Electronic Supplementary Material (ESI) for Analyst.

Supplementary Information to

Glass Capillary Based Microfluidic ELISA for Rapid Diagnostics

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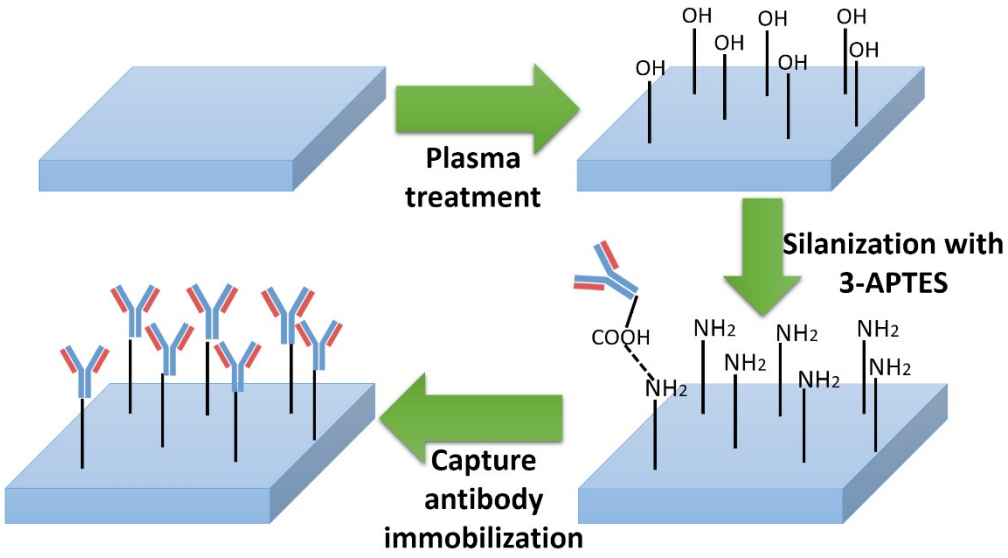

Figure S1 Mechanism of surface chemical activation. Air plasma treatment can promote hydroxylation on glass surface, thus facilitates silanization with 3-APTES. The silanized glass surface will have a monolayer of free amino group, which has a high affinity toward free carboxyl groups on the proteins (can be found on Aspartic acid, Glutamic acid and c-terminus of proteins).

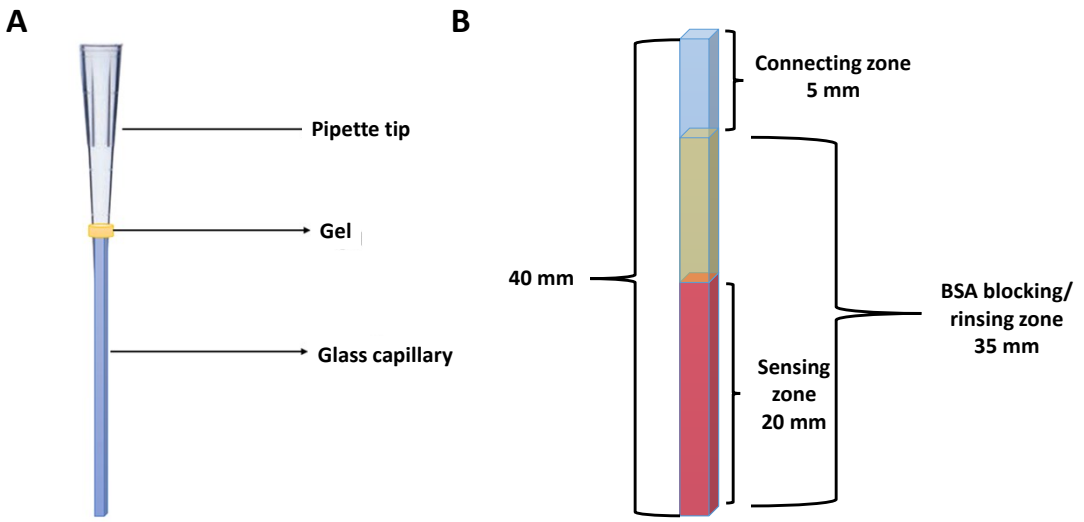

Figure S2 A. Basic structure of a capillary reactor unit. Which is composed by a capillary and an adaptor (pipette tip in this case). B. Illustration of space allocation in each capillary reactor. The sensing zone is 20 mm long, which means the sample volume is 20μL. The blocking/ rinsing zone is 35 mm long, which means 35 μL of liquid is required for blocking and rinsing.

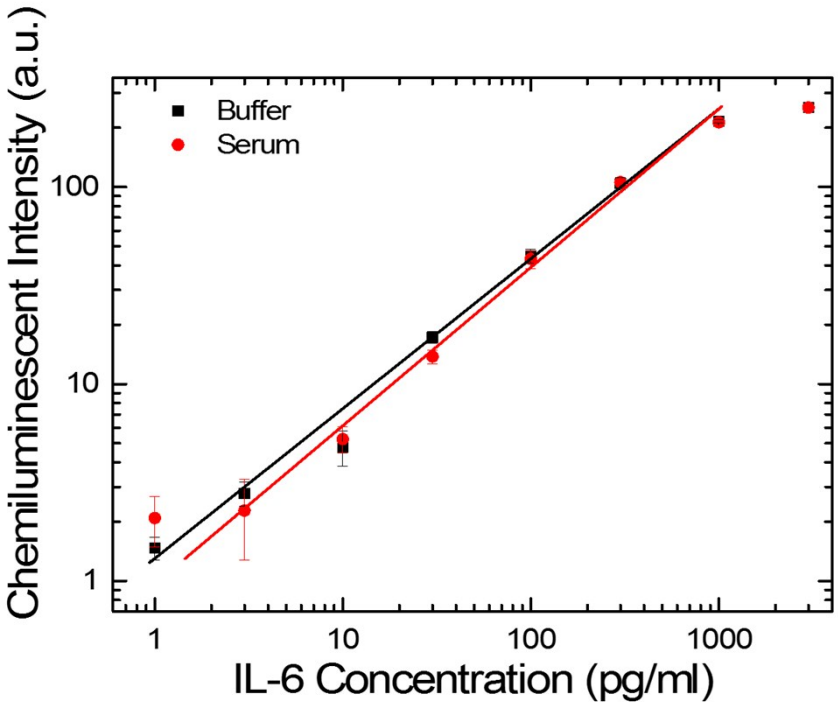

Figure S3 The response curves of IL-6 ELISA with capillary reactors. There is a strong consistency between the measurements in buffer solution and the measurements in human serum. Both curves are mostly linear on the log-log scale between 3 pg/ml and 1000 pg/ml IL-6 with a slope of 0.7578 and 0.8117 for buffer and serum, respectively.

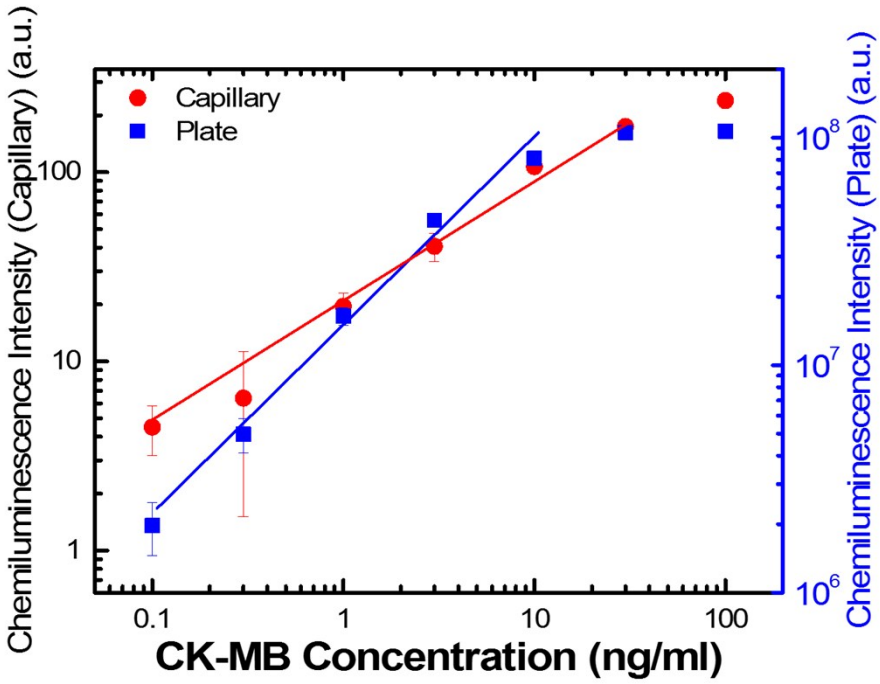

Figure S4 Background subtracted response curves of CK-MB ELISA in human serum with capillary reactors and 96-well plate.

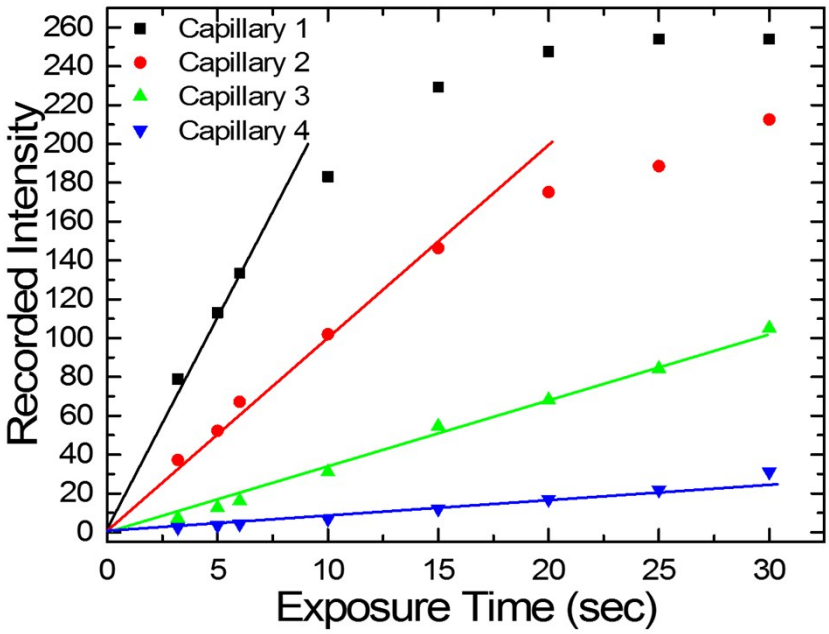

Figure S5 The response of the CMOS sensor to different light intensities. Calibration data points show the CMOS sensor in Canon 80D camera has a good response linearity when integrated light intensity is not close to saturation (<160 out of 254). For readings below 160, the recorded light intensity is mostly proportional to exposure time.

	Analytes	LOD	Dynamic range	Assay Time	Sample Volume
Capillary Chemiluminescent ELISA	IL-6	1 pg/ml	1 – 1000 pg/ml	40 minutes	20 μl
	CK-MB	0.03 ng/ml	0.03 – 250 ng/ml	16 minutes	
96 - Well Plate Chemiluminescent ELISA	IL-6	1 pg/ml	1 – 1000 pg/ml	320 minutes	100 μl
	CK-MB	0.03 ng/ml	0.03 – 10 ng/ml	200 minutes	
96 - Well Plate Fluorescent ELISA	IL-6	10 pg/ml	10 – 1000 pg/ml	335 minutes	100 μl
	CK-MB	0.1 ng/ml	0.1 – 30 ng/ml	215 minutes	

Table S1 Comparison among capillary based chemiluminescent ELISA, traditional 96-well plate based chemiluminescent ELISA and traditional 96-well plate based fluorescent ELISA.

Protocol for 96-well plate based chemiluminescent ELISA

The procedure of chemiluminescent IL-6 assay with 96-well plate is: 1. Incubate 100 μL of capture antibody working solution in each well overnight. 2. Rinse the plate three times with washing buffer. 3. Incubate the blocking buffer (300 μL) in each well for 90 minutes. 4. Repeat step two. 5. Add 100 μL of sample and standard solution to each well and incubate for 120 minutes. 6. Repeat step two. 7. Add 100 μL of detection antibody solution to each well and incubate for 120 minutes. 8. Repeat step two. 9. Add 100 μL of Streptavidin-HRP (SAv-HRP) solution to each well and incubate for 30 minutes. 10. Repeat step two. 11. Add 100 μL of chemiluminescent substrate into each well. 12. Take measurement immediately with chemiluminescence plate reader (PMT associated luminometer). The procedure of chemiluminescent IL-6 assay with capillary reactors was described in detail in the result section.

The procedure of chemiluminescent CK-MB assay with 96-well plate is: 1. Incubate 100uL of capture antibody working solution in each well overnight. 2. Rinse the plate three times with washing buffer. 3. Incubate the blocking buffer (300 μL) in each well for 90 minutes. 4. Repeat step two. 5. Add 100 uL of sample and standard solution to each well and incubate for 90 minutes. 6. Repeat step two. 7. Add 100 μL of detection antibody solution to each well and incubate for 90 minutes. 8. Repeat step two. 9. Add 100 uL of chemiluminescent substrate into each well. 10. Take measurement immediately with chemiluminescence plate reader. The procedure of chemiluminescent CK-MB assay with capillary reactors was described in detail in the result section.