Electronic Supplementary information (ESI) for Nanoscale

Liquid Exfoliation of 2D-MoS2 nanosheets and their utilization as a label-free electrochemical immunoassay for subclinical ketosis

Satish K. Tuteja[1], Todd Duffield[2], Suresh Neethirajan[1]*

[1]BioNano Laboratory, School of Engineering, University of Guelph, Guelph, ON, N1G 2W1, Canada

[2]Population Medicine, Ontario Veterinary College, University of Guelph, ON, N1G 2W1, Canada

*Corresponding author:

E-mail address: sneethir@uoguelph.ca (S. Neethirajan).

1. Experimental

1.1 Sample preparations: Before an assay, the serum sample was thawed at room temperature and then diluted 10 times in PBS buffer (pH 7.4). Three replicates of each standard and diluted serum sample were prepared and measured; the average absorbance readout by the micro plate reader was calculated to evaluate the sample concentration. The βHBA standard solution ranging from 0.7 mM to 10 mM was made by diluting the 3-hydroxybutyric acid with PBS buffer and was used for the βHBA standard curve. Serum samples with known βHBA concentrations were analyzed using a Roche Cobas 6000 c501 automated chemistry analyzer (Roche Canada, Laval, QC, Canada), the values from which were provided by our collaborator (Ontario Veterinary College, Canada); the detailed sample preparation procedure can be found elsewhere.[1] Briefly, cow's blood was firstly collected from the coccygeal blood vessels in a vacuum tube without anticoagulant and stored in a cool place. Within 6 h of collection, the collected sample was then centrifuged to harvest serum at 4 °C at 2990g and stored at −20 °C for further use. Before assaying, the serum sample was thawed at room temperature and diluted to a series of concentrations ranging from 0.7 mM to 10 mM in PBS buffer. Milk and blood sample solutions were prepared by spiking them with βHBA concentrations ranging from 0.7 to 10 mM.

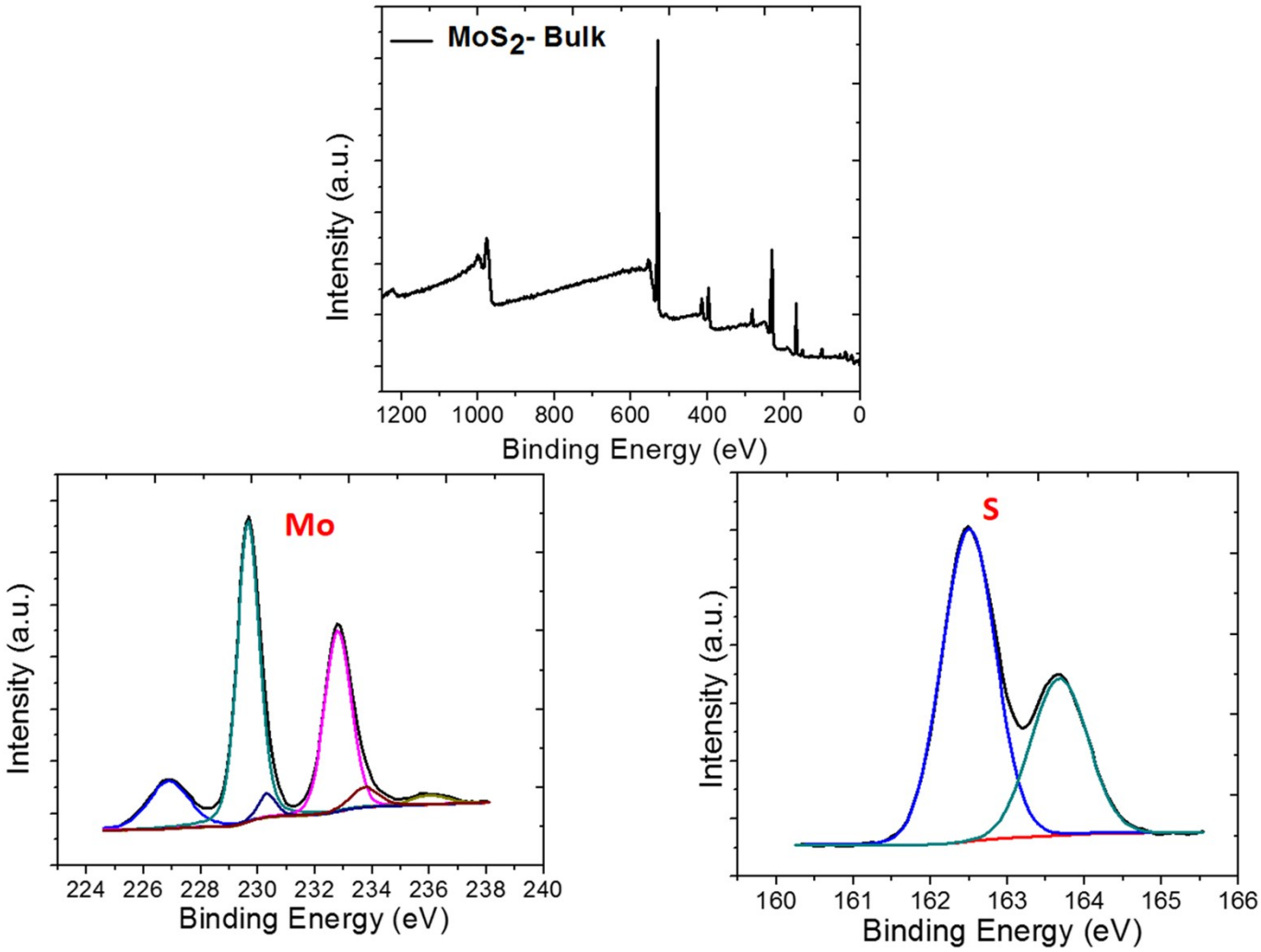


Figure S1. XPS spectral analysis of Bulk MoS_2 along with high resolution scan of Mo 3d and and S 2p.

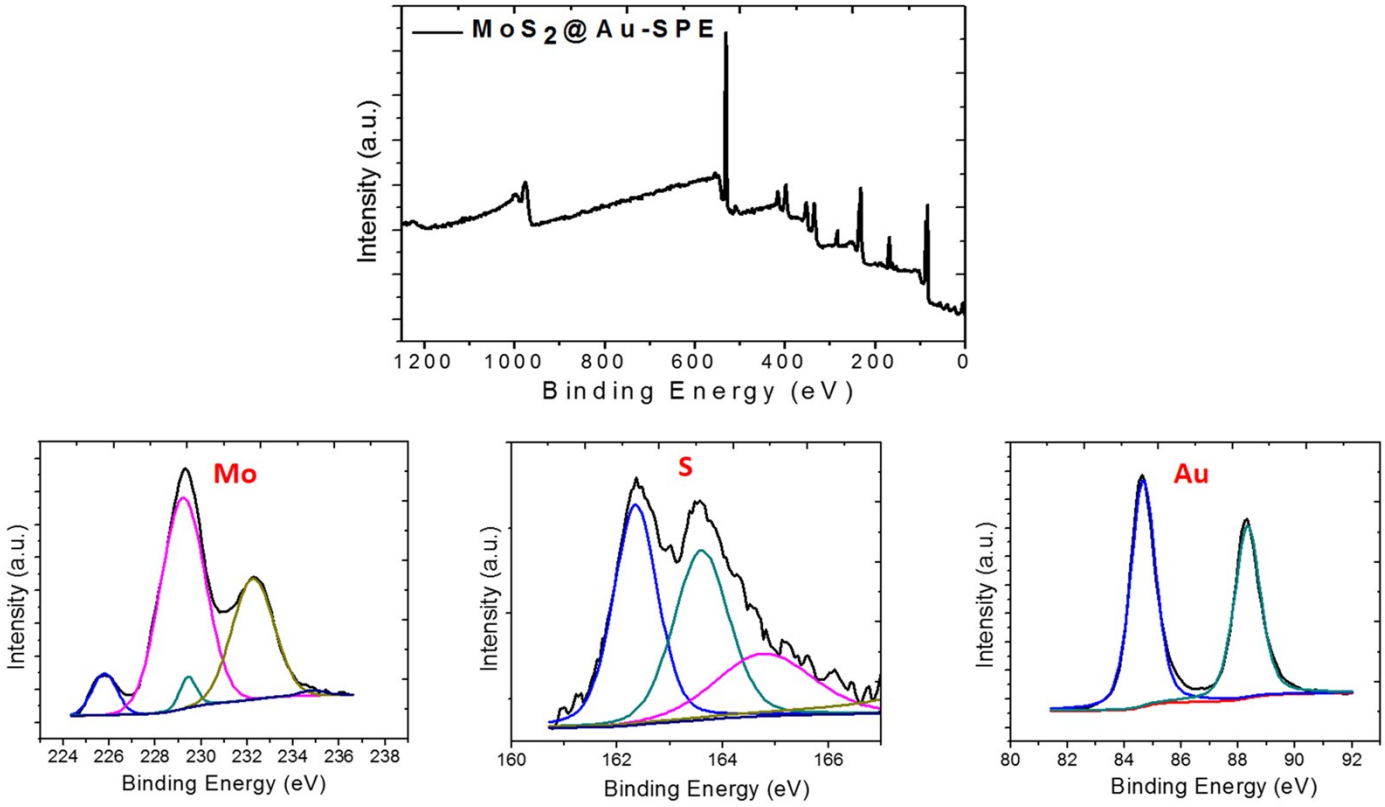
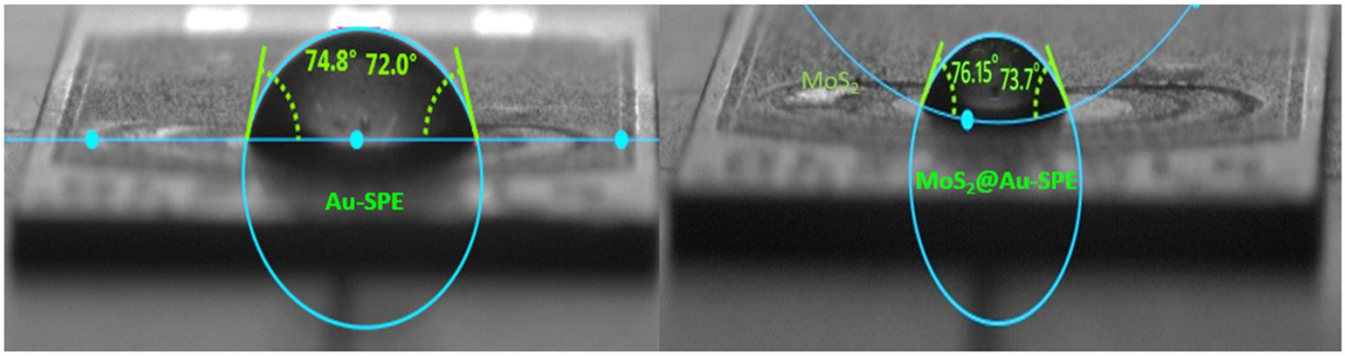


Figure S2. XPS spectral analysis of MoS_2@Au-SPE along with high resolution scan of Mo 3d, S 2p and Au 4f.



Material	Left Angle	Right Angle	Mean Angle
Au-SPE	74.8°	72.0°	73.4°
MoS_2@Au-SPE	76.15°	73.7°	74.92°

Figure S3. Contact angle measurements of SPE modified with gold and SPE modified with MoS_2.

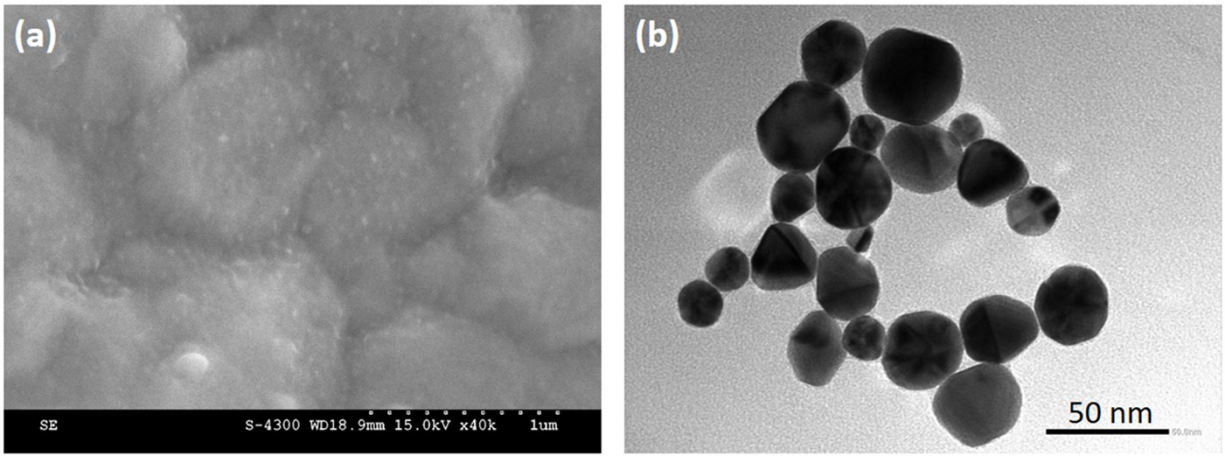


Figure S4. (a) A SEM image of electrodeposited gold colloids on SPEs and **(b)** a TEM image of electrodeposited gold nanocolloids on a copper grid using the same electrochemical technique as adopted for the SPEs.

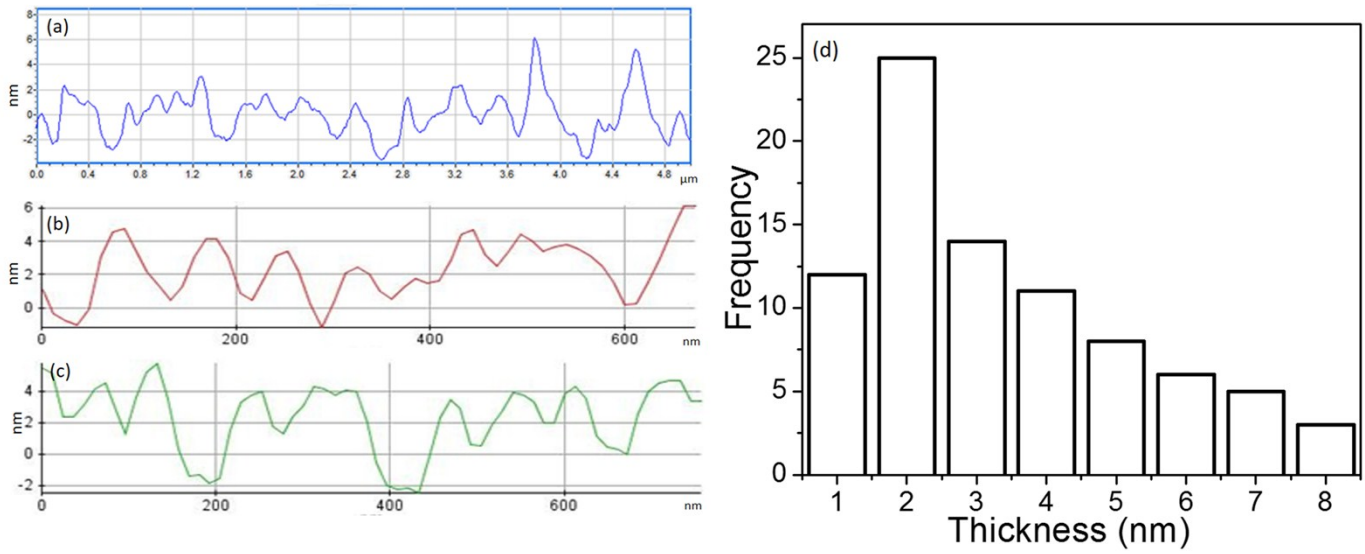


Figure S5. (a) Atomic Force Miscroscopy image (AFM) line profile analysis of MoS_2 nanostrutures, **(b. & c.)** show line profiling of electrodeposited gold nanocolloids.(d) AFM statistics of the thickness of MoS_2 nanosheets.

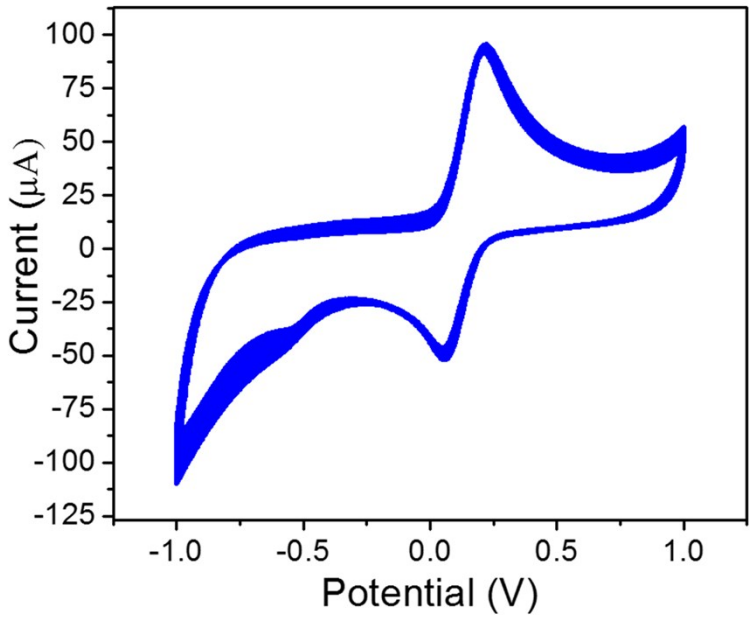


Figure S6. The stability response of Ab/MoS_2@Au-SPE checked over the 25 CV cycles .

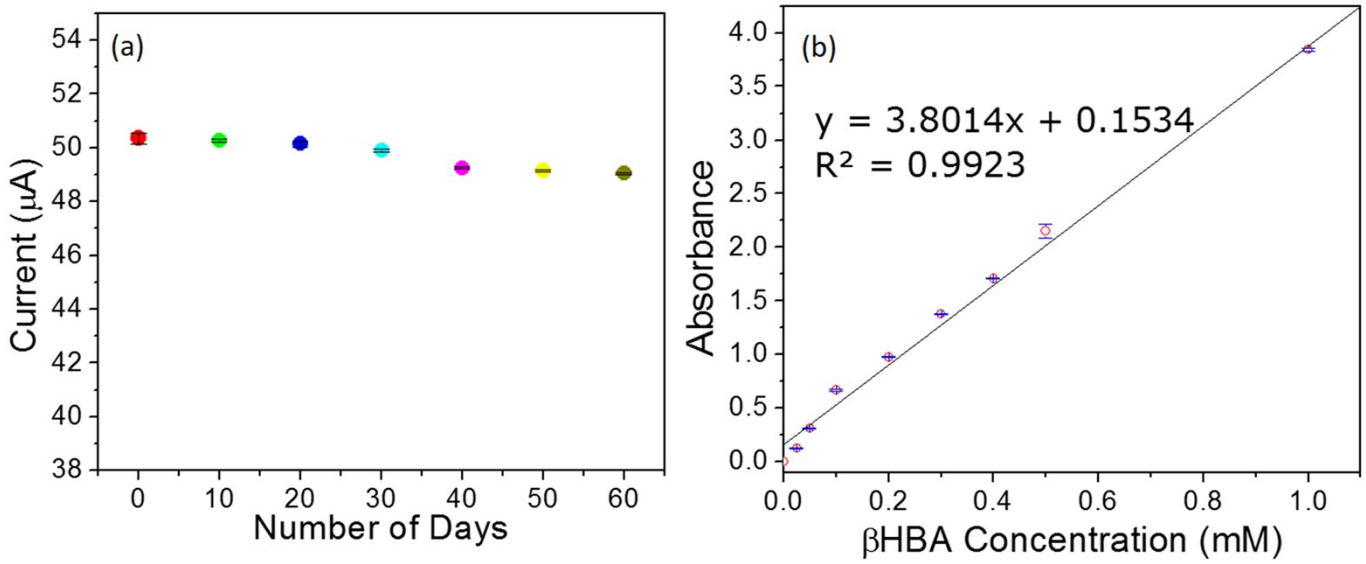


Figure S7. (a) Stability of Ab/MoS_2@Au-SPEs during storage and the corresponding variation in response was measured at different time intervals and **(b)** Standard calibration plot using a commercial colorimetric kit.

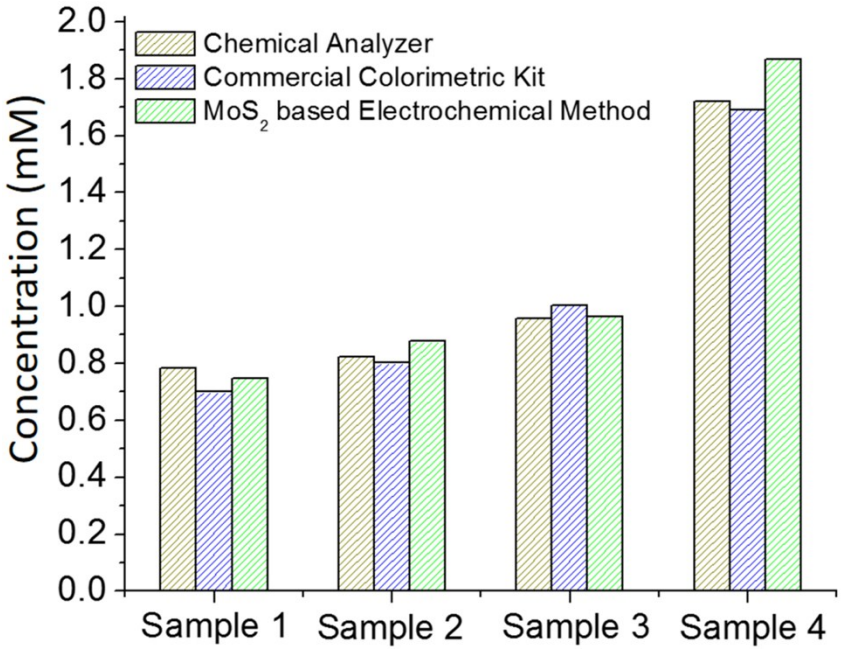


Figure S8. βHBA concentration determination in clinical samples of unknown βHBA antigen concentration, using 3 different techniques.

Table S1: Analysis of standard, spiked serum, spiked blood, spiked milk (containing known βHBA concentrations) and clinical serum samples (containing unknown βHBA concentrations using DPV (Differential Pulse Voltammetry) technique.

βHBA Conc. (mM)	**Current (µA) in Standard (n=3)**	**Current (µA) in Spiked Serum (n=3)**	**Conc. Calculated & % Recovery (mM)**	**Current (µA) in Milk Samples (n=3)**	**Conc. Calculated & % Recovery (mM)**	**Current (µA) in Blood Samples (n=3)**	**Conc. Calculated & % Recovery (mM)**	**Real Clinical Samples**	**Current (µA) Measured (n=3)**	**Estimated Conc. (mM)**
0.7	83.67	85.43	0.607 (86.7)	82.41	0.719 (102.7)	86.11	0.571 (81.5)	Unknown Sample- 1	80.55	0.748
0.8	77.226	79.53	0.764 (95.5)	75.25	0.885 (10.6)	80.53	0.748 (93.5)	Unknown Sample- 2	75.45	0.877
1	72.626	76.57	0.828 (82.8)	71.92	1.017 (101.7)	76.11	0.848 (84.8)	Unknown Sample- 3	73.53	0.96
1.2	64.666	67.41	1.13 (94.1)	63.77	1.22 (101.6)	67.41	1.131 (94.2)	Unknown Sample- 4	45.21	1.869
1.4	58.346	55.81	1.43 (102.1)	55.36	1.43 (102.1)	62.55	1.266 (90.4)			
1.5	50.866	52.93	1.47 (98)	51.53	1.49 (99.3)	53.44	1.465 (97.6)			
2	43.213	48.41	1.66 (83)	42.78	2.15 (107.5)	48.49	1.655 (82.7)			
3	40.396	41.56	2.58 (86)	41.77	2.51 (83.6)	43.11	2.03 (67.6)			
4	35.453	33.42	4.27 (106.7)	35.99	3.89 (97.2)	37.21	3.64 (91)			
5	28.146	30.49	4.67 (93.4)	30.45	4.68 (93.6)	31.41	4.55 (91)			
7.5	18.303	16.63	7.97 (106.2)	20.25	7 (93.3)	22.53	6.42 (85.6)			
10	9.466	11.15	9.52 (95.2)	9.56	9.97 (99.7)	11.95	9.29 (92.9)			

Table S2: Analysis of standard and spiked serum samples with known concentration of βHBA using chronoamperometry.

Sr. No.	Conc. in Standard (mM)	Current Measured (µA) (n=3)	Conc. in Spiked Serum (mM)	Current Measured (µA) (n=3)	Calculated conc. of antigen using standard calibration (mM)	% Recovery
1	0	7.5533	0	7.55		
2	0.7	7.353333	0.7	7.36	0.676	96.5
3	0.8	7.04	0.8	6.96	0.933	116.6
4	1	6.92	1	6.93	0.983	98.3
5	1.2	6.863333	1.2	6.86	1.2	100
6	1.3	6.753333	1.3	6.71	1.328	102.1
7	1.4	6.6	1.4	6.56	1.424	101.7
8	1.5	6.433333	1.5	6.44	1.496	99.7
9	2	6.403333	2	6.4	2.1428	107.1
10	5	6.333333	5	6.35	4.285	85.7
11	10	6.153333	10	6.11	11.203	112

Table S3: Analysis of standard and spiked serum samples with known concentrations of βHBA using color based absorbance (at 450 nm) with a commercial colorimetric kit. The clinical samples of unknown concentrations were also measured, and the concentrations were estimated by using the linear plot equation shown in Figure 7d.

Sr. No.	BHBA Conc. in Standard (mM)	Absorbance Measured (O. D.) (n=3)	BHBA Conc. in spiked Serum (mM)	Absorbance Measured (O. D.) (n=3)	Calculated Conc. of BHBA using standard calibration plot (mM)	% Recovery	Real Serum Sample (Diluted 10 times)	Absorbance Measured (n=3)	Conc. Calculation
1	0.025	0.128	0.025	0.132	0.0255	102	Sample-1	0.455	0.7
2	0.05	0.312	0.05	0.335	0.0531	106.2	Sample-2	0.527	0.802
3	0.1	0.667	0.1	0.684	0.105	105	Sample-3	0.669	1.005
4	0.2	0.975	0.2	0.974	0.199	99.5	Sample-4	0.882	1.69
5	0.3	1.378	0.3	1.45	0.321	107			
6	0.4	1.710	0.4	1.85	0.431	107.75			
7	0.5	2.152	0.5	2.41	0.576	115.2			
8	1	3.844	1	3.98	1.04	104			

Table S4: The comparison of results obtained by using chemical analyzer, a colorimetric kit, and by our proposed electrochemical method.

Sr. No.	Clinical Serum Samples of Unknown conc. of βHBA	Detection by Chemical Analyzer	Detection by Commercial Colorimetric Kit	Our Proposed Electrochemical Method
1	Sample-1	0.784 mM	0.7 mM	0.748 mM
2	Sample-2	0.824 mM	0.802 mM	0.877 mM
3	Sample-3	0.958 mM	1.005 mM	0.966 mM
4	Sample-4	1.721 mM	1.69 mM	1.869 mM

References

(1) Gohary, K.; LeBlanc, S. J.; Lissemore, K. D.; Overton, M. W.; Von Massow, M.; Duffield, T. F. *J. Dairy Sci.* **2014**, *97* (10), 6231–6241.