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

Supplementary Material (ESI)

Rapid molecular infectious disease diagnostics on a fully automated centrifugal-microfluidic LabDisk system using highly sensitive nested PCR with integrated sample preparation

SUPPLEMENTARY INFORMATION

Content

1) Oligonucleotide sequences - Supplemental Table S1 and Table S2

Table S1 – Primer sequences used for consensus multiplex PCR pre-amplification

Oligonucleotide	Type	Gene locus	Nucleotide Sequence 5' to 3'	T_M [°C] *
Consensus primer Gram -	Forward primer	Subunits B of the gyrase gene *(gyrB)*	GGT ATG TAT ATC GGC GA	50.0
Consensus primer Gram -	Reverse primer	Subunits B of the gyrase gene *(gyrB)*	ACT TTA TAG GAG TTA TCi TC	50.0
Consensus primer Gram +	Forward primer	Subunits B of the gyrase gene *(gyrB)*	GTA TGG GAA ATT GTi GAT AA	50.0
Consensus primer Gram +	Reverse primer	Subunits B of the gyrase gene *(gyrB)*	CCi CCA CCG AAT TTA C	48.0

** Melting Temperature T_M calculated using the suppliers (Metabion) webtool: http://www.metabion.com/support/biocalc.php*

Table S2 – Primer and hydrolysis probe sequences for real-time PCR

Oligonucleotide	Type	Gene locus	Nucleotide Sequence 5' to 3'	TM [°C] *
(*Staphylococcus warneri*-specific amplification)	Forward primer	Subunits B of the gyrase gene *(gyrB)*	GCT ATG CTG AYG AAA TTA ACG TAA CA	62.0
	Reverse primer	Subunits B of the gyrase gene *(gyrB)*	CAA CTG CTG GAC GTC CCA T	60.0
	Hydrolysis Probe	Subunits B of the gyrase gene *(gyrB)*	6FAM-TAC TGA CAA TGG TCG TGG TAT CCC AGT AGA TA-*BBQ1*	71.0
(*Haemophilus influenzae*-specific amplification)	Forward primer	Subunits B of the gyrase gene *(gyrB)*	TAT TTG AAG TGG TGG ATA ATG CCA TT	62.0
	Reverse primer	Subunits B of the gyrase gene *(gyrB)*	TGC AGA AAC GCC TTC TTC AGG A	62.0
	Hydrolysis Probe	Subunits B of the gyrase gene *(gyrB)*	6FAM-AAG CCC TCG CTG GCC ATT G-BBQ1	62.0
(*Escherichia coli*-specific amplification)	Forward primer	Subunits B of the gyrase gene *(gyrB)*	CGG GTC ACT GTA AAG AAA TTA TCG T	63.0
	Reverse primer	Subunits B of the gyrase gene *(gyrB)*	CCG ATA CGC CCT CTT CCG	61.0
	Hydrolysis Probe	Subunits B of the gyrase gene *(gyrB)*	6FAM-TAC AGG ATG AYG GGC GCG GCA TTC-BBQ1	69.0
(*Streptococcus agalactiae*-specific amplification)	Forward primer	Subunits B of the gyrase gene *(gyrB)*	CAG GAC ATA TTA AAG TTT ACA TTG AAC	61.0
	Reverse primer	Subunits B of the gyrase gene *(gyrB)*	GTC TCT ACA GCT GGT CTT C	57.0
	Hydrolysis Probe	Subunits B of the gyrase gene *(gyrB)*	6FAM-ACA GTT GTT GAT GAT GGT CGC GG-BBQ1	65.0

** Melting Temperature T_M calculated using the suppliers (Metabion) webtool: http://www.metabion.com/support/biocalc.php*

2) Rotational frequency protocol - Supplemental Table S3

For full automation of the assay on the LabDisk player all liquid handling steps and biochemical reactions are automatically conducted by application of the specific rotational processing protocol.

Table S3 – Primer and hydrolysis probe sequences for real-time PCR

#	Rotation [Hz]	Time [s]	Notes	Action
1	15	5	Centrifugal force acts upon liquids	The DNA extraction buffers are transferred from the inlet chambers to the lysis/- binding chamber, washing chamber HS, washing chamber LS and elution chambers, respectively.
2	7 Hz and 15 Hz		Alternation between step 2-3 for 15 min with acceleration and deceleration rate of 15 Hz/s.	Repetitive acceleration and deceleration result in constant mixing of the lysate solution.
3	0	30	Defined position of LabDisk	Capillary siphon of binding buffer is activated
4	15	30	Centrifugal force acts upon liquids	Binding buffer is released to the lysis-/binding chamber
5	5 Hz and 12 Hz	300	Alternation between step 5 Hz and 12 Hz for 5 min with acceleration and deceleration rate of 15 Hz/s. At 5 Hz, the magnetic force dominates upon the magnetic beads. At 12 Hz, the centrifugal force dominates upon the magnetic beads.	Repetitive acceleration and deceleration result in constant mixing of the solution with magnetic beads. At 12 Hz magnetic beads sediment, at 5 Hz magnetic beads move radially inwards in the solution again.
6	0	60	GTM-mediated transport of magnetic beads	Transport of magnetic particles from lysis-/binding chamber into washing chamber HS
7	7 Hz and 15 Hz	60	Alteration between step 7 Hz and 15 Hz (acceleration 15 Hz/s) repeated for 1 min.	Mixing of beads in washing buffer HS
8	0	60	GTM-mediated transport of magnetic beads	Transport of magnetic particles from washing chamber HS into washing chamber LS
9	7 Hz and 15 Hz	60	Alteration between step 7 Hz and 15 Hz (acceleration 15 Hz/s) repeated for 1 min.	Mixing of beads in washing buffer HS
10	0	50	Bead-Transport (GTM)	Transport of magnetic particles from washing chamber LS into elution chamber
11	7 Hz and 15 Hz	300	Alteration between step 7 Hz and 15 Hz (acceleration 15 Hz/s) repeated for 5 min.	Mixing of beads in elution chamber. The DNA is released into the liquid phase.
12	15	30	-	Sedimentation of magnetic beads in elution chamber
13	30	20	-	Transfer of eluate to preamplification chamber
14	30	2400	Thermocycling starts under rotation. Polymerase activation: 95°C for 120s; 12 thermocycles: Denaturation: 95°C for 20 s Annealing: 52°C for 60 s Elongation: 70°C for 30 s	PCR preamplification. In parallel, release of RNAse free water to mixing chamber from stickpack.
19	7	4	-	Pumping of preamplification product to mixing chamber by centrifugo-dynamic inward pumping.
20	15 Hz and 25 Hz	60	Alteration between step 15 Hz and 25 Hz (acceleration 15 Hz/s) for 1 min.	Mixing of preamplification product with buffer for Realtime PCR in mixing chamber
21	3 Hz	15	-	Activation of mixing chamber siphon
22	18 Hz	10	The mixture is separated into 13 subvolumes of 20 µL each.	Metering of real-time PCR reaction volumes
23	19 Hz	10		
24	20 Hz	10		
25	25 Hz and 45 Hz	20	Alteration between step 25 Hz and 45 Hz (acceleration 15 Hz/s) for 20 s	Transfer of reaction volumes into real-time PCR cavities
24	7 Hz	9000	Thermocycling starts under rotation. Denaturation: 95°C for 20 s Annealing: 60°C for 60 s Elongation: 69°C for 60 s	Real-time PCR amplification with optical detection of fluorescence signal in real-time PCR cavities.

3) LabDisk Player – Prototype centrifugal point-of-care analyzer

Processing of the LDAA was performed in the prototype "LabDisk Player" (QIAGEN Lake Constance, Stockach, Germany), depicted in Figure 2c. The portable analyser with a size of approximately 18 x 28 x 15 cm^3 and a weight of ~ 2 kg features four channel fluorescence detection (e.g. FAM, ROX, Cy5 etc.), PCR-thermocycling and the possibility to run predefined protocols with high flexibility on defined rotational frequencies (0 Hz – 90 Hz), accelerations (0.1 Hz s^{-1} – 50 Hz s^{-1}) and a precise positioning accuracy (< 0.1°). Stationary neodymium iron boron (NdFeB) magnets (#S-06-06-N and #S-03-01-N, Supermagnete.de, Germany) were integrated in the device for automated transportation of super-paramagnetic beads in the DNA extraction module of the LabDisk using GTM. The LabDisk is mounted on a rotational axis within a processing chamber. The temperature of the processing chamber can be controlled with a heating and cooling mechanism that is based on convection of hot air and ambient air, respectively. Heating of circulating air is realized with heating wires whereas ambient air is used for cooling. The temperature is controlled with a rotary valve that regulates influx of ambient air and release of internally heated air.

4) Supplemental video: Processing of LLDA on the LabDisk player

The video demonstrates the complete automated workflow of the LLDA in the LabDisk player. To visualize the liquid-flow in the rotating LabDisk, the LabDisk player was equipped with a customized stroboscopic image acquisition system (Biofluidix GmbH, Germany).

5) False positive signal generation during development phase for *Escherichia coli (E.coli)*

During the development phase false positive signal generation (see Figure S1) occurred for control serum samples. Within this phase a different batch of the dry PCR beads were used compared to the results presented in the manuscript. The likely cause of the false positive signals are *E.coli* DNA contamination of the PCR beads, which is a *Taq*-polymerase produced by an *E. coli* strain.

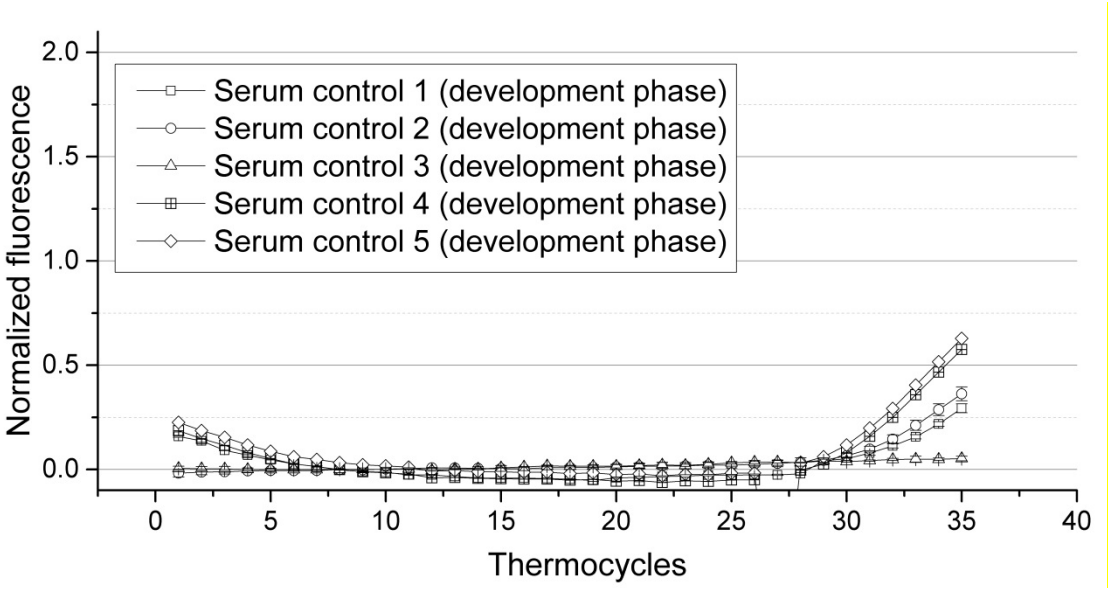

Figure S1 – False positive signals in control serum samples during the development phase