Electronic Supplementary Material (ESI) for RSC Advances.

Magnetic chemiluminescent immunoassay for human C-reactive protein on the centrifugal microfluidics platform

G.Czilwik[1], S.K.Vashist[1,2], V.Klein[1], G.Roth[3,4], A.Buderer[1], F. von Stetten[1,2], R. Zengerle[1,2,3] and D.Mark[1]*

[1] Hahn-Schickard, Georges-Koehler-Allee 103, 79110 Freiburg, Germany

[2] Laboratory for MEMS Applications, IMTEK - Department of Microsystems Engineering, University of Freiburg, Georges-Koehler-Allee 103, 79110 Freiburg, Germany

[3] BIOSS – Center for Biological Signalling Studies, University of Freiburg, 79110 Freiburg, Germany.

[4] Laboratory for Microarray Copying, Centre for Biological Systems Analysis (ZBSA), University of Freiburg, 79104 Freiburg, Germany

*Corresponding author's e-mail: gregor.czilwik@hsg-imit.de; Tel.: +49 761 2037231; Fax: +49 761 20373299

Table S1 – Frequency protocol being used for the automated LabDisk-based hCRP MCIA

Step	Rotation [Hz]	Time [s]	Notes	Action
1	20	15	Centrifugal force acts upon the liquids	All reagents are transferred from the inlet chambers to the reaction, washing and detection chambers.
2	20	10	**Immunoreaction (Step 2-4)**	Shake mode mixing of liquids and dynabeads by acceleration and deceleration which induce inertial forces
3	10	10		
4	0	15	Defined position of LabDisk with reference to magnet 2. Alteration between steps 2-4 repeated 25 times	Dynabeads are collected by magnet 2 to circumvent sedimentation during 15 min of incubation.
5	0	15	Defined position of LabDisk with reference to magnet 2	Dynabeads are collected in immunoreaction chamber
6	0	15	Defined position of LabDisk with reference to magnet 1	Dynabeads are moved out of the immunoreaction chamber into the air split
7	0	1	Incremental rotation of 0.5°C repeated 25 times	Dynabeads are moved from immunoreaction chamber to the first washing chamber through the air gap.
8	20	10	Centrifugal force acts upon the dynabeads	Dynabeads are centrifuged into first washing chamber
9	10	2	**Washing 1 (Step 9-10):**	Shake mode mixing of liquids and dynabeads by acceleration and deceleration which induce inertial forces.
10	20	2	Alternation between steps 9-10 repeated 15 times	
11	0	15	Defined position of LabDisk with reference to magnet 2	Dynabeads are collected in the first washing chamber
12	0	15	Defined position of LabDisk with reference to magnet 1	Dynabeads are moved out of the first washing chamber into the air split
13	0	1	Incremental rotation of 0.5°C repeated 25 times	Dynabeads are moved from the first washing chamber into the second washing chamber through the air gap.
16	20	10	Centrifugal force acts upon the dynabeads	Dynabeads are centrifuged into second washing chamber
17	10	2	**Washing 2 (Step 17-18):**	Shake mode mixing of liquids and dynabeads by acceleration and deceleration which induce inertial forces.
18	20	2	Alternation between steps 17-18 repeated 15 times	
19	0	15	Defined position of LabDisk with reference to magnet 2	Dynabeads are collected in the second washing chamber
20	0	15	Defined position of LabDisk with reference to magnet 1	Dynabeads are moved out of the second washing chamber into the air split
21	0	1	Incremental rotation of 0.5°C repeated 25 times	Dynabeads are moved from the second washing chamber into the detection chamber through the air gap.
22	15	10	Centrifugal force acts upon the dynabeads	Dynabeads are transferred into the detection chamber
23	0	2	Defined position of LabDisk with reference to mixing magnet	Dynabeads are collected with mixing magnet
24	0	1	**Detection:** Defined position of LabDisk with reference to luminescence detector	Chemiluminescence signal is acquired

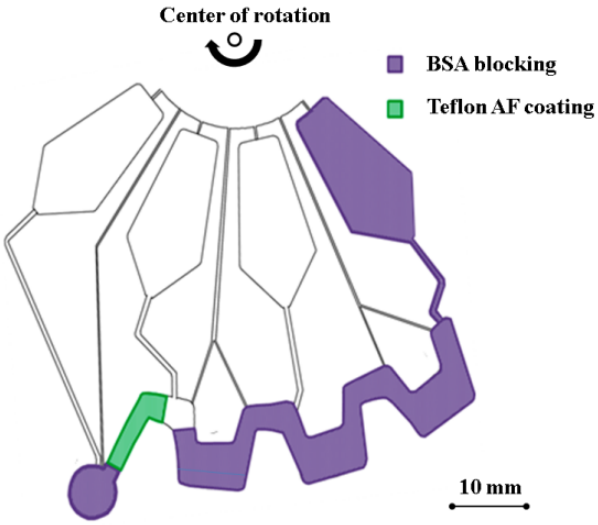
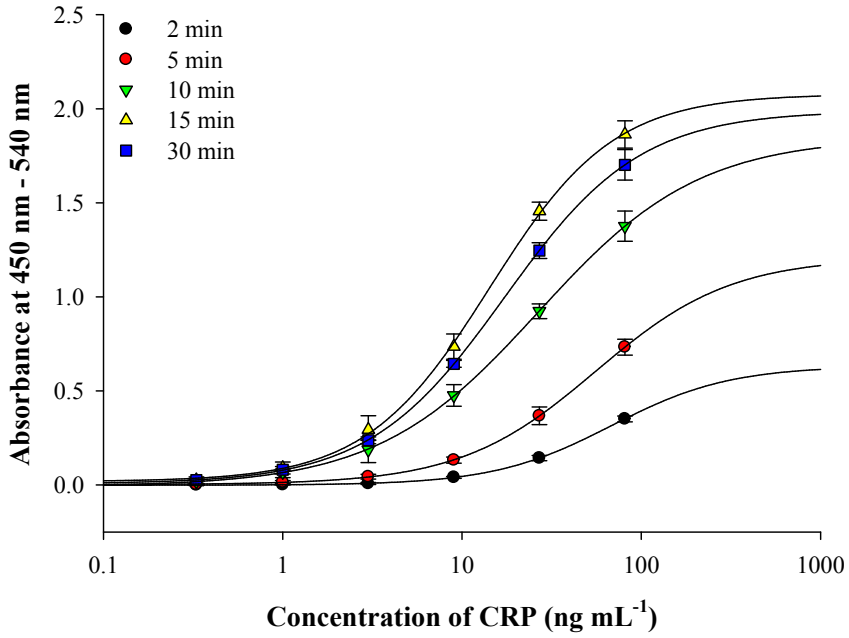
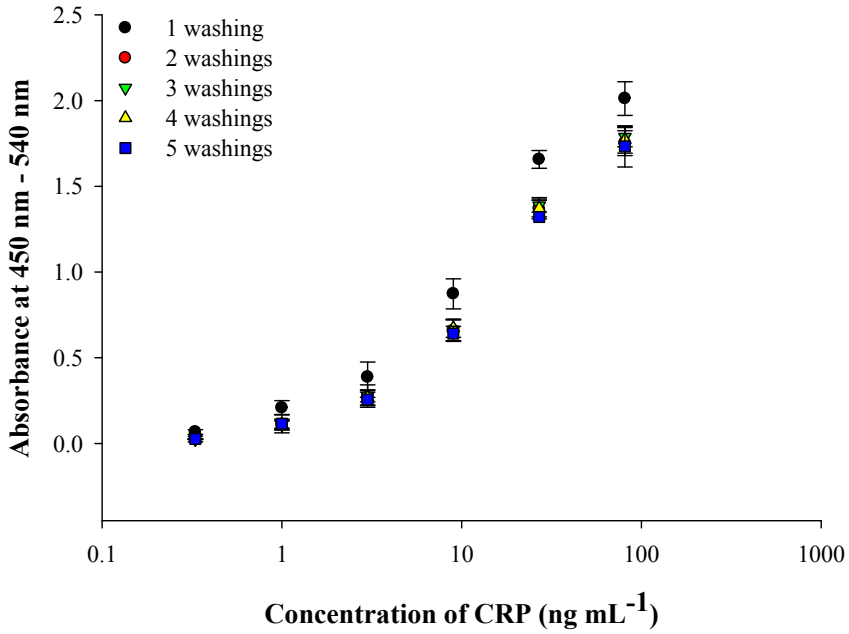


Figure S1 - Surface modification of the LabDisk. The relevant compartments (marked as purple) of the bead transport structure were blocked by incubating with 300 µl of PBS containing 5.0% (w/v) BSA for 30 min. The BSA-blocked LabDisk was then rinsed with ultrapure H2O and dried with N2. Subsequently, the Teflon coating was applied by pipetting 20 µl of 0.5 % Teflon AF solution at the final ridge (marked green) and dried by evaporation of volatile diluent (FC-770).

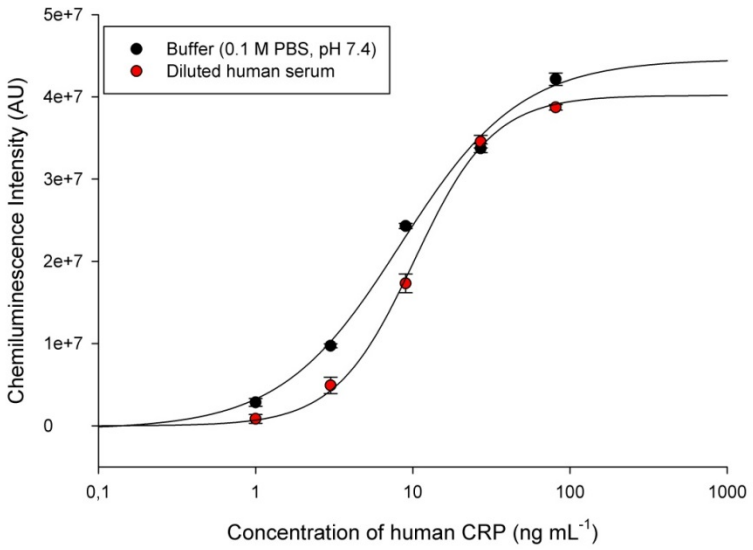
(A)


2 min
5 min
10 min
15 min
30 min
Absorbance at 450 nm - 540 nm
Concentration of CRP (ng mL-1)
0.0
0.5
1.0
1.5
2.0
2.5
0.1
1
10
100
1000

(B)


1 washing
2 washings
3 washings
4 washings
5 washings
Absorbance at 450 nm - 540 nm
Concentration of CRP (ng mL-1)
0.0
0.5
1.0
1.5
2.0
2.5
0.1
1
10
100
1000

(C)



(D)

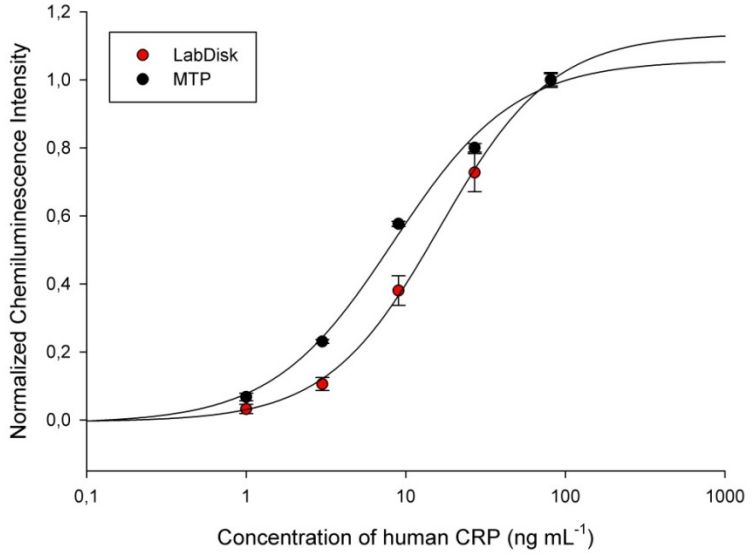


Figure S2. (A) Optimization of incubation time and **(B)** number of washings for the developed one-step kinetics-based MCIA. All experiments were done in triplicate and the error bars represent the standard deviation. **(C)** Assay curve of the manual MCIA for hCRP spiked to buffer and human serum. **(D)** Normalized signals of the LabDisk-based MCIA and the manual MCIA for hCRP spiked to buffer. The datasets of manual and automated LabDisk-based MCIA were normalized for more effective comparison as the chemiluminescence signals of these formats cannot be compared directly due to the different optical detectors used in these formats. Therefore, the normalizations were conducted by dividing the signal obtained for each hCRP concentration in the assay with the maximum signal obtained at the highest hCRP concentration in the respective assay.

Table S2. Analytical parameters of the developed LabDisk-based hCRP MCIA for human serum

Characteristics	LabDisk-based hCRP MCIA
Immunoassay duration	25 min
Number of process steps	Only one initial loading step of reagents
Number of washings required	Only 2
Detects the clinically-relevant hCRP range (3-80 µg mL^{-1})	Yes
Detection range	3-81 ng mL^{-1}
Linear range	3-27 ng mL^{-1}
LOD (ng mL^{-1}) in human serum	1.5
LOD (ng mL^{-1}) in buffer	0.2
LOQ (ng mL^{-1}) in human serum	1.8
LOQ (ng mL^{-1}) in buffer	0.3
R^2 (human serum)	0.99
R^2 (buffer)	0.99
Half-effective concentration EC_{50} (human serum)	8.1
Half-effective concentration EC_{50} (buffer)	16.3
Hill slope (human serum)	2.5
Hill slope (buffer)	1.2

Table S3. Process steps involved in the developed LabDisk MCIA procedure and the manual MCIA in MTPs.

LabDisk MCIA	Manual MCIA
Load immunoassay components at inlets of LabDisk and start centrifugation protocol (2 min.)	Block MTP with BSA (2 hours)
Incubation 15 min (automated)	Pipette immunoassay components on MTP (2 min)
Wash 2 times – 5 min (automated)	Incubate for 15 min (manual handling required)
Incubation of enzyme-substrate reaction– 1 min (automated)	Wash 2 times using magnetic holder (10 min, manual handling required)
Optical detection on LabDisk player (automated)	Incubation of enzyme-substrate reaction (1 min, manual handling required)
	Read on MTP reader (5 min, manual handling required)