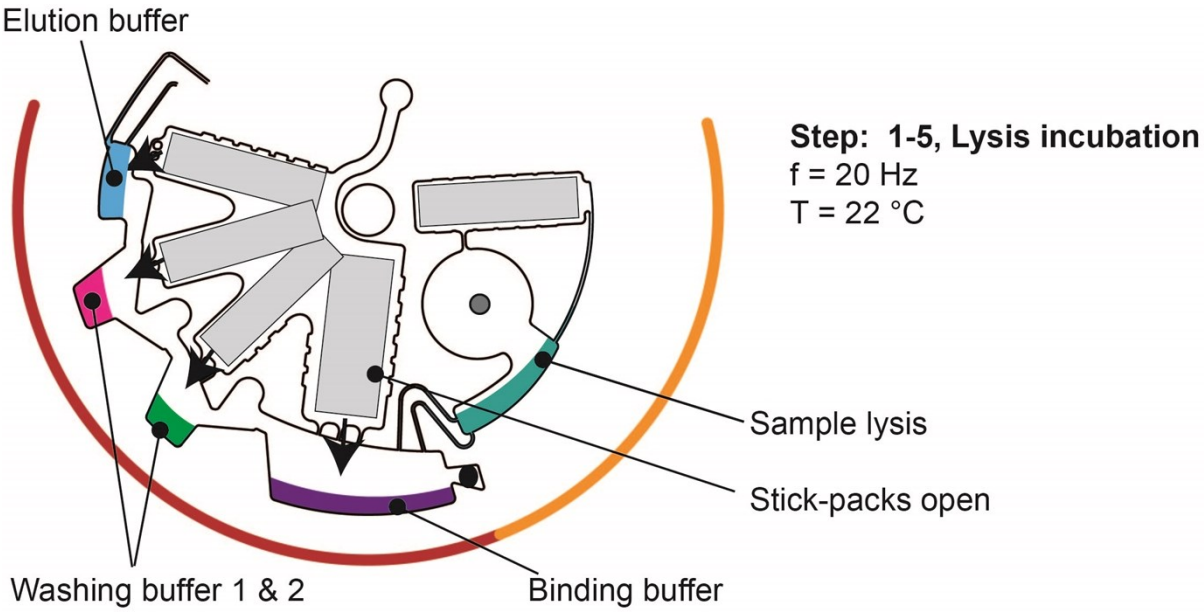
[6] Hin S et al., *Lab Chip*, 2018, **18**, 362-370.

[7] Mark D et al., *Microfluidics and Nanofluidics*, 2011, **10**, 1279-1288.

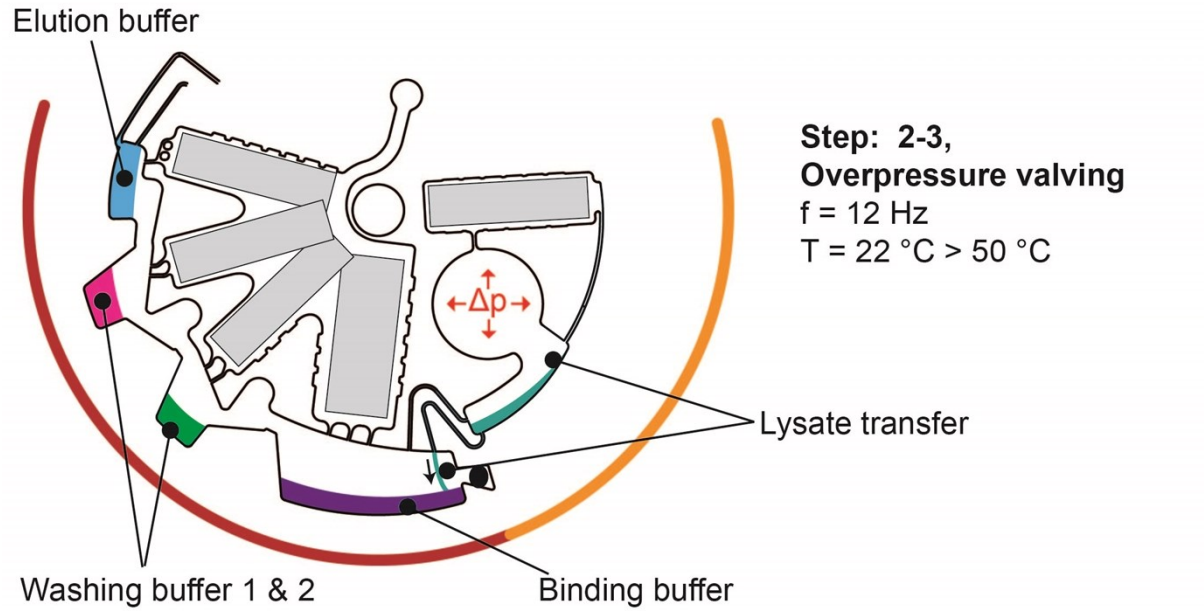
3. Illustration of liquid flow through RespiDisk



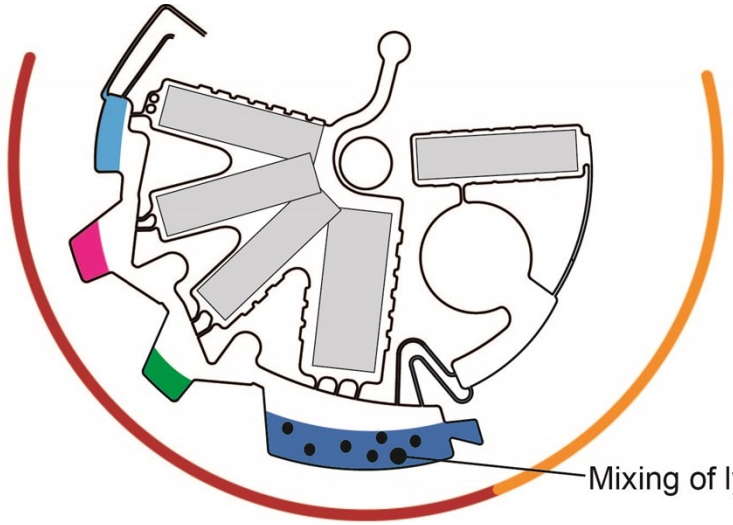




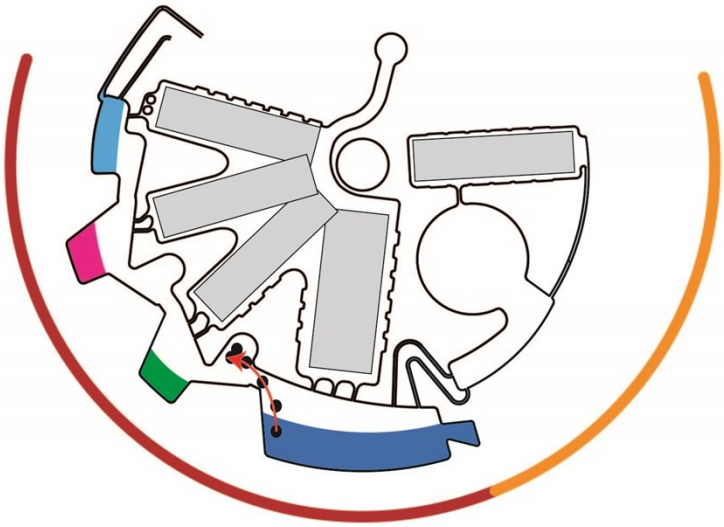
Step: 1-5, Lysis incubation
f = 20 Hz
T = 22 °C



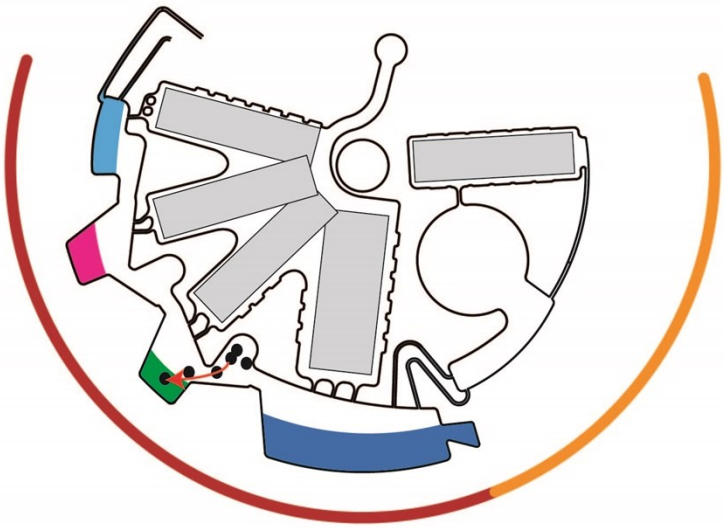
Step: 2-3,
Overpressure valving
f = 12 Hz
T = 22 °C > 50 °C



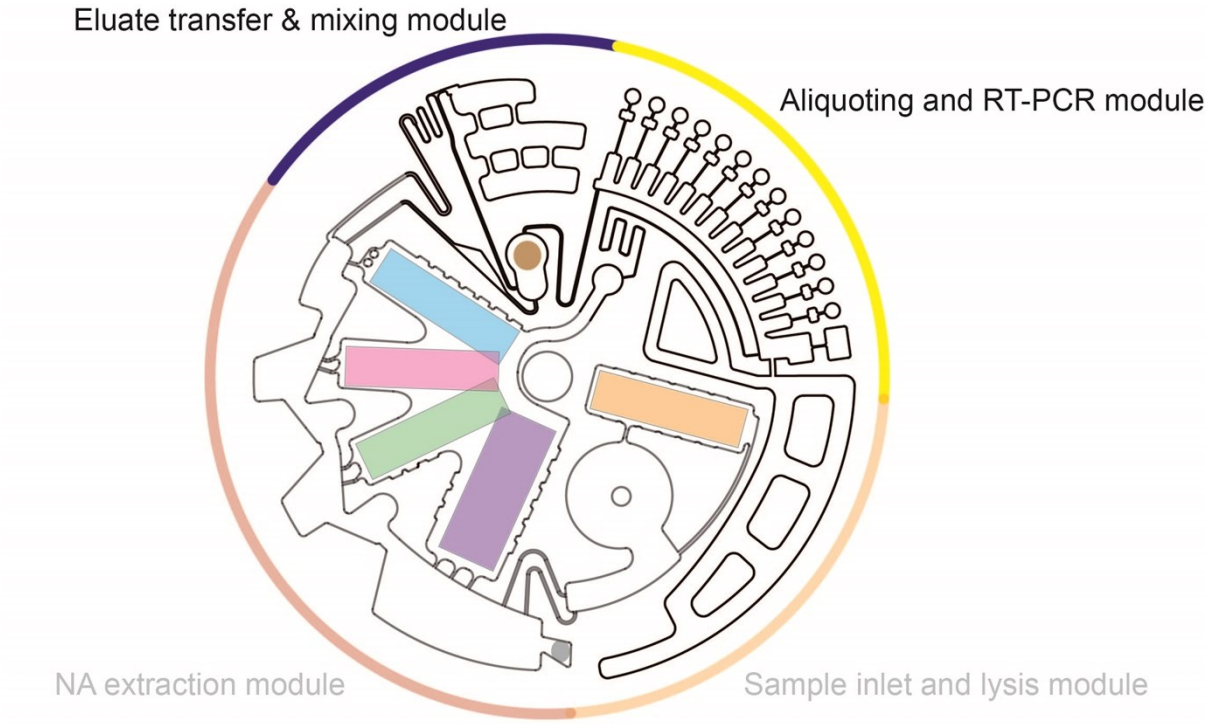
Step: 4-1, Binding step
f = 8-15 Hz
T = 22 °C



Step: 4-2, Transfer beads
f = 10 Hz ... 3 Hz
T = 22 °C



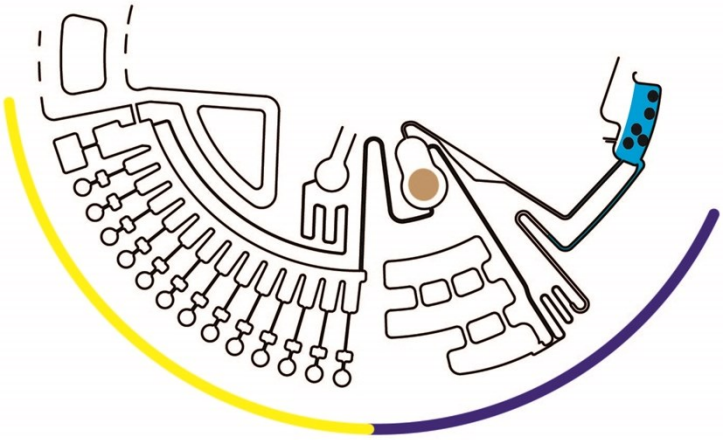
Step: 5-1, Sediment beads
f = 30 Hz
T = 22 °C



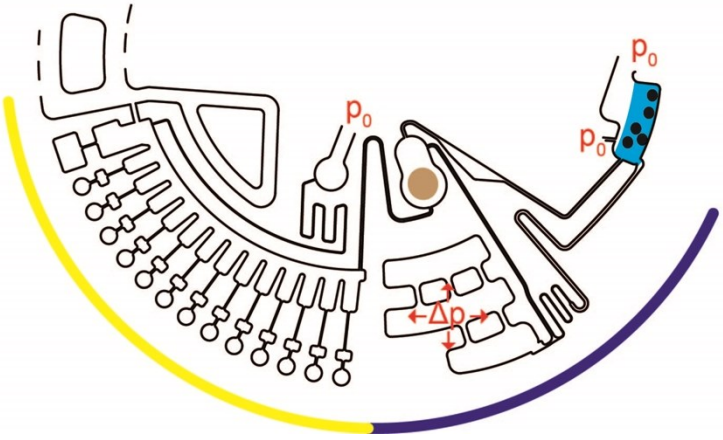
...

2 x bead transfer

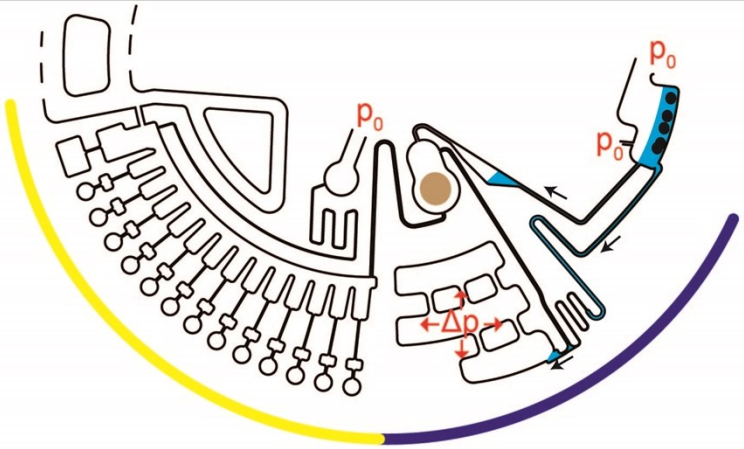
...



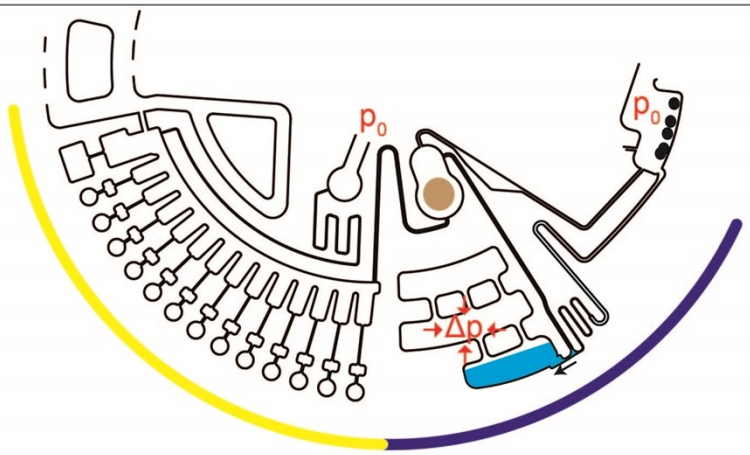
Step: 7-4, Elution
f = 13 Hz ... 18 Hz
T = 60 °C



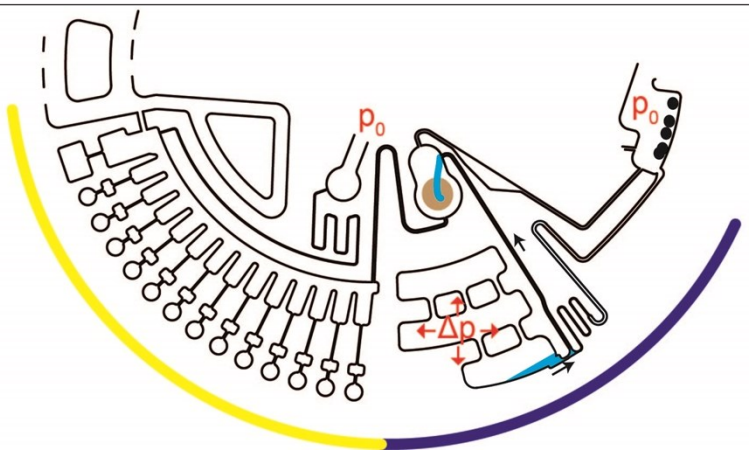
Step: 8-1,
TCR actuated valving: Heat
f = 20 Hz
T = 22 °C > 60 °C



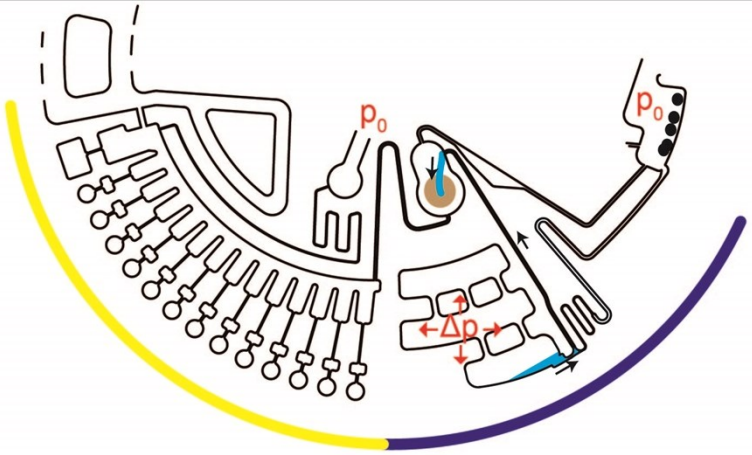
Step: 8-1,
TCR actuated valving: Cool
f = 20 Hz > 9 Hz
T = 60 °C > 35 °C



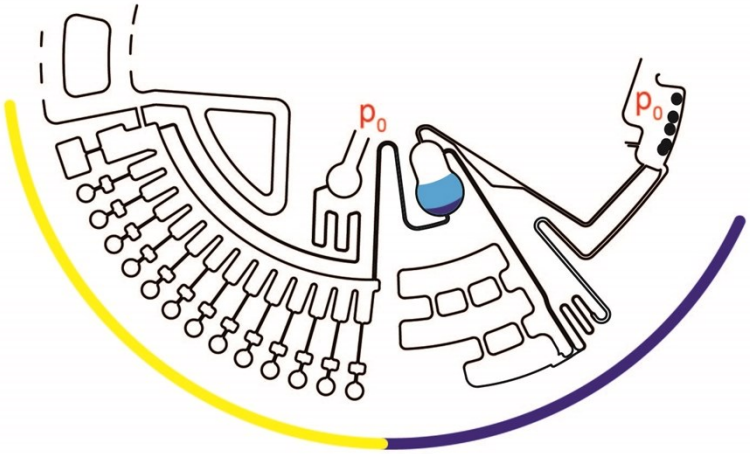
Step: 8-5,
Load compression chamber
f = 40 Hz
T = 35 °C



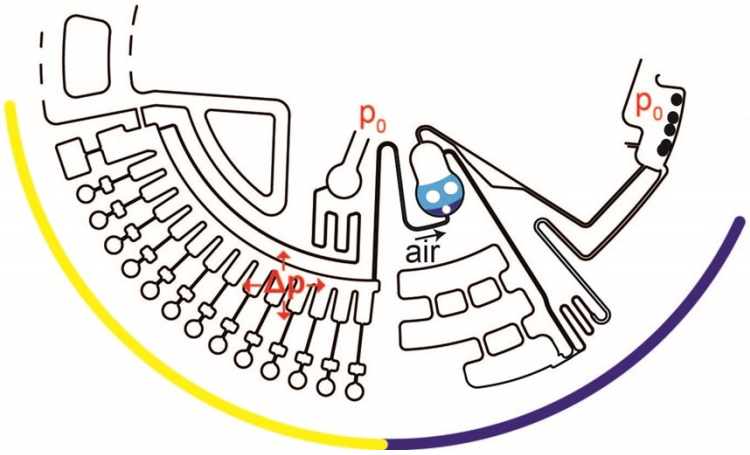
Step: 8-5,
Inward pumping
f = 5 Hz
T = 35 °C



Step: 8-5,
Inward pumping
f = 5 Hz
T = 35 °C



Step: 9-3,
Lyocake rehydrated, unmixed
f = 5 Hz
T = 35 °C



Step: 10-3,
TCR actuated mixing
f = 6 Hz
T = 60 °C

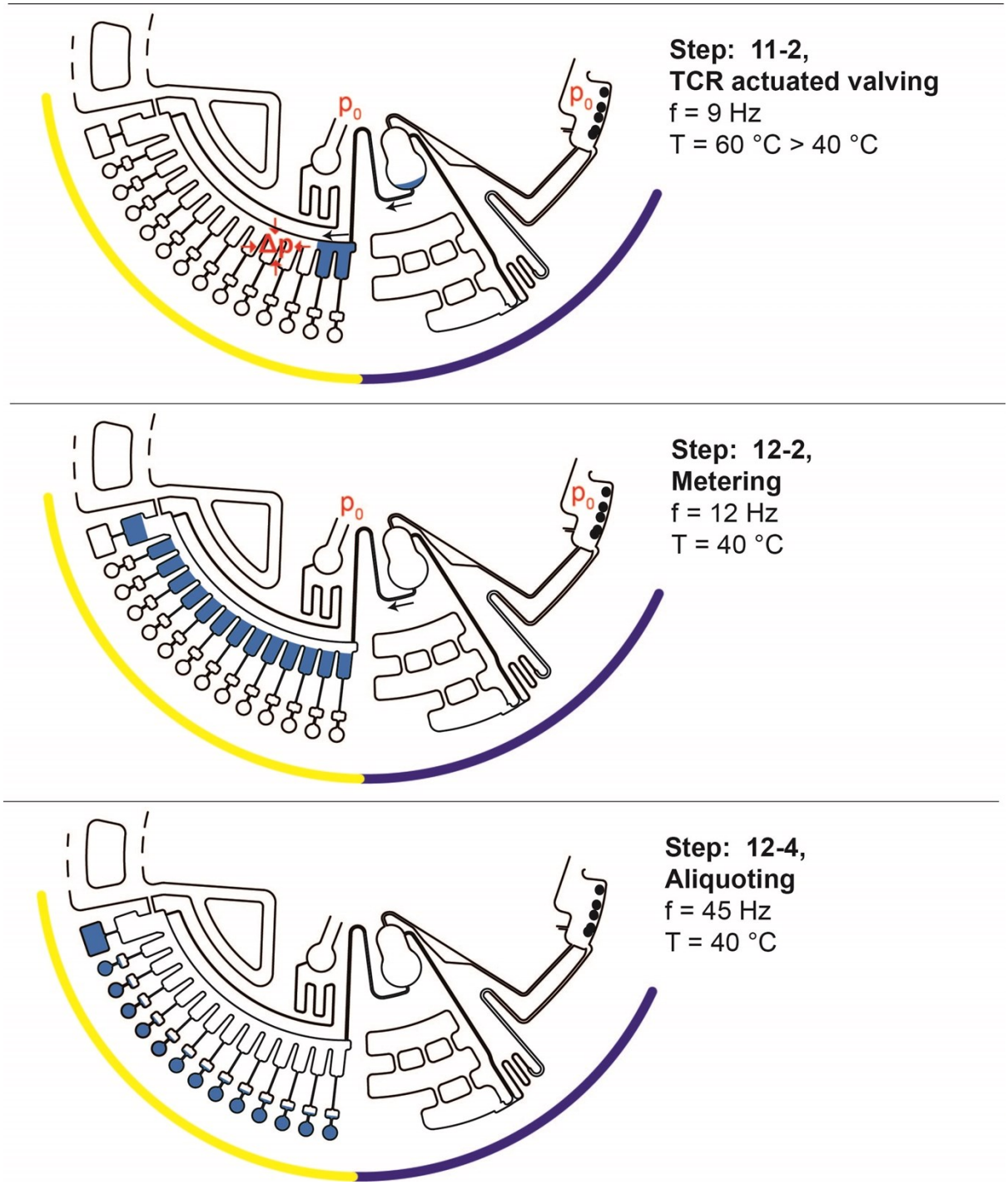


Figure S2: Illustration of the liquid flow through the RespiDisk.

The figures show all steps of the fluidic processing from the opening of the stick-packs through to the aliquoted RT-PCR reaction mix with step references to the protocol shown in table S1. The failure rate of the system was at 30 % due to fabrication issues, as the fabrication of the disks was done using prototyping processes, where variations in quality are always present. If a disk failed, the run was repeated. This rate can be reduced when transferring processes to a production line.