Electronic Supplementary Material (ESI) for Lab on a Chip

Supplementary Information

Microfluidic barcode assay for antibody-based confirmatory diagnostics

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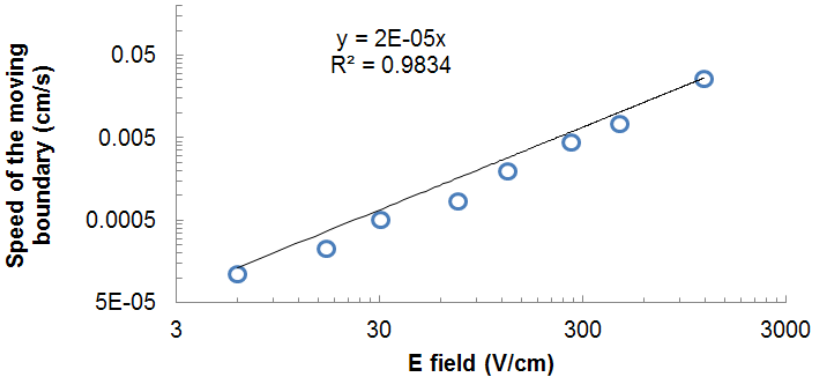

Figure S1, Speed of the moving boundary increases linearly with the electric field ($v_i=\mu_i E/n$), where slope $2.2\text{x}10^{-5}$ $cm^2V^{-1}s^{-1}$ is the ratio of mobility (μ_i) to the enhancement factor (n). At 30V/cm patterning of 800µm long zone takes 160 seconds whereas at 1180 V/cm it takes about 3 seconds.

Table S1. Antigen patterning parameters for a four sets of antigen bands interlaced with biotin spacers in a channel. Depending on the mobility and the concentration of the antigen-biotin, duration of the loading step and corresponding loading voltage can be adjusted for the desired band length. Before any step involving a new solution, wells were washed with TG buffer 3 times.

	Step	Sample	Duration (min)	Voltage (V)	Current (μA)	Energy (Watt-hour)
1	Load	20uM Biotin	1	1.5	0.15	3.75E-09
2	Incubate		1	0	0	
3	Reverse Wash	TG buffer	2	-18	-2	1.20E-06
4	Load	5uM c100p-biotin	2	9	1	
5	Incubate		1	0	0	
6	Reverse Wash	TG buffer	2	-18	-2	1.20E-06
…	Repeat 1-6 for the next agents					
49	Reverse Load	20uM Biotin	2	18	2	1.20E-06
50	Incubate		1	0	0	
51	Final wash	TG buffer	3	18	2.8	2.52E-06
		Total duration for 4 Ag zones	**60 minutes**		**Total energy (Watt hour)**	**1.81E-05**

Table S2. Serological assay parameters. 21 minutes of the 30 minute assay time was automated. 5-10 minutes of manual intervention included sample loading and washing of the wells. In the first step, 3μl sample (HCV-c100p human antibodies spiked in 2% serum in TG buffer) was refreshed at the end of 5 minute loading. Similarly the wash buffer (TG) at the final stage (step 4) was refreshed at the 2.5 minute interval. Before each wash step run and 2ndary antibody load, wells were cleaned with TG buffer 3 times. Here, 3 independent assays were run together. For detection, AF-568 labeled antihuman goat antibodies were used. Total energy consumption was 4.04×10^{-5} Watt-hours. 3x9V batteries hold 15 Watt-hour of energy leading to 3×10^{5} possible assays.

	Step	Sample	Duration (min)	Voltage (V)	Current (μA)	Energy (Watt-hour)
1	Sample Load	2% serum sample	5	30	3.5	8.75E-06
	Sample Load	2% serum sample	5	30	3.5	8.75E-06
2	Wash	TG buffer	2.5	-30	-2.8	3.50E-06
3	2ndary AB (AF-568) Load	40μg/ml in TG	3.5	-40	-5.3	1.24E-05
4	Wash	TG buffer	2.5	30	2.8	3.50E-06
	Wash	TG buffer	2.5	30	2.8	3.50E-06
		Total duration	**21 minutes**		**Total energy (Watt hour)**	**4.04E-05**

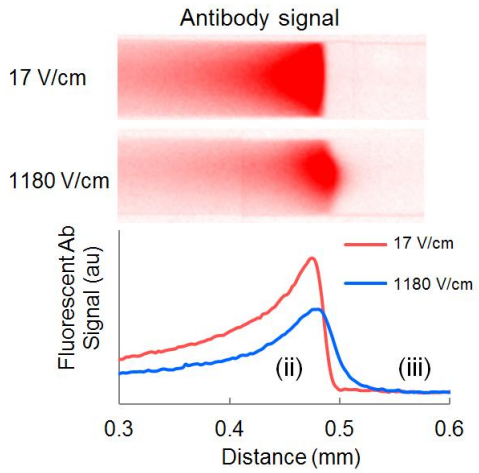

Figure S2, **Effect of immobilizatin conditions over antibody capture.** Channels, in which antigens were immobilized at different electric fields (17V/cm and 1180V/cm), were tested with loading of c100p (AF-568) antibodies from the right direction. Figure shows the antibody capture profile of two distinct channels immobilized under the two specified loading conditions. In both channels, antibody loading parameters were the same (25V) and capture only occurred in the antigen immobilized region (ii), confirming region (iii) remained antigen free in all cases. Similar to the immobilized antigen distribution, a smoother boundary with increased transverse signal variation at the boundary is observed in the antibody signal in the channel where the antigen immobilization was performed at 1180 V/cm.

Figure S3, Gel deformation under high electric field. Here AF-568 labeled c100p antibodies are loaded against c100p antigen band at 450V (1800V/cm). In the first few minutes of the loading, deformation was along the loading direction (convex to the left). With the loss of the buffer in the left well, deformation changed direction towards the right. Loss of the buffer in the left well can be attributed to the electroosmotic pumping of the fluid from left well to the right well through the deformations within the gel matrix or gel-glass wall interface after the introduction of high voltage. When the applied voltage was turned off, the gel profile regained its original form.