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

Supplementary Information for:

LabDisk with complete reagent prestorage for sample-to-answer nucleic acid based detection of respiratory pathogens verified with Influenza A H3N2 virus

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Content:

1) RealAccurate Quadruplex Respiratory qPCR panels

Table S1 Targets of the seven prestored respiratory pathogen panels. Primers and fluorescence probe for the interal control only was prestored in reaction cavity 1.

Respiratory pathogen panel	Prestored in reaction cavity #	Targets
PF0960-R RealAccurate Quadruplex Influenza	2	Influenza A virus
		Influenza B virus
		Influenza A H1N1
		Internal Control
PF0961-R RealAccurate Quadruplex Corona	3	Coronavirus 229E
		Coronavirus OC43
		Coronavirus NL63/HKU1
		Internal Control
PF0962-R RealAccurate Quadruplex Parainfluenza	4	Parainfluenza virus 1
		Parainfluenza virus 2
		Parainfluenza virus 3
		Parainfluenza virus 4
PF0963-R RealAccurate Quadruplex RSV/hMPV	5	Respiratory Syncytial Virus A
		Respiratory Syncytial Virus B
		Human Metapneumovirus A+B
		Internal Control
PF0964-R RealAccurate Quadruplex Rhinovirus	6	Rhinovirus/Enterovirus
		Internal Control
PF0965-R RealAccurate Quadruplex Adeno/Boca	7	Adenovirus
		Bocavirus
		Internal Control
PF0966-R RealAccurate Quadruplex Atypical Bacteria	8	*Mycoplasma pneumoniae*
		Chlamydophila pneumononiae
		Legionella pneumophila
		Bordetella pertussis

2) Rotational frequency protocol - Supplemental Table S2

For fully automated sample-to-answer detection of respiratory diseases in the LabDisk player all liquid handling steps and biochemical reactions are automatically processed by applying the specific rotational processing protocol.

Table S2 Applied rotational frequency protocol

#	Rotation	Time	Notes	Action
1	10 Hz	30 sec	Centrifugal force acts upon sample	The Sample is transferred from the inlet chamber to the lysis/- binding chamber and rehydrates the prestored magnetic beads
2	55 Hz	60 sec	Centrifugal force induces burst pressure on 4 out of 5 stick-pack prestored liquid reagents	4 of the 5 nucleic acid extraction buffers (lysis, washing 1, washing 2 and elution) are transferred from stick-packs to their corresponding chambers of the nucleic acid extraction structure
3	2 Hz and 14 Hz		Alternation between 2 Hz and 14 Hz for 10 min with acceleration and deceleration rates of 7 Hz/s and 3 Hz/s	Repetitive acceleration and deceleration result in constant mixing during lysis procedure
4	80 Hz	60 sec	Centrifugal force induces burst pressure of the stick-pack containing the binding buffer	Binding buffer is transferred to the lysate
5	0 Hz – 10 Hz	300 sec	Alternation between 4 sec stops under stationary magnet to collect beads on one end of binding chamber (magnetic force dominates). At subsequent rotation of 10 Hz for 3 sec centrifugal force dominates and induces bead mixing.	5 minutes binding procedure facilitated by magnet induced bead mixing comprising repetitive stops, acceleration and deceleration
6	0	60 sec	GTM-mediated[23] transport of magnetic beads	Transfer of the magnetic beads from the lysis-/binding chamber to the washing chamber 1
7	2 Hz and 14 Hz		Alternation between 2 Hz and 14 Hz for 3 min with acceleration and deceleration rates of 7 Hz/s and 3 Hz/s	Repetitive acceleration and deceleration result in constant mixing of the magnetic beads in the washing buffer 1
8	0	60 sec	GTM-mediated[23] transport of magnetic beads	Transfer of the magnetic beads from the washing chamber 1 to the washing chamber 2
9	2 Hz and 14 Hz		Alternation between 2 Hz and 14 Hz for 3 min with acceleration and deceleration rates of 7 Hz/s and 3 Hz/s	Repetitive acceleration and deceleration result in constant mixing of the magnetic beads in the washing buffer 2
10	0	60 sec	GTM-mediated[23] transport of magnetic beads	Transfer of the magnetic beads from the washing chamber 2 to the elution chamber
11	0	1 sec	Heat up and hold temperature at 50 °C	Nucleic acid release is done at 50 °C
12	2 Hz and 14 Hz		Alternation between 2 Hz and 14 Hz for 5 min with acceleration and deceleration rates of 7 Hz/s and 3 Hz/s	Mixing of beads in elution chamber. The nucleic acid is released into the liquid phase
13	0	60 sec	GTM-mediated[23] transport of magnetic beads	Transfer of the magnetic beads from the elution chamber to the washing chamber 2 so that magnetic beads don't affect following microfluidic procedure
14	0	1 sec	Cool down to room temperature	Cool down after nucleic acid release is finished
15	80 Hz – 0 Hz	60 sec	Process is described in detail in our previous work[52]	Pumping of nucleic acid containing elution buffer to the collection chamber by centrifugo-dynamic inward pumping
16	10 Hz	40 sec	Centrifugal force acts upon elution buffer	Elution buffer is transferred to aliquoting structure and separated into 8 subvolumes of 10 µl each
17	20 Hz	30 sec	Centrifugal force acts upon elution buffer	Aliquots of 10 µl are transferred into the eight reaction cavities inducing rehydration of the therein prestored lyophilisates and primer and fluorescence probes.
24	6 Hz	8100 sec	Thermocycling starts under rotation. Reverse transcription: 50 °C for 1800 sec Initial denaturation: 95 °C for 300 sec 45 thermocycles: Denaturation: 94 °C for 5 sec Annealing and elongation: 55 °C for 40 sec	Real-time RT-PCR amplification with optical detection of fluorescence signal in the reaction cavities.