Accessible Telemedicine Diagnostics with ELISA in a 3D Printed Pipette Tip

Mohamed Sharafeldin,[a,b,1] Karteek Kadimisetty,[a,1] Ketki R. Bhalerao,[a] Itti Bist,[a] Abby Jones,[a] Tianqi Chen,[a] Norman H. Lee,[f] and James F. Rusling*[a,c,e]

[a] Department of Chemistry, University of Connecticut, Storrs, CT 06269, USA.

[b] Analytical Chemistry Department, Faculty of Pharmacy, Zagazig University, Zakazik, Sharkia, Egypt

[c] Institute of Material Science, Storrs, CT 06269, USA.

[d] Department of Surgery and Neag Cancer Center, UConn Health, Farmington, CT 06032.

[e] School of Chemistry, National University of Ireland at Galway, Ireland.

[f] Department of Pharmacology & Physiology, George Washington University, Washington, DC 20037, United States.

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[1] These authors contributed equally to this work.

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Pipette tip 3D design and dimensions

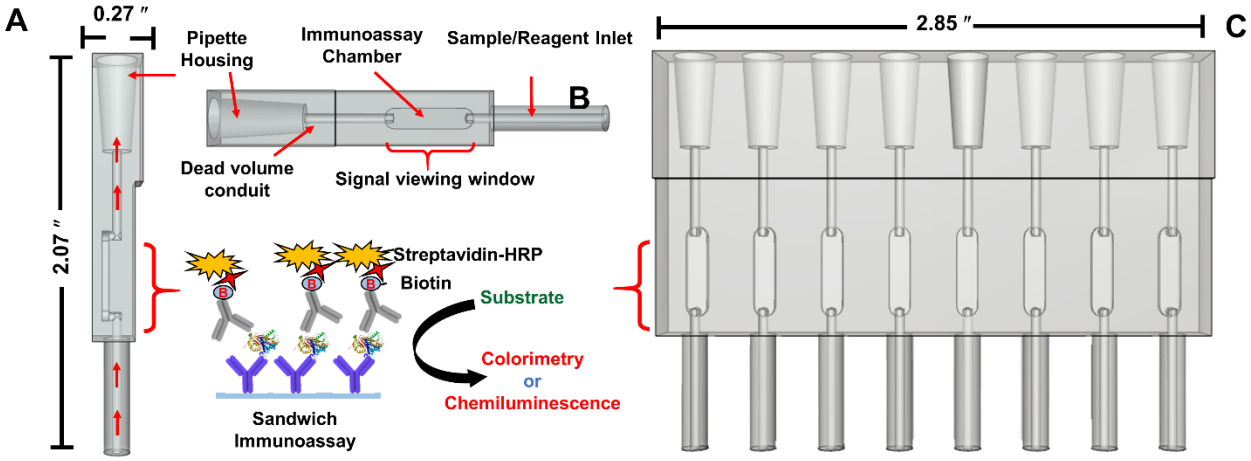

Figure S1. 3D Design of ELISA tip platform. (A) A single pipette ELISA tip array, side view showing flow of reagents from bottom into the immunoassay chamber derived by vacuum suction applied through pipette attached at pipette housing on the top. (B) Front view, immunoassay chamber as a colorimetric/chemiluminescent viewing window. Inset showing sandwich immunoassay on the surface of immunoassay chamber. (C) Multi-chamber pipette ELISA tip used via a multichannel pipette for multianalyte or multiplex detection.

Photographs of the single and multichannel printed tips

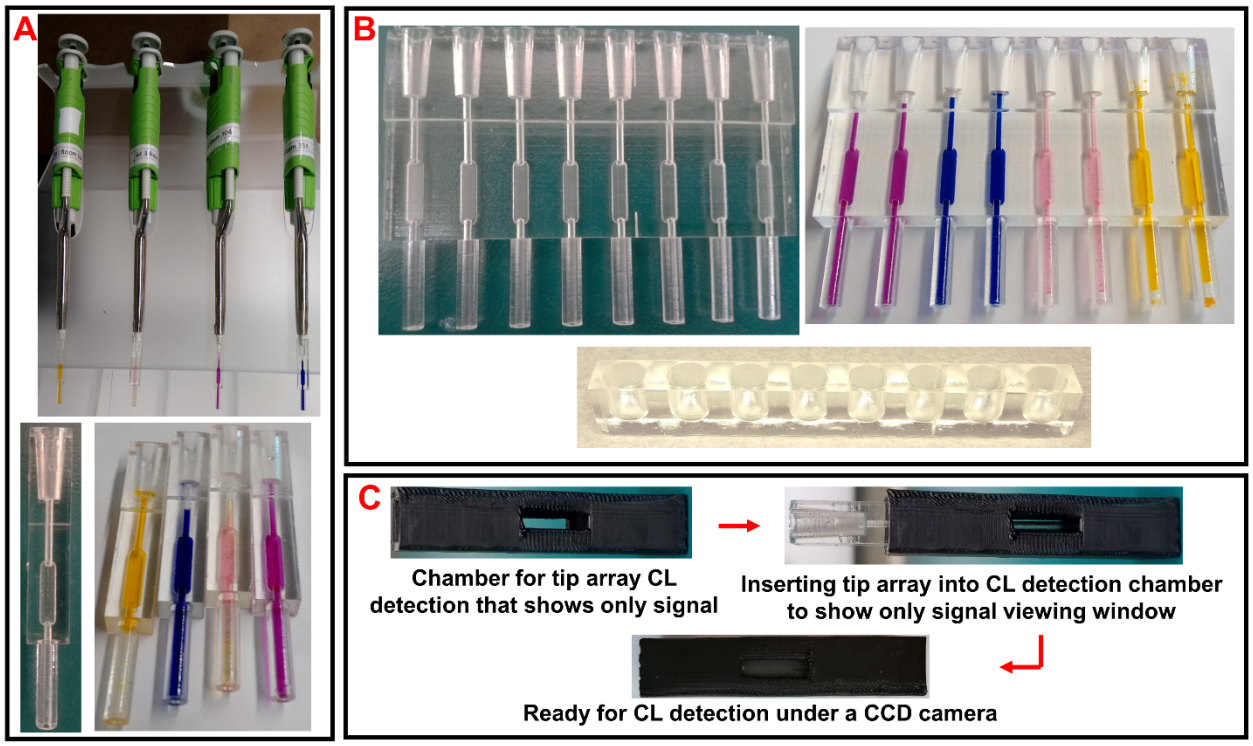

Figure S2. (A) Photographs of the single chamber ELISA in a tip array, as printed and filled with different colored dyes. (B) Multi chamber/ 8 channel ELISA in a tip platform, as printed and colored dye filled showing 4 biomarker detection application. Inset 8 well 3D printed add-on for use in multi analyte analysis. (C) A non-transparent chamber for CL detection that blocks unwanted CL from the sampling conduit and dead volume conduit.

Optimized capture and detection antibodies concentrations

Table S1. Selected optimized capture and detection antibody concentrations for sandwich immunoassay

Protein Biomarker	Capture [Ab_1] (µg/mL)	Detection [Ab_2] (µg/mL)
PSA	10	1.0
VEGF	6	0.6
IGF-1	7.2	1.0
CD-14	10	1.0

Color Grab on android smartphone image analysis

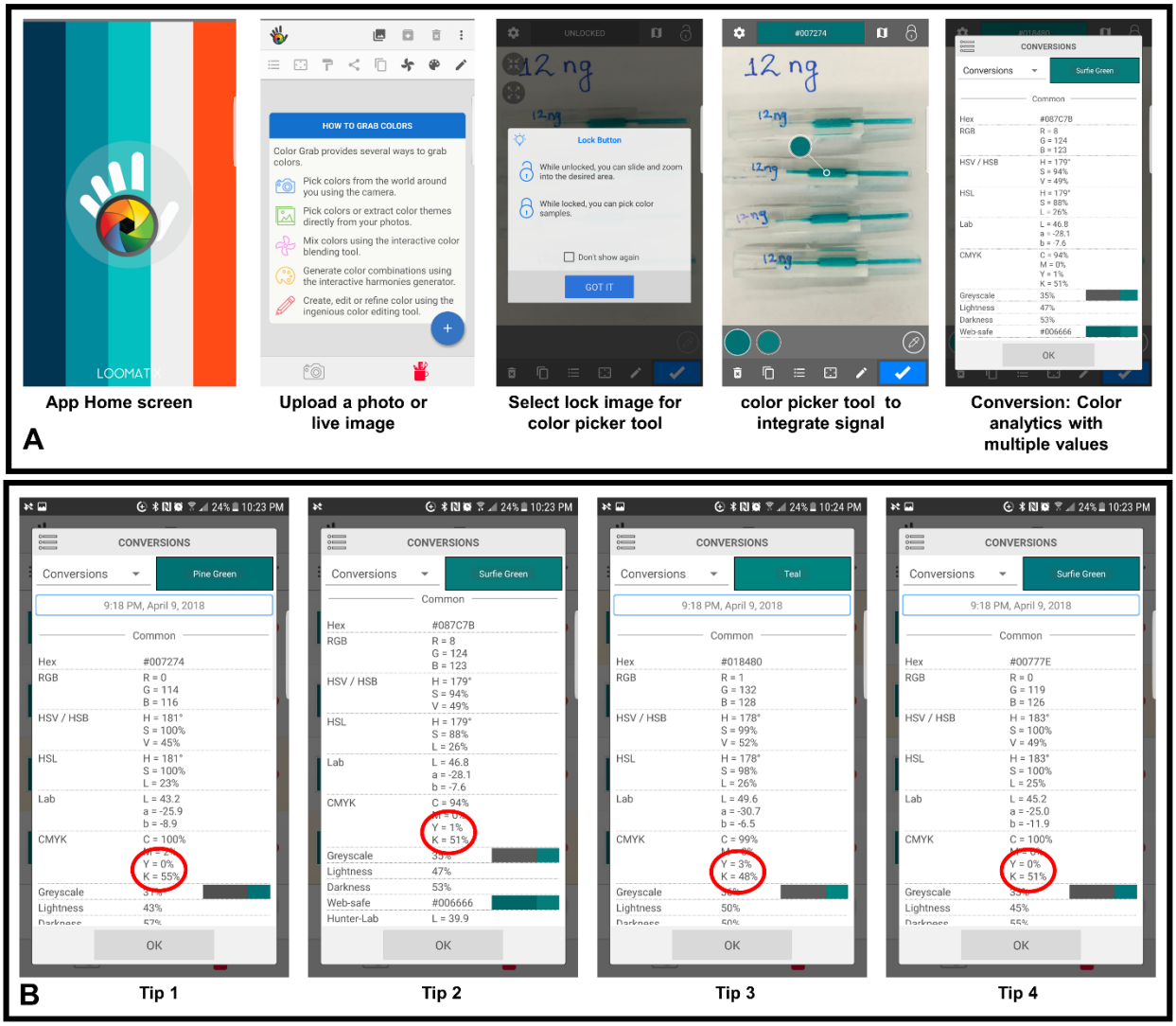

Figure S3. Screen shots from smartphone color analysis software "ColorGrap" of the ELISA in a Tip array. (A) Simple color analysis is showed step wise from upload to a photo to picking color to provide color analytics. (B) Color analysis sample output for 4 tip arrays as a part of reproducibility experiments and highlighted in the red circles is the selected parameter of developing calibration curves.

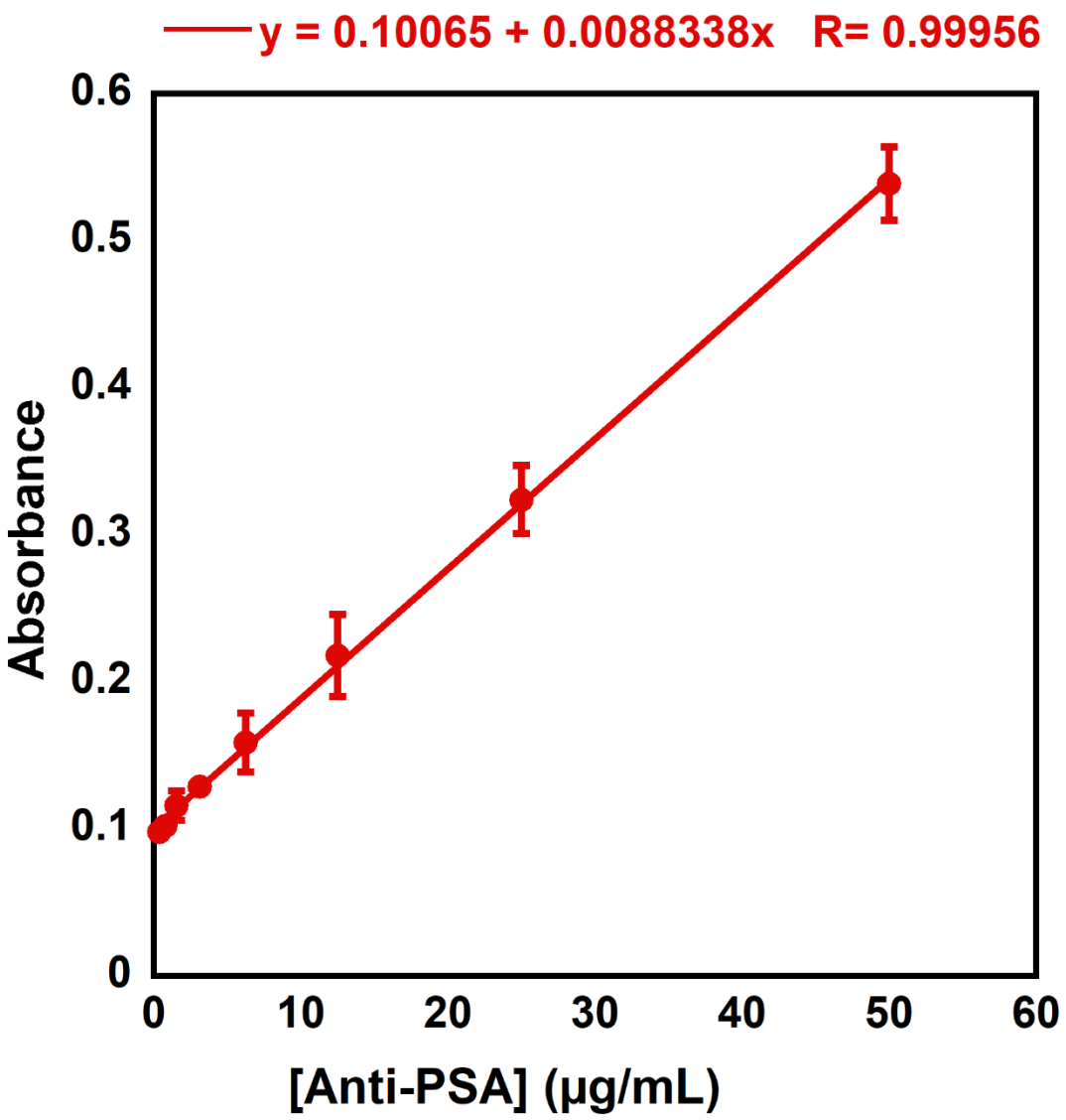

Fig. S4: Bicinchoninic acid (BCA) assay for prostate specific antigen (PSA) detection antibody quantification.[1] 100 µL of different concentrations of Ab_2 were mixed with 100 µL of the BCA reagent and incubated at 37˚ C for 2 hrs. Absorbance of the mixture at 562 nm was plotted against Ab_2 concentration to construct calibration curve. Calibration curve was used to estimate the concentration of antibody bound to 3D printed chips with one cm^2 surface area. Antibody coverage was estimated to be 10 ± 5% µg/cm^2 which is approximately $1.0X10^{13}$± 5% antibodies/cm^2.

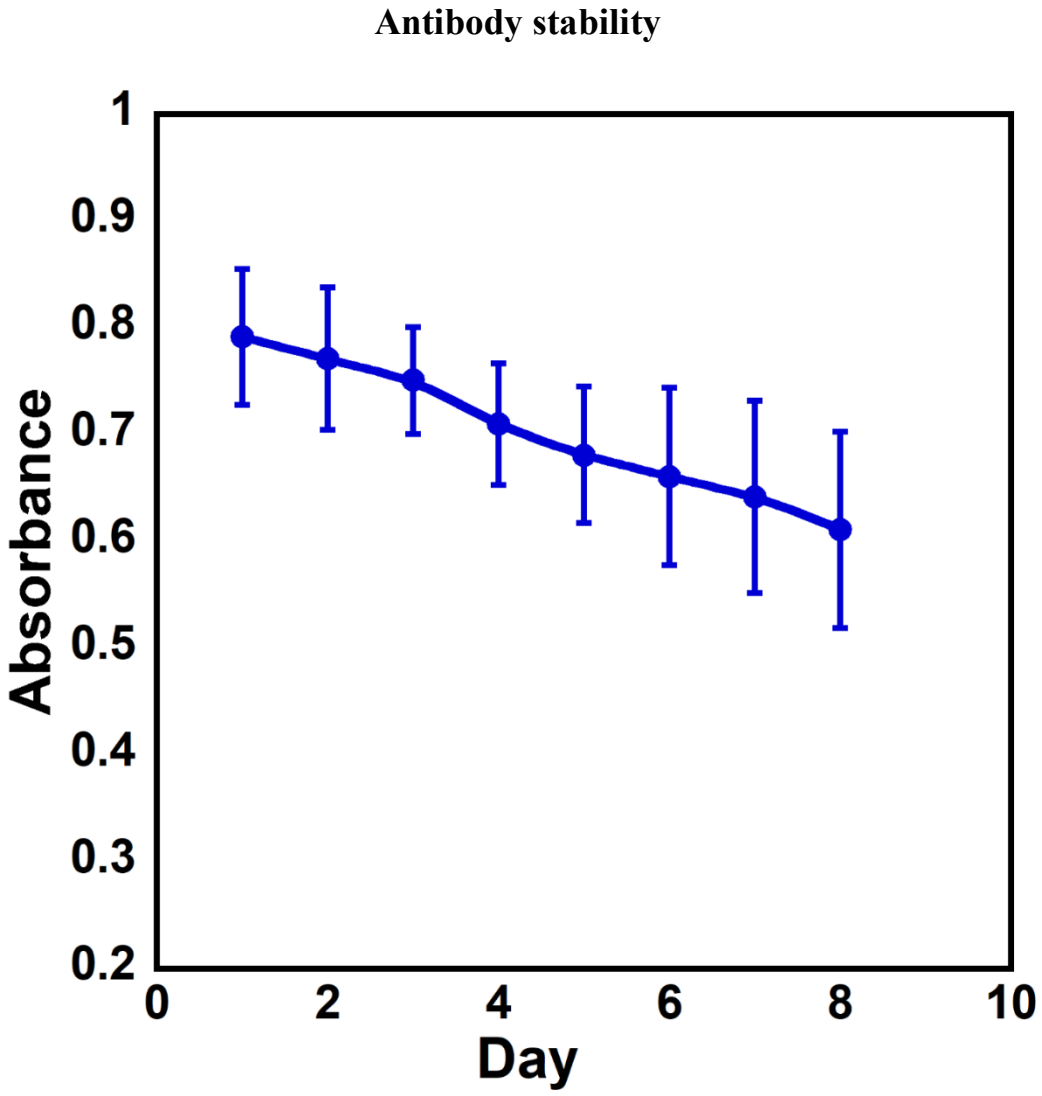

Fig. S5: Antibody stability study. Colorimetric absorbance at 450 nm measured after running assay in tips stored at 4˚C for up to 7 days. Tips maintained 80% of its original activity after 7 days of storage (n=3)

Receiver operating characteristics (ROC) curve analysis

While the number of samples analyzed is much too small to make definitive conclusions about diagnostic value, we determined ROC curves to obtain a preliminary prediction.[2] ROC curves demonstrated that PSA has the best positive predictive value for prostate can cancer among the three biomarkers as shown by the area under the curve (AUC) of 0.967 compared to 0.700 for IGF-1 and 0.833 for CD14 (Fig. S5). Adding up the values of the three biomarkers showed a stronger correlation than any as demonstrated by AUC of 1.000.

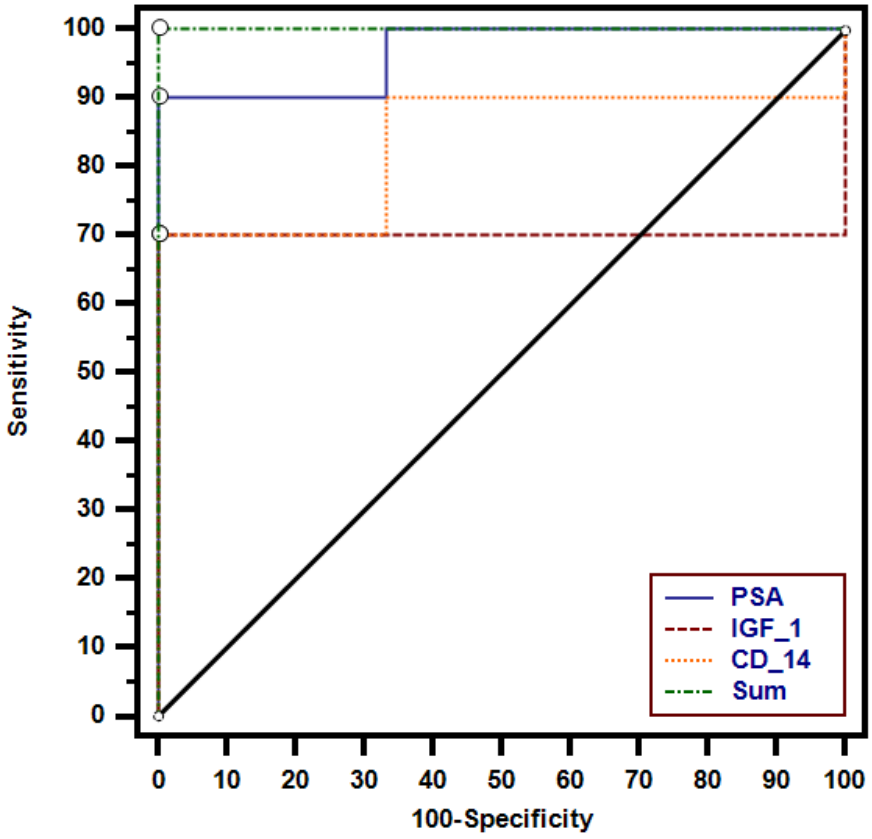

Fig. S6: Receiver operating characteristic (ROC) curve for PSA (blue), IGF-1 (red), CD-14 (orange) and sum of the values of the 3 biomarkers (green). Data collected from analysis of 13 real human samples consisting of 3 controls and 10 prostate cancer samples. AUC were 0.967 for PSA, 0.700 for IGF-1, 0.833 for CD-14 and 1.000 for the sum of the concentrations of the 3 biomarkers.

Patient sample Analysis/ micro-well ELISA

Procedure: ELISA kits for Prostate Specific Antigen (PSA) (DY1344), Insulin-Like Growth Factor 1 (IGF-1) (DY291) and Cluster of Differentiation-14 (CD-14) (DY383) were from R&D systems. Micro-well ELISAs were performed following protocol and concentrations specified by the vendor. Briefly, micro-well plates were coated with Ab_1 by incubating them with Ab_1 solutions overnight at RT, plates decorated with Ab_1 were washed 3X with PBS-T20, blocked with 1% BSA in PBS and again washed 3X with PBS-T20. 100 µL of standards/diluted patient samples were incubated for 2hr at RT, washed 3X with PBS-T20 and then incubated with 100 µL of Ab_2 for 2hr at RT. Plates were washed 3X with PBS-T20 and allowed to incubate with 100 µL ST-HRP for 20 min, washed 3X with PBS-T20 and incubated for 15 min with TMB and color developed was measured at 450 nm. Patient samples were diluted with PBS 10X for PSA and IGF-1 and 100X for CD-14 to bring target analytes to working concentration of the assay.

Results: calibrations between analyte concentration and absorbance at 450 nm (Fig. S7) were used to calculate target antigen concentration in analyzed patient samples. Found concentrations were compared to concentration calculated using Tip-assay (Table S1-Table S3). Correlation coefficients were 0.999 for PSA, 0.986 for IGF-1 and 0.981 for CD-14 and slopes were 0.775 for PSA, 0.945 for IGF-1 and 0.917 for CD-14. These results indicated a very good correlation between developed Tip-ELISA and reference standard micro-well ELISA.

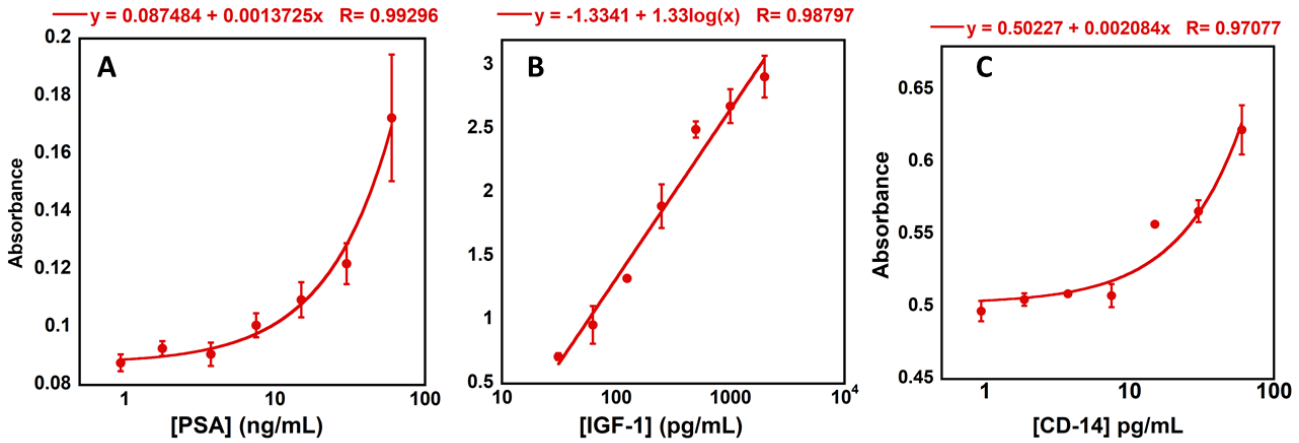

Fig. S7: Calibration curves for (A) PSA, (B) IGF-1, and (C) CD-14 obtained by plotting absorbance at 450 nm against standard antigen concentration. Absorbance at 540 nm was subtracted and n=3.

Table S2: Comparison between calculated conc. of PSA in patient samples between Micro-well ELISA and Tip-ELISA

Cancer Status	Patient Sample Code	PSA			
		Tip-ELISA		Micro-well ELISA	
		Found Conc. ng/mL	St. Dev.	Found Conc. ng/mL	St. Dev.
Controls	**C3**	0.21	0.00	0.72	0.08
	C8	0.49	0.04	0.45	0.03
	C18	0.28	0.01	1.23	0.17
Indolent Prostate cancer (Gleason score <7)	**23**	1.01	0.45	8.87	1.15
	24	0.31	0.03	0.23	0.02
	25	2.81	0.03	0.72	0.06
	27	4.77	0.75	10.70	1.18
	28	1.27	0.01	1.58	0.16
Aggressive Prostate cancer (Gleason score >7)	**1114**	3.09	0.14	2.54	0.15
	1115	9.45	17.20	10.81	1.41
	1123	7.90	18.10	2.34	0.05
	1136	1030.67	33.74	800.05	56.00
	1138	64.25	1.80	62.61	5.01

Table S3: Comparison between calculated conc. of IGF-1 in patient samples between Micro-well ELISA and Tip-ELISA

Cancer Status	Patient Sample Code	IGF-1			
		Tip-ELISA		Micro-well ELISA	
		Found Conc. ng/mL	St. Dev.	Found Conc. ng/mL	St. Dev.
Controls	**C3**	6.99	0.04	4.27	0.05
	C8	5.97	0.07	4.78	0.04
	C18	4.81	0.00	5.02	0.07
Indolent Prostate cancer (Gleason score <7)	**23**	2.77	0.12	2.35	0.02
	24	31.45	0.40	26.83	0.13
	25	2.42	0.01	2.28	0.00
	27	1.83	0.00	2.07	0.01
	28	2.32	0.03	2.13	0.01
Aggressive Prostate cancer (Gleason score >7)	**1114**	24.84	0.15	24.65	0.35
	1115	29.36	0.85	29.37	0.21
	1123	11.13	0.00	9.60	0.07
	1136	2.46	0.71	2.16	0.02
	1138	3.24	0.47	2.30	0.18

Table S4: Comparison between calculated conc. of CD-14 in patient samples between Micro-well ELISA and Tip-ELISA

Cancer Status	Patient Sample Code	CD-14			
		Tip-ELISA		Micro-well ELISA	
		Found Conc. ng/mL	St. Dev.	Found Conc. ng/mL	St. Dev.
Controls	**C3**	0.17	0.01	0.35	0.03
	C8	0.26	0.01	0.53	0.07
	C18	0.76	0.07	0.87	0.07
Indolent Prostate cancer (Gleason score <7)	**23**	0.66	0.01	1.07	0.10
	24	0.62	0.03	1.25	0.04
	25	1.14	0.03	1.20	0.08
	27	4.90	0.20	4.82	0.05
	28	1.48	0.10	1.12	0.13
Aggressive Prostate cancer (Gleason score >7)	**1114**	2855.21	6.75	2220.26	310.84
	1115	3006.53	9.82	3053.69	335.91
	1123	2530.48	20.15	2434.10	121.70
	1136	602.40	33.24	610.16	42.71
	1138	532.71	34.37	630.76	94.61

Table S5: Comparison between calculated conc. of IGF-1 in patient samples between Micro-well ELISA and Tip-ELISA using smart phone imaging with ColorGrab®

Cancer Status	Patient Sample Code	IGF-1			
		Tip-ELISA		Micro-well ELISA	
		Found Conc. ng/mL	St. Dev.	Found Conc. ng/mL	St. Dev.
Controls	**C3**	4.78	0.15	4.27	0.05
	C8	4.90	0.10	4.78	0.04
	C18	5.26	0.16	5.02	0.07
Indolent Prostate cancer (Gleason score <7)	**23**	1.81	0.06	2.35	0.02
	24	28.68	0.78	26.83	0.13
	25	2.29	0.03	2.28	0.00
	27	1.46	0.19	2.07	0.01
	28	2.29	0.13	2.13	0.01
Aggressive Prostate cancer (Gleason score >7)	**1114**	19.39	0.84	24.65	0.35
	1115	31.16	2.67	29.37	0.21
	1123	8.45	0.24	9.60	0.07
	1136	1.60	0.10	2.16	0.02
	1138	2.29	0.13	2.30	0.18

Electrochemical Assay Procedure

Multiplexed quantification of 8 protein biomarkers panel was performed on a 16-electrode system array recently developed in our lab. Only 3 biomarkers were chosen to establish a comparison between the performances of the two systems based on the results obtained from the analysis of 13 patient samples. The electrochemical assay was completed using Kanichi® screen printed carbon electrode decorated with capture antibodies following a layer-by-layer method previously reported.[3] Prior to insertion into the double microfluidic device the arrays were incubated at 4°C for 1 hour with 2% (BSA) in PBS buffer. Arrays were then washed in the microfluidic device at a flow rate of 100 µL min^{-1}. A mixture of detection antibody from either the set of high or low concentration biomarkers, streptavidin poly-HRP, and either antigen or 100 times diluted patient sample was immediately injected at the same flow rate. The high concentration biomarkers were incubated for 10 minutes whereas the low concentration biomarkers were incubated for 20 minutes. Both sides were washed with 0.05% Tween 20 in PBS following each incubation. A solution of 1 mM hydroquinone was then flowed through the channel before detection and amperometric responses were recorded at -0.2 V vs Ag/AgCl, accomplished by injecting a mixture of 100 mM H_2O_2 and 1 mM HQ in PBS.

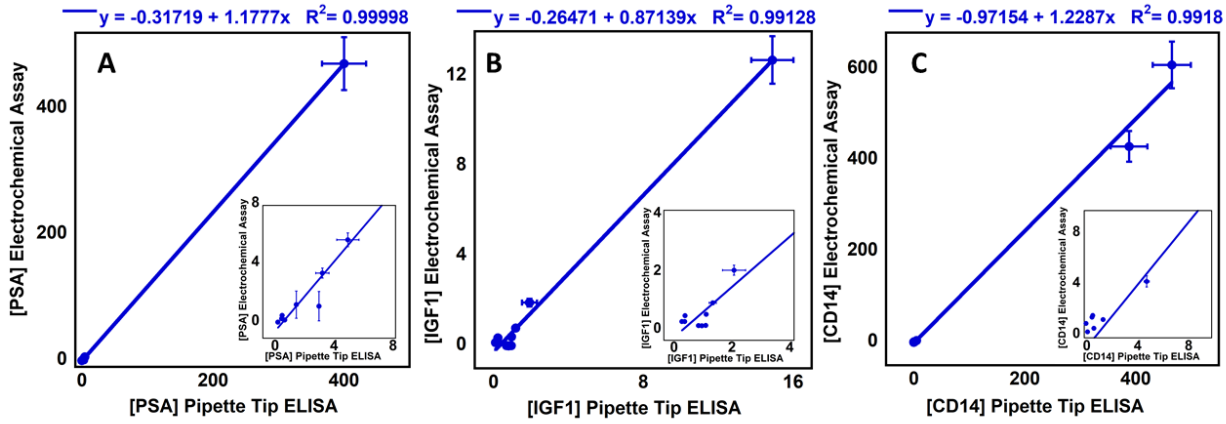

Fig. S8: Linear correlations for pipette tip ELISA vs. referee assay for (A) PSA, (B) IGF1, and (C) CD14, quantified in ng/mL (n=3) in patient samples. Insets show low concentration ranges.

Spike recovery validation of Electrochemical Assay

Electrochemical assay was validated by calculating percent recoveries of selected biomarkers from spiked human serum. Each biomarker was run at three different levels, low, medium and high in order to insure assay performance. Percent recoveries varied between 90-120 % indicating good assay performance (Table S5).

Table S5: Calculated percent recoveries from spiked human serum samples using electrochemical assay

Biomarker	Percent recovery		
	Low concentration (0.002-0.009 ng/mL)	**Mid concentration (0.01- 0.6 ng/mL)**	**High concentration (0.4- 9.7 ng/mL)**
PSA	91.9	115.9	116.28
IGF-1	113.3	98.6	120.8
CD-14	96.3	105.1	96.9

IGF-1 smart phone imaging colorimetric Tip-ELISA assay correlation to conventional ELISA

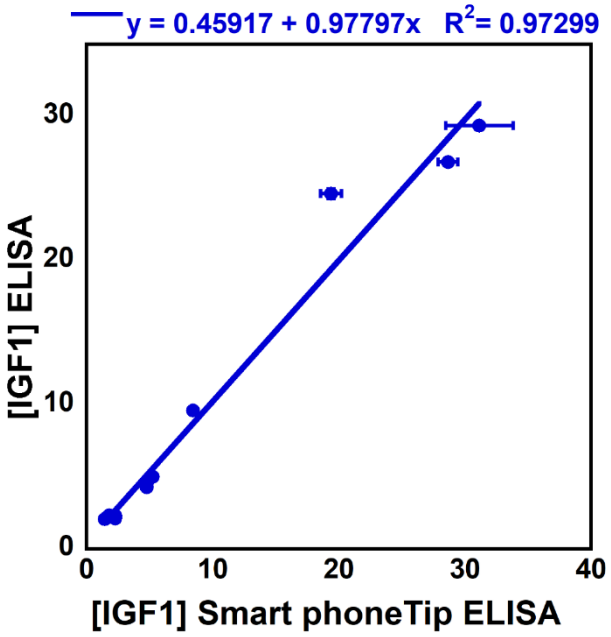

Fig. S9: Linear correlations for smart phone pipette tip ELISA vs. referee assay for IGF-1, quantified in ng/mL (n=3) in patient samples.

IGF-1 concentration in 10X diluted serum samples was also calculated using smart phone imaging colorimetric assay and had correlation coefficient of 0.973 to conventional ELISA with slope of 0.978.

Box-and-whisker plots

Clustered multiple variables box plots [4] show the found expressions of the three biomarkers in the human serum samples, with Fig. 6A showing cancer-free, indolent cancer patient, and aggressive cancer patient groups and Fig. 6B shows cancer-free and combine cancer patient groups on a log scale so that low concentrations can be visualized. Each box includes values that lie between upper and lower quartile (25th to 75th percentile). Vertical lines include maximum and minimum concentration of each biomarker and the horizontal line in each box represents the median value

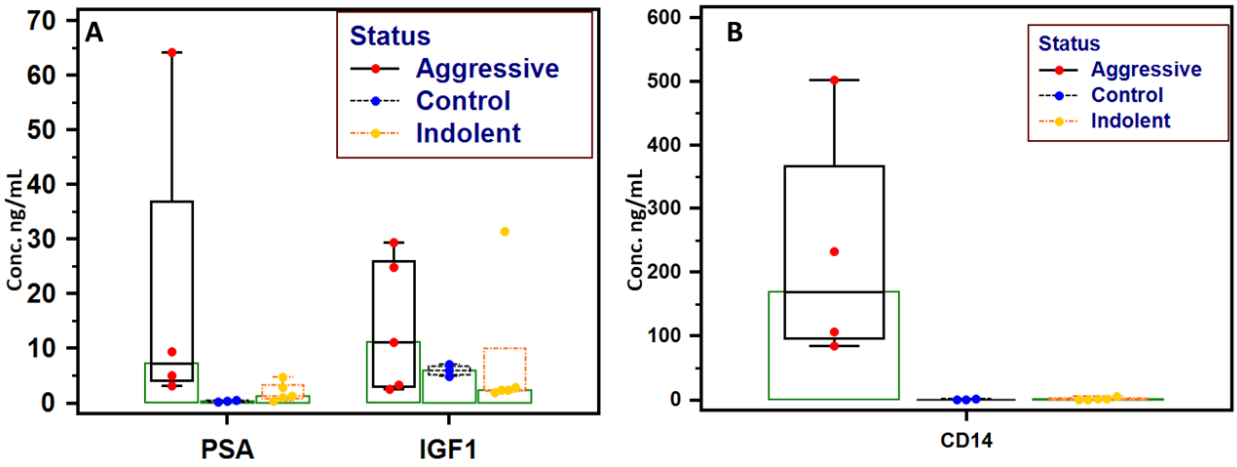

Fig. S10: Box-and-whisker plots representing amounts of each biomarker in the human samples: (A) PSA and IGF-1 serum level comparison; (B) CD-14 serum level comparison between control group (blue), indolent prostate cancer group (yellow), and aggressive prostate cancer group (red). Results obtained from tip-based assay using colorimetric procedures (n=3).

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