

Article 9 Supplementary Material (Quintas, B. et al.)

Smartphone-Based Colourimetric Protein Quantification in Human Urine Using Gold Nanoparticles

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Supplementary Table

Table SM1 | Limits of detection (LOD) for various techniques for protein detection and quantification.

Method		Advantages	Disadvantages	Limit of detection (LOD)	Ref
Dipsticks		Low cost Availability Rapid results	Low sensitivity No reusability Occurrence of false positives or false negatives due to urine complexity Semi-quantitative analysis	15-30 mg/L	[1]
Mass Spectrometry		Sensitivity of the equipment	Expensive equipment. Requires skilled personnel and complex instrumentation	-	[2]
Immunoassay	Fluoroimmunoassay	High sensitivity and specificity. Allows for a quantitative analysis. Can evaluate several samples simultaneously	Requires specific antibodies. Has some propensity for cross-reactivity. Expensive material and equipment	0.5 mg/L	[3]
	Chemiluminescence			0.01 mg/L	[4]
	Electrochemical immunosensor			5×10^{-4} mg/L	[5]
Fluorescence spectroscopy	Time resolved Fluorescence Resonance Energy Transfer	High sensitivity. Non-destructive. Allows for real time evaluation	Requires fluorescent labelling. Can have interference from other fluorophores in the sample	5.5 mg/mL	[6]
	Spectrofluorimetry			0.5 mg/mL	[7]
RAMAN spectroscopy		Non-destructive. No need for labelling	Low sensitivity. Requires specialized expensive equipment	0.1-0.6 μ g/mL	[8]
.Bradford		Fast, simple and inexpensive.	Can have interference from molecules in solution. Lower sensitivity	0.006 mg/mL	[9]
BCA		High-sensitivity. Compatible with detergents.	More expensive than Bradford. Is sensitive to reducing agents.	0.0005 mg/mL	[10]

Table SM2 | Response % data obtained for each smartphone model and each specific condition.

Smartphone model: Samsung Galaxy S21 FE 5G							
Individual	Red Channel	SD	[BSA] _{real}	[BSA] _{calc}	SD	%Recovery	SD
1	152.043	5.925	1	1.4	0.1	144%	6%
	155.427	8.518	2	2.3	0.1	116%	6%
	160.377	6.689	5	5.1	0.2	102%	4%
	165.85	7.365	10	10.5	0.5	105%	5%
	172.814	5.609	25	28	1	110%	4%
	176.907	6.923	50	49	2	99%	4%
	181.917	5.639	97	101	3	104%	3%
2	149.013	5.262	1	0.93	0.03	93%	3%
	155.195	5.111	2	2.3	0.1	112%	4%
	161.083	7.249	5	5.2	0.2	104%	5%
	166.1	10.223	10	10.6	0.7	106%	7%
	172.126	12.062	25	25	2	100%	7%
	177.563	7.037	50	54	2	108%	4%
	182.418	10.119	97	108	6	112%	6%
3	151.3	2.273	1	1.29	0.02	129%	2%
	154.794	2.199	2	2.13	0.03	106%	2%
	160.741	2.657	5	4.95	0.08	99%	2%
	166.507	2.684	10	11.3	0.2	112%	2%
	172.353	2.801	25	25.9	0.4	103%	2%
	177.074	5.498	50	50.6	1.6	101%	3%
	181.907	5.911	97	101	3	104%	3%
4	152.919	5.911	1	1.6	0.1	163%	6%
	156.692	4.231	2	2.8	0.1	139%	4%
	163.583	5.804	5	7.4	0.3	148%	5%
	165.355	6.79	10	9.6	0.4	95%	4%
	172.67	8.758	25	27.0	1.4	108%	5%
	177.973	3.474	50	58	1	115%	2%
	182.213	4.036	97	105	2	108%	2%
5	151.14	11.122	1	1.3	0.1	126%	9%
	154.98	5.022	2	2.2	0.1	109%	4%
	161.367	6.291	5	5.4	0.2	108%	4%
	165.25	11.255	10	9.4	0.6	94%	6%
	171.971	11.837	25	24	2	98%	7%
	176.855	10.929	50	49	3	98%	6%
	181.853	12.774	97	100	7	103%	7%
6	152.234	7.349	1	1.5	0.1	148%	7%
	154.174	3.619	2	1.95	0.05	97%	2%
	161.433	5.748	5	5.5	0.2	109%	4%
	166.211	8.955	10	10.8	0.6	108%	6%
	173.187	5.934	25	29	1	116%	4%
	176.934	2.778	50	49.6	0.8	99%	2%
	181.755	5.049	97	98	3	102%	3%

Smartphone model: iPhone 14 Pro							
Individual	Red Channel	SD	[BSA] _{real}	[BSA] _{calc}	SD	%Recovery	SD
1	150.842	8.03	1	1.2	0.1	121%	6%
	153.924	3.379	2	1.88	0.04	94%	2%
	159.629	3.435	5	4.2	0.1	85%	2%
	165.524	1.497	10	9.8	0.1	98%	1%
	170.765	9.38	25	21	1	82%	5%
	177.037	4.832	50	50.3	1.4	101%	3%
	180.52	10.42	97	83	5	85%	5%
2	149.529	7.117	1	1.01	0.05	100%	5%
	156.569	13.197	2	2.7	0.2	137%	12%
	162.547	4.453	5	6.4	0.2	128%	4%
	166.459	4.133	10	11.2	0.3	112%	3%
	172.463	3.611	25	26.3	0.5	105%	2%
	177.25	5.258	50	51.9	1.5	104%	3%
	182.216	6.772	97	105	4	108%	4%
3	150.673	3.165	1	1.18	0.02	118%	2%
	154.258	7.509	2	2	0.1	98%	5%
	162.053	5.669	5	6	0.2	119%	4%
	166.866	6.145	10	11.8	0.4	118%	4%
	173.148	9.979	25	29	2	116%	7%
	176.738	7.242	50	48	2	96%	4%
	182.807	2.419	97	114.4	1.5	118%	2%
4	150.583	4.334	1	1.17	0.03	117%	3%
	156.62	8.119	2	2.8	0.1	138%	7%
	162.044	15.397	5	6	0.6	119%	11%
	164.512	3.26	10	8.5	0.2	85%	2%
	173.12	6.135	25	29	1	115%	4%
	177.802	5.749	50	56	2	112%	4%
	181.971	7.185	97	102	4	105%	4%
5	150.999	10.417	1	1.2	0.1	124%	9%
	155.096	9.611	2	2.2	0.1	111%	7%
	160.414	7.89	5	4.7	0.2	95%	5%
	166.148	6.04	10	10.7	0.4	107%	4%
	172.778	5.224	25	27.5	0.8	110%	3%
	178.301	7.595	50	60.3	2.6	120%	5%
	183.807	7.063	97	132	5	136%	5%
6	151.332	7.556	1	1.3	0.1	130%	6%
	154.638	3.532	2	2.08	0.05	104%	2%
	161.286	6.411	5	5.4	0.2	107%	4%
	165.821	2.577	10	10.2	0.2	102%	2%
	172.45	6.153	25	28	1	111%	4%
	177.45	5.502	50	53.4	1.7	107%	3%
	182.012	6.35	97	102.2	3.6	105%	4%

Smartphone model: OPPO Reno 8 Lite							
Individual	Red Channel	SD	[BSA] _{real}	[BSA] _{calc}	SD	%Recovery	SD
1	150.314	4.199	1	1.12	0.03	112%	3%
	155.432	4.752	2	2.33	0.07	116%	4%
	160.374	5.64	5	4.7	0.2	94%	3%
	165.419	8.004	10	9.6	0.5	96%	5%
	171.63	5.15	25	23.3	0.7	93%	3%
	177.6	6.041	50	54.5	1.9	109%	4%
	182.211	3.551	97	105	2	108%	2%
2	149.061	5.425	1	0.94	0.03	94%	3%
	154.401	5.1	2	2.01	0.07	100%	3%
	161.74	9.06	5	5.7	0.3	114%	6%
	166.249	6.759	10	10.8	0.4	108%	4%
	171.415	10.428	25	22.6	1.4	90%	5%
	178.01	11.884	50	58	4	116%	8%
	181.98	5.493	97	102	3	105%	3%
3	154.022	3.131	1	1.9	0.04	190%	4%
	155.821	3.154	2	2.46	0.05	123%	2%
	161.252	3.719	5	5.3	0.1	107%	2%
	165.029	4.252	10	9.12	0.2	91%	2%
	171.374	4.504	25	22.5	0.6	90%	2%
	177.115	6.829	50	51	2	102%	4%
	181.198	2.401	97	91	1	94%	1%
4	151.199	5.15	1	1.27	0.04	127%	4%
	156.946	4.6	2	2.89	0.08	144%	4%
	160.937	6.882	5	5.1	0.2	102%	4%
	165.891	6.898	10	10.3	0.4	103%	4%
	171.597	5.182	25	23.2	0.7	93%	3%
	175.772	4.655	50	42	1	84%	2%
	182.185	5.457	97	105	3	108%	3%
5	151.229	6.579	1	1.28	0.06	128%	6%
	155.266	6.209	2	2.3	0.1	114%	5%
	160.677	7.121	5	4.9	0.2	98%	4%
	165.557	11.85	10	9.8	0.7	98%	7%
	171.007	4.489	25	21.4	0.6	86%	2%
	179.007	4.489	50	66.6	1.7	133%	3%
	182.446	8.906	97	108.7	5.3	112%	5%
6	150.053	6.252	1	1.08	0.04	108%	4%
	154.975	3.33	2	2.18	0.05	109%	2%
	160.525	7.834	5	4.8	0.2	96%	5%
	165.857	7.369	10	10.3	0.5	103%	5%
	171.468	4.104	25	22.8	0.5	91%	2%
	176.947	2.423	50	49.7	0.7	99%	1%
	180.835	4.522	97	86	2	89%	2%

References

- [1] S. Sahu, J. John, A. Augusty, Estimation of 24 h Urine Protein Versus Spot Urine Protein Creatinine Ratio in Patients with Kidney Disease, *Indian J Clin Biochem* 37 (2022) 361–364. <https://doi.org/10.1007/S12291-020-00943-0>.
- [2] X. Yang, Y. Tang, R.R. Alt, X. Xie, F. Li, Emerging techniques for ultrasensitive protein analysis, *Analyst* 141 (2016) 3473–3481. <https://doi.org/10.1039/C6AN00059B>.
- [3] B.M. Chavers, J. Simonson, A.F. Michael, A solid phase fluorescent immunoassay for the measurement of human urinary albumin, *Kidney Int* 25 (1984) 576–578. <https://doi.org/10.1038/ki.1984.57>.
- [4] J.K. Horton, M. Davies, J.S. Woodhead, I. Weeks, A rapid and sensitive method for estimating low concentrations of albumin in human urine, *Clinica Chimica Acta* 186 (1989) 45–51. [https://doi.org/10.1016/0009-8981\(89\)90202-7](https://doi.org/10.1016/0009-8981(89)90202-7).
- [5] G. Ning, W. Lu-Yan, X. Wei-Min, L. Tian-Hua, J. Qian-Li, Electrochemical Immuno-Biosensor for the Rapid Determination of Nuclear Matrix Protein 22 (NMP22) antigen in Urine Samples by Co(III) Phthalocyanine/Fe₃O₄/Au Collide Coimmobilized Electrode, *Chinese Journal of Analytical Chemistry* 35 (2007) 1553–1558. [https://doi.org/10.1016/S1872-2040\(07\)60093-0](https://doi.org/10.1016/S1872-2040(07)60093-0).
- [6] Q.-P. Qin, O. Peltola, K. Pettersson, Time-resolved Fluorescence Resonance Energy Transfer Assay for Point-of-Care Testing of Urinary Albumin, *Clin Chem* 49 (2003) 1105–1113. <https://doi.org/10.1373/49.7.1105>.
- [7] Z. Xu, W. Yang, C. Dong, Determination of human serum albumin using an intramolecular charge transfer fluorescence probe: 4'-Dimethylamino-2,5-dihydroxychalcone, *Bioorg Med Chem Lett* 15 (2005) 4091–4096. <https://doi.org/10.1016/j.bmcl.2005.06.014>.
- [8] N. Chamuah, A. Saikia, A.M. Joseph, P. Nath, Blu-ray DVD as SERS substrate for reliable detection of albumin, creatinine and urea in urine, *Sens Actuators B Chem* 285 (2019) 108–115. <https://doi.org/10.1016/j.snb.2019.01.031>.
- [9] S. Chutipongtanate, K. Watcharatanyatip, T. Homvises, K. Jaturongkakul, V. Thongboonkerd, Systematic comparisons of various spectrophotometric and colorimetric methods to measure concentrations of protein, peptide and amino acid: Detectable limits, linear dynamic ranges, interferences, practicality and unit costs, *Talanta* 98 (2012) 123–129. <https://doi.org/10.1016/j.talanta.2012.06.058>.
- [10] A.N. Ngo, Optimal Concentration of 2,2,2-Trichloroacetic Acid for Protein Precipitation Based on Response Surface Methodology, *J Anal Bioanal Tech* 5 (2014). <https://doi.org/10.4172/2155-9872.1000198>.