Electrochemical carbamazepine aptasensor for therapeutic drug monitoring at the point of care

Supporting Information

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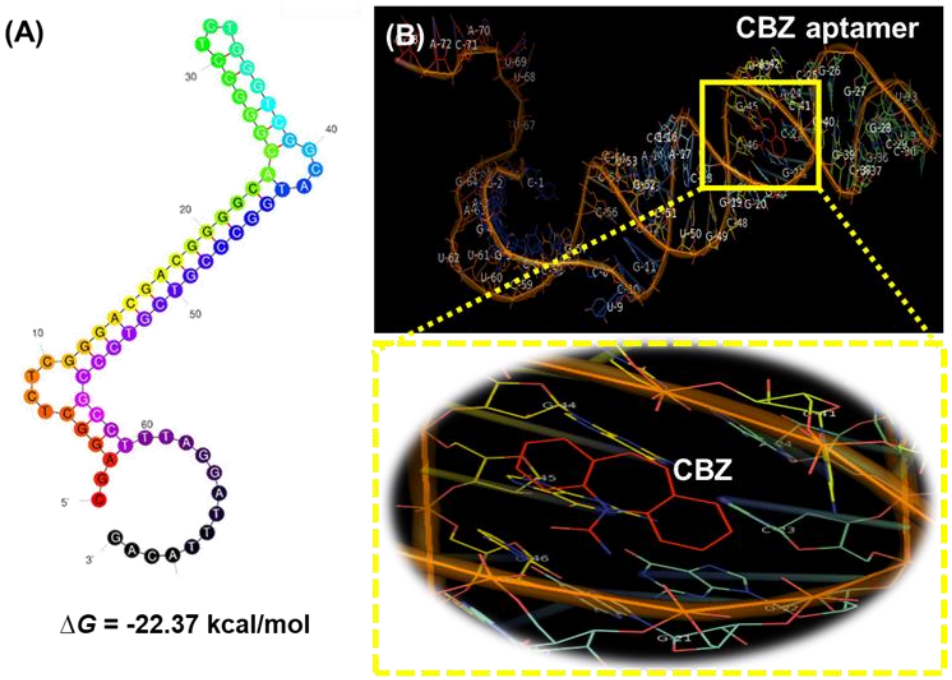

Figure S1. CBZ aptamer structure and folding study. (A) M-fold simulation of aptamer secondary structure and **(B)** *in silico* docking simulation of aptamer and CBZ (red).

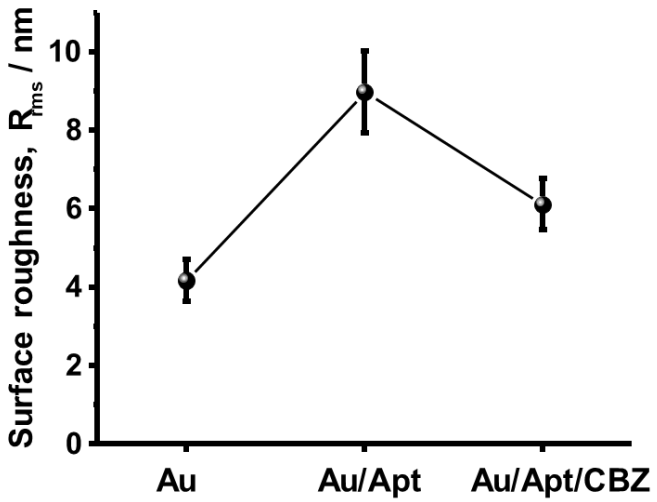

Figure S2. **AFM surface roughness.** Variation of the root-mean-square surface roughness (R_{rms}) at different stages of aptasensor fabrication.

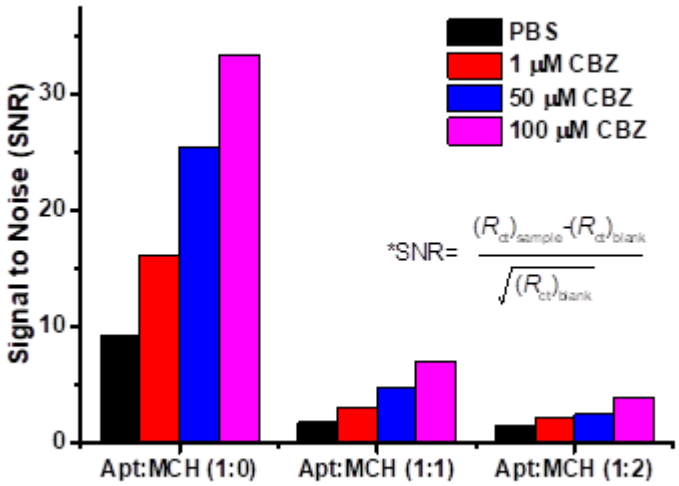

Figure S3. Aptamer loading density optimization. Measured aptamer to mercaptohexanol (MCH) density with 1, 50, and 100 μM CBZ. (SNR calculated from the equation inserted[56]).

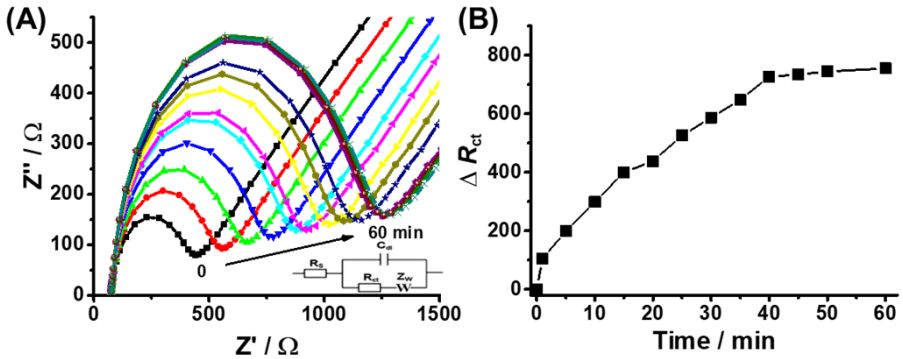

Figure S4. CBZ incubation time. (A) Measured EIS spectra at various CBZ incubation times (0 to 60 min). **(B)** Plot of ΔR_{ct} vs. time compiled from EIS data.

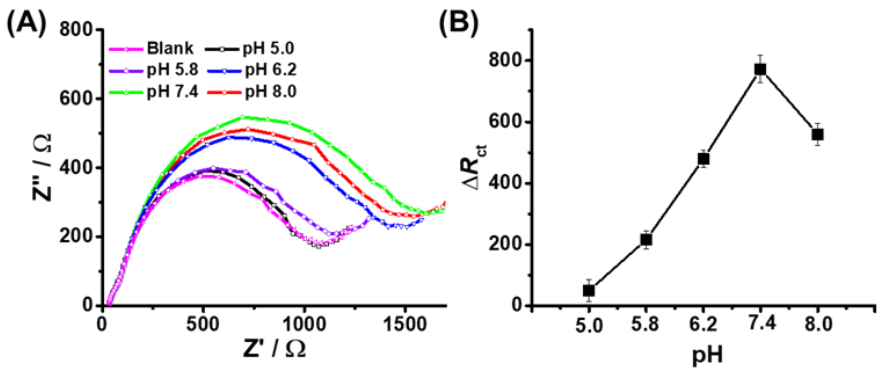

Figure S5. pH optimization. **(A)** EIS spectra at different pH of sample solution. **(B)** Plot of ΔR_{ct} vs. pH obtained from EIS data.

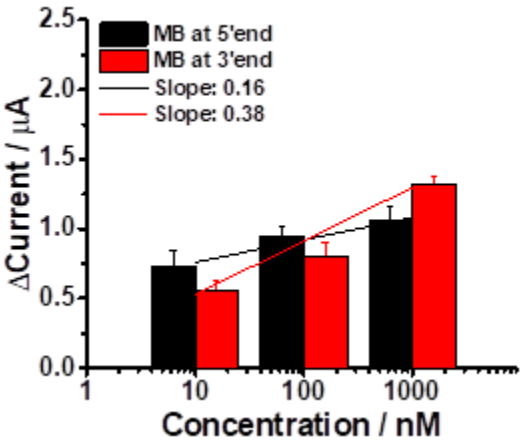

Figure S6. **Aptamer label position.** Optimization of label location (3' vs. 5') MB modified aptamers.

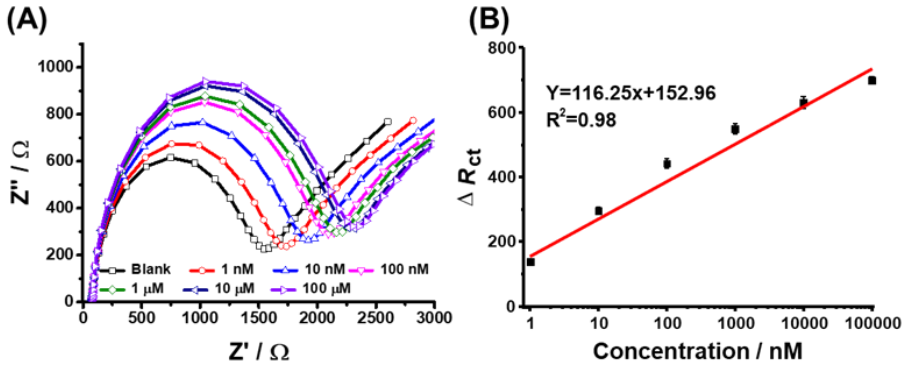

Figure S7. **Label-free aptasensor using EIS. (A)** Nyquist plot and **(B)** calibration curve.

Table S1. **Recovery experiments**.

Assay Time (min)	Sample concentration (nM)	Sample #1		Sample #2		Sample #3	
		Calculated Concentration (nM)	Recovery (%)	Calculated Concentration (nM)	Recovery (%)	Calculated Concentration (nM)	Recovery (%)
5	100	84.3	84.3	94.5	94.5	102.9	102.9
	5000	4026.6	80.5	5054.8	101.1	3804.1	76.1
	10000	12589.3	125.9	7525.6	75.3	8925.2	89.3
30	15	14.0	93.4	12.4	82.7	13.8	92.0
	1000	1092.9	109.2	872.7	112.7	1144.8	114.5
	5000	5242.4	104.8	4189.0	83.3	4857.1	97.1

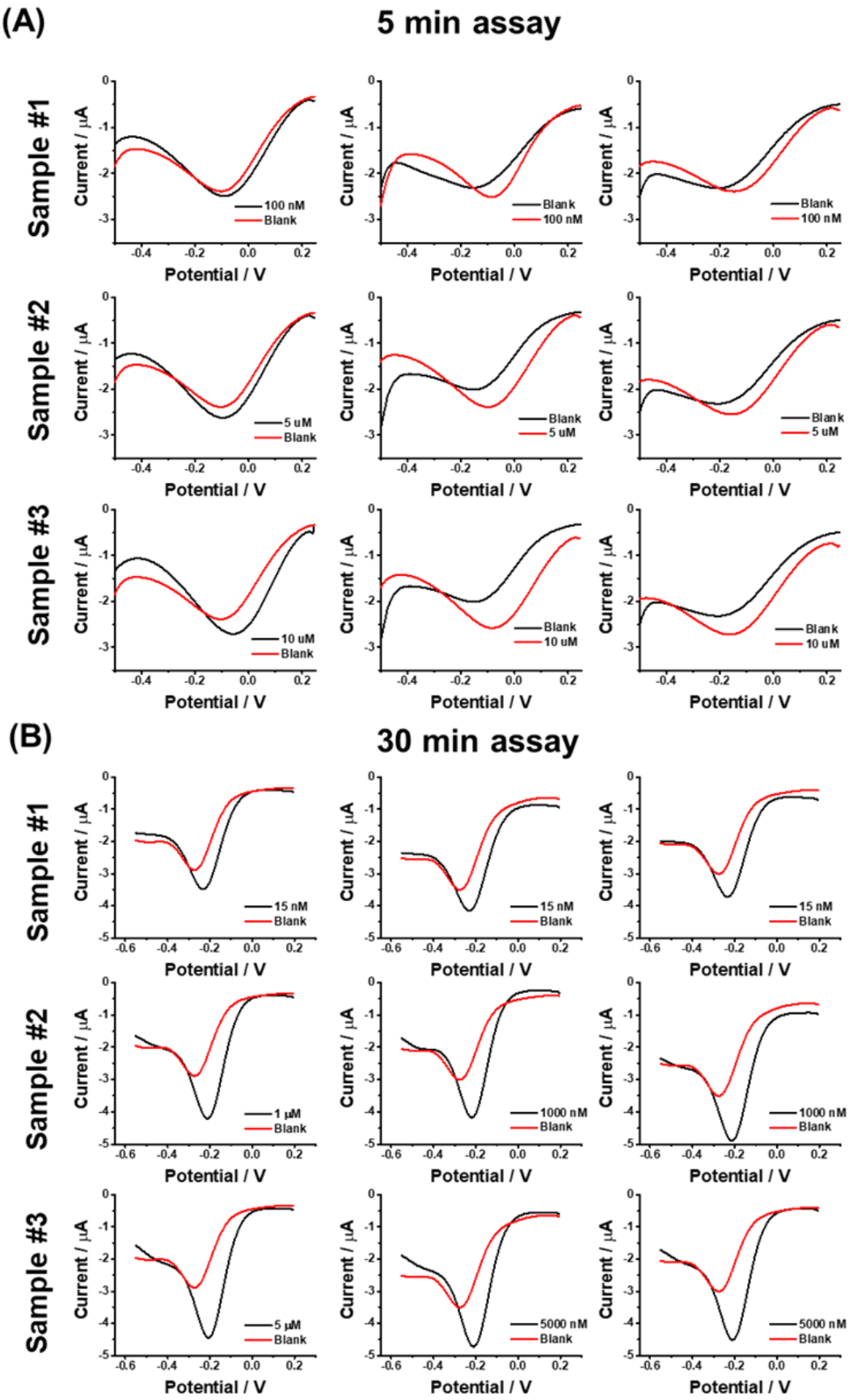

Figure S8. **Recovery tests.** (A) Voltammograms were measured for **(A)** 30-minute assay and **(B)** 5-minute assay with 15 nM, 1 µM, and 10 µM of CBZ spiked in undiluted human serum.

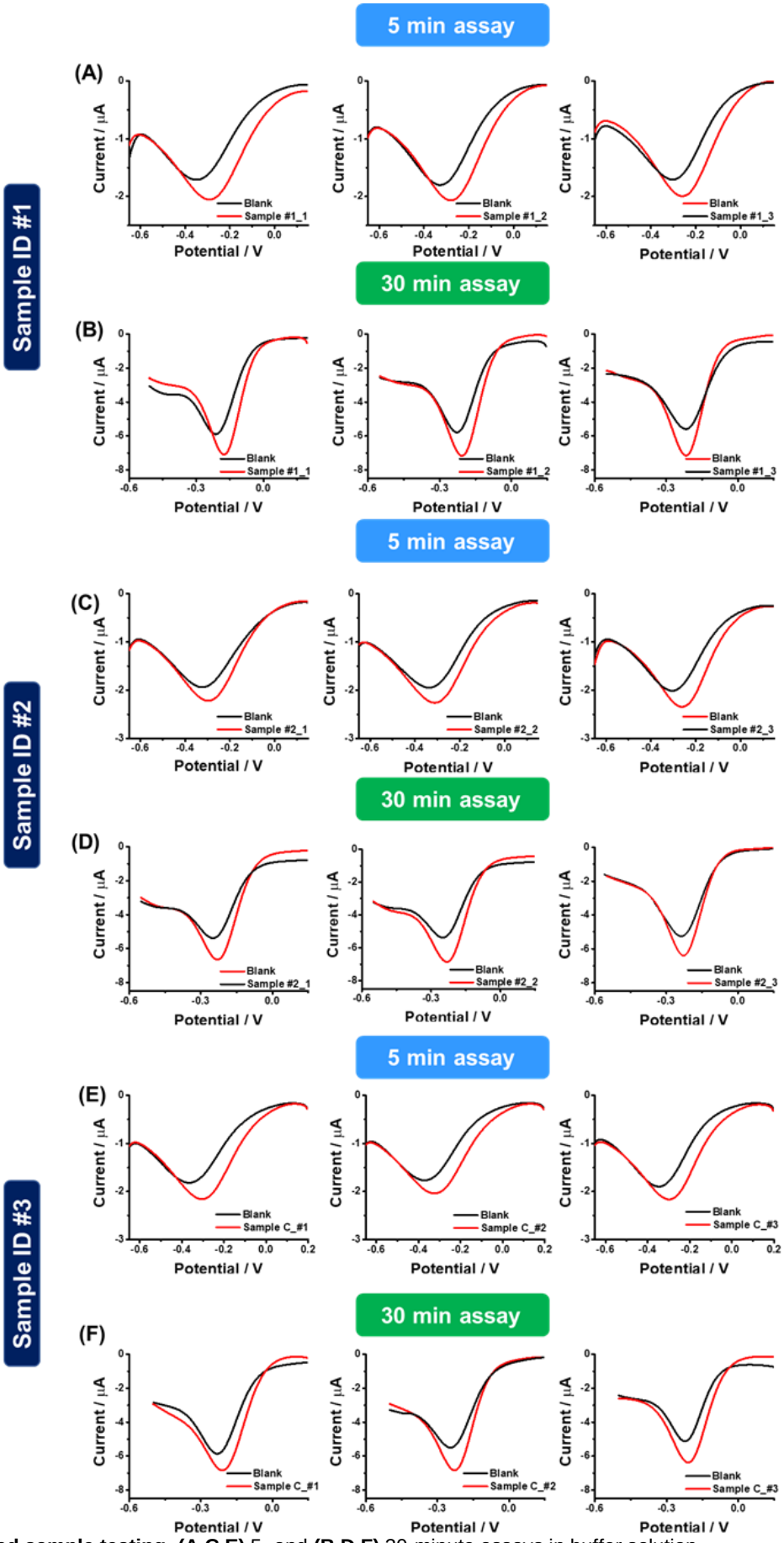

Figure S9. Blinded sample testing. (A,C,E) 5- and **(B,D,F)** 30-minute assays in buffer solution.

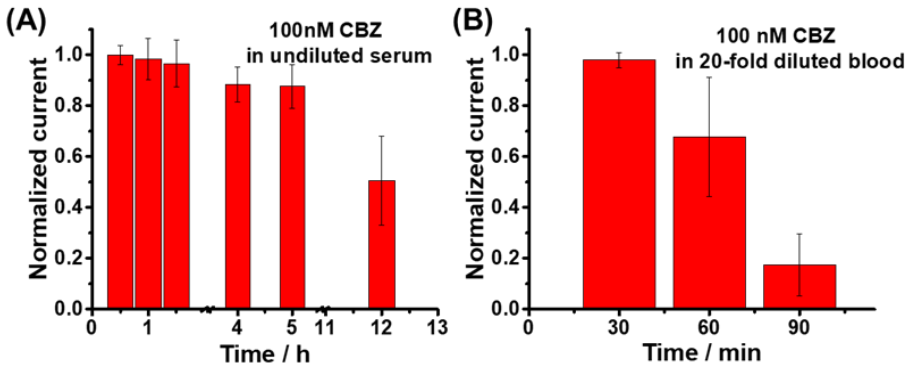

Figure S10. **Aptamer stability to nucleases. (A)** Peak current for 100 nM CBZ spiked in undiluted serum at 4 °C (on a tube cooler). **(B)** Peak current for 100 nM CBZ spiked in 20-fold diluted blood at ambient temperature.

Table S2. Blinded verification tests using CBZ spiked in buffer solution.

Sample	True Concentration (μM)	30-minute assay		5-minute assay	
		Calculated Concentration (μM)	CV (%)	Calculated Concentration (μM)	CV (%)
#1	50	45.5, 58.6, 47.3 (μ = 50.5)	14.1	47.7, 42.1, 54.9 (μ = 48.2)	13.3
#2	15	13.3,16.9,12.6 (μ = 14.3)	16.1	9.9,13.1,14.5 (μ = 12.5)	18.9
#3	20	21.1, 24.3, 18.0 (μ = 21.1)	14.9	17.9,18.5,25.6 (μ = 20.7)	20.1

Table S3. Analytical performance of CBZ detection methods.

Method		Probe	Selective	Pre-treatment?	Dynamic Range (μM)	LOD (μM)	Assay Time (min)	Ref.
Electrochemical (direct)	SWV, CV	GCE/MIPEDOT (Molecular imprinting sensor)	Yes	No	100-2000	980	15	[57]
	EIS	Au/Gr/AuNPs	No	No	10-1000	3.03	8	[3]
	DPV	GCE	No	No	N/A	0.59	5	[8]
Fluorescence polarization immunoassay		Glass column	No	Yes	N/A	0.85	30	[8]
		GC column	No	Yes	10.6-105.8	N/A	90	[58]
LC-MS/MS		Synergi 4μ Fusion column	No	Yes	4.2-169.3	105.81	90	[59]
Dispersive liquid-liquid microextraction		Eurospher -100 C18 column	No	Yes	0.002-0.8	0.0009	60	[60]
Electrochemical aptasensor	SWV	MB-aptamer	Yes	No	0.01-100	0.001	30	This study
					0.01-100	0.001	5	

Table S4. Assay comparison table.

	Rapid Testing	Routine Testing
Sample incubation time	5 min	30 min
Purpose	Emergency care decisions for fast and appropriate medical care	Regular medical checkups for long-term administrated patients
Electrode type	Disposable screen-printed electrode	Disk electrode
Required Sample volume	50 µL	50 µL
Measurement solvent volume	50 µL	5 mL
Washing step	No	Yes
Dynamic range	10 nM to 100µM	10 nM to 100µM
LOD	1.25nM	1.82nM
Linear regression	I(µA)=0.080[CBZ(nM)]+0.150 in serum	I(µA)=0.389[CBZ(nM)]+0.336 in serum
RSD % (Accuracy)	16.2	5.46
Uncertainty in concentration	25.07	5.82