Supporting Information

Integrated Biosensor for Rapid and Point-Of-Care Sepsis Diagnosis

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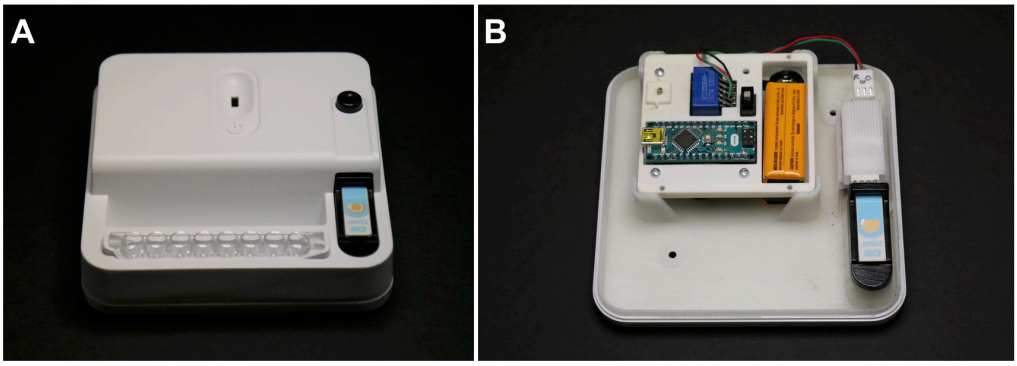

Figure S1. (A-B) IBS device exterior and interior.

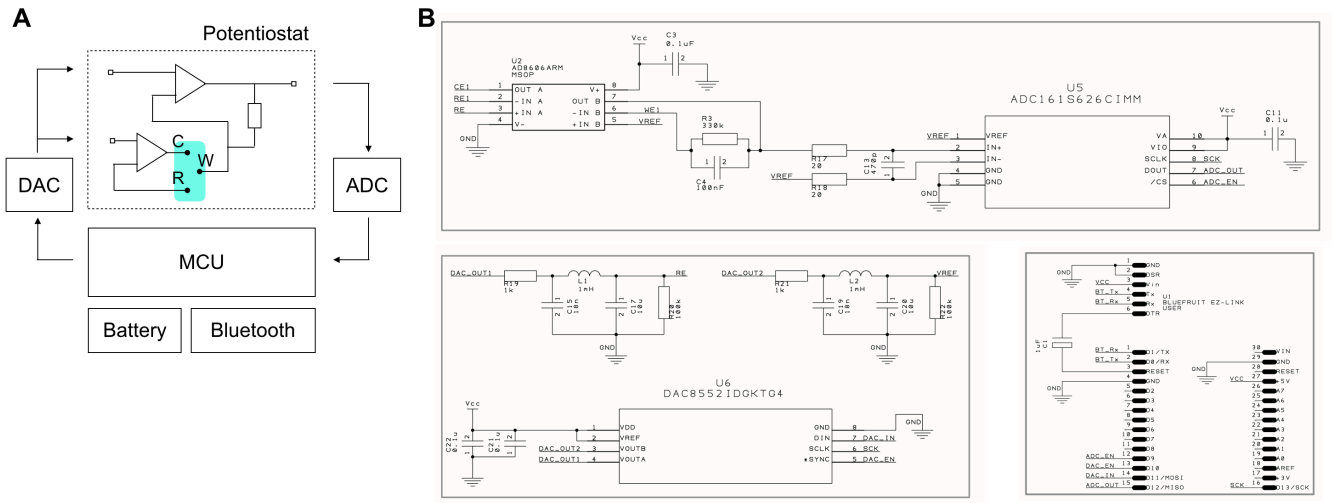

Figure S2. Circuit schematic. (A) The device housed a custom-designed potentiostat, an analog-to-digital converter (ADC), a digital-to-analog converter (DAC), and a microcontroller unit (MCU). Electrical currents fro working (W) to counter (C) electrodes were measured with a constant potential was applied between working and reference (R) electrodes. (**B**) (**Top**) A potentiostat with an analog-to-digital converter. The operational amplifier (AD8606 , Analog Devices) was connected to the parallel circuit of R3 (330 kΩ) and C4 (100 nF), which formed a transimpedance amplifier with a low-pass filter with a cut-off frequency 5 Hz. The signal from the working electrode flowed through the transimpedance amplifier and the low-pass filter, and became the input (IN+) of the differential analog-to-digital converter (ADC161S626, Texas Instruments). Another input (IN-) of the analog-digital converter was connected to the VREF, a set value for the electric potential WE1. The difference between two electric potentials (WE1 - VREF) was converted. (**Bottom left**) A digital-to-analog converter (DAC8552, Texas Instruments). The set values for the electric potentials applied to the reference electrode (RE) and the working electrode (VREF) were generated through the digital-to-analog converter. (**Bottom right**) A microcontroller unit (ATmega328, Microchip). It was connected to a bluetooth communication module (Blue fruit EZ-Link, Adafruit).

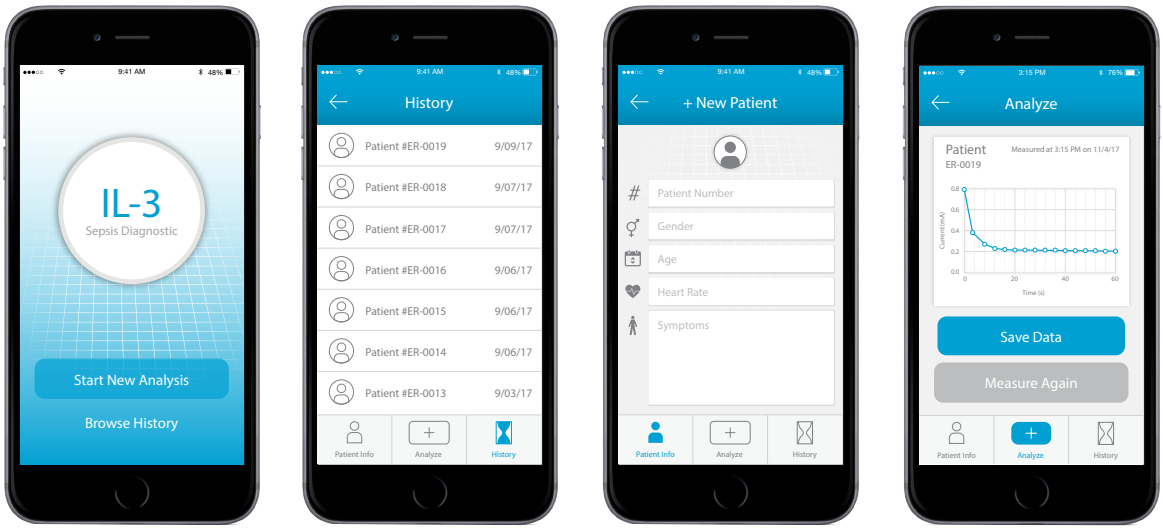

Figure S3. Screenshots of IBS App user interface. The smartphone App communicates with the sensor *via* Bluetooth and automatically uploads data to a cloud server. Functions include i) registering new patient information, ii) displaying measurement results, iii) storing measurement value and date/time, and iv) tracking patient history.

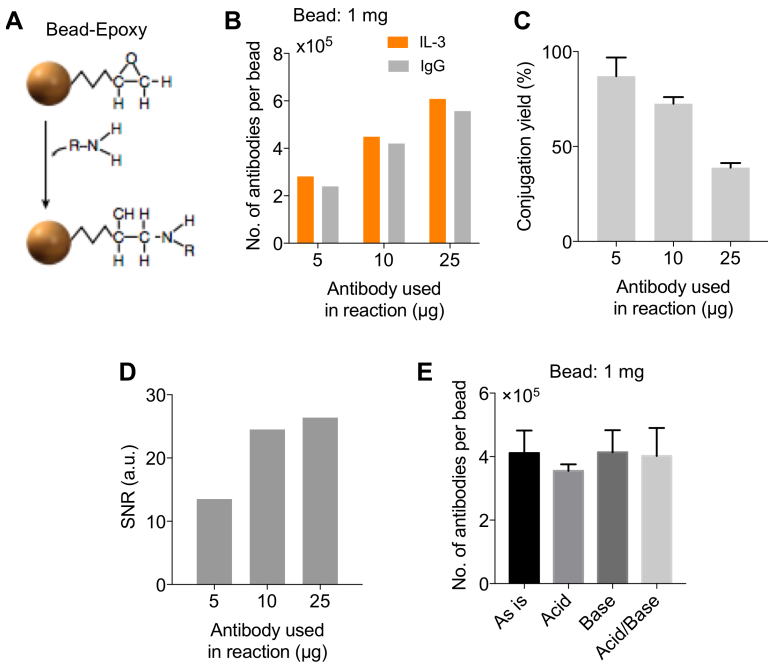

Figure S4. Optimization of bioconjugation. (A) Conjugation chemistry for attaching antibodies to magnetic beads. **(B)** The number of antibodies per bead was estimated. For a given bead number, antibody concentrations in the reaction were increased. **(C)** The conjugation yield [= antibody bound to beads) / (antibody used in the reaction)] were estimated. The yield was <50% when large amount of antibodies were used. **(D)** Antibody-bead conjugates from (B) were used for electrochemical detection. Higher signal-to-noise ratio (SNR) was observed when magnetic beads with higher number of antibodies were used. But the rate of SNR increase plateaued. **(E)** Antibody-conjugated beads were subject to basic (0.1 M NaOH), acidic (0.1 M HCl), or both type of buffers, and resuspended in PBS. Such pH challenges would break non-covalent bonding including electrostatic or hydrophobic interactions. Antibody amounts on beads, measured by the BCA assay, remained the same after each pH challenge; this results confirmed the covalent interactions between epoxy-coated beads and antibodies.

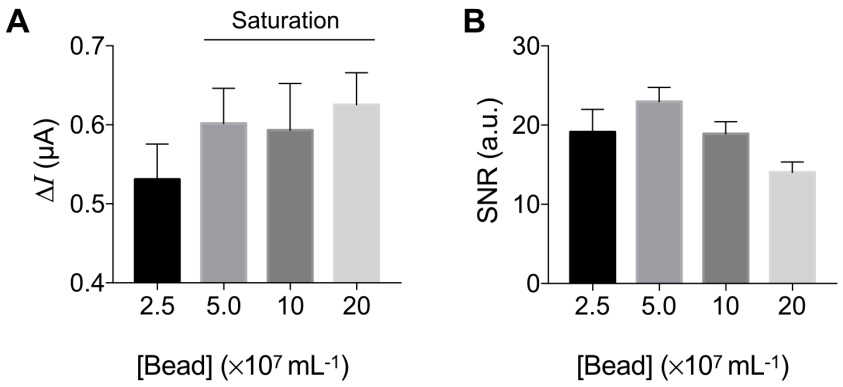

Figure S5. Optimization of the IBS assay. (A) Magnetic beads targeting IL-3 were used to detect 1 ng/mL IL-3 in human serum. Bead concentrations > 2.5 × 10^7 mL^{-1} led to the signal saturation. **(B)** The optimal bead concentration was set to ~5.0 x 10^7 mL^{-1} (*i.e.*, ~ 5.0 × 10^6 beads per test) as it resulted in the best signal-to-noise ratio (SNR). The data are displayed as mean ± SD from triplicate measurements.

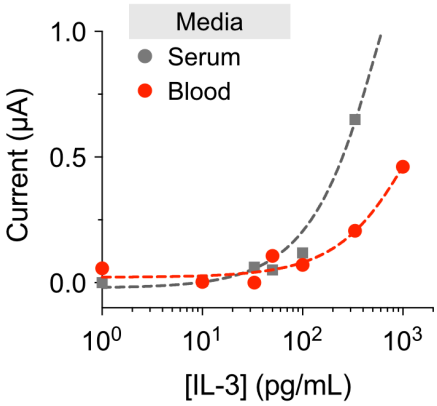

Figure S6. IBS assay with whole blood. Varying concentrations of IL-3 were spiked into human whole blood, assayed by IBS, and compared with the serum data.

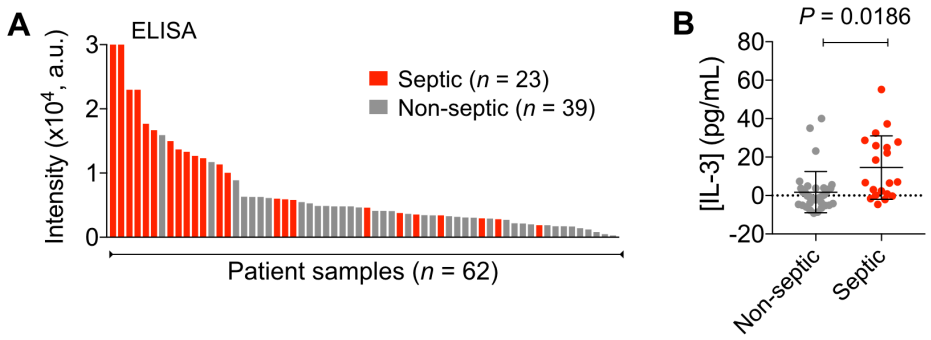

Figure S7. ELISA analysis. Patient samples from 23 septic patients and 39 non-septic patients were analyzed with ELISA. **(A)** A waterfall plot shows the ELISA signals sorted from high (left) to low (right). Each column represents a different patient sample (red, septic; grey, non-septic). **(B)** Compared to IBS, ELISA was not as sensitive in distinguishing septic from non-septic controls. *P* value was determined by unpaired t-test. **P* > 0.01.

Table S1. Device Cost and Assay Cost.

Component	Cost ($)
Analog to digital converter (ADC161S626)	7.18
Microcontroller unit (ATmega328)	2.07
Digital to analog converter (DAC8552)	3.78
Amplifier (AD8606)	2.03
Bluetooth module (Bluefruit EZ-link)	22.50
9V Battery	1.19
Magnetic pipette (a magnet and 3D printed body)	5.00
Others (resistor, capacitance, electric board, case)	5.00
Total	**48.75**

Reagent & Disposable	Price ($)	Total amount	Amount /test	# of tests	Cost/ test ($)
Dynabead M-270 Epoxy (Thermo, 14301)	394.00	60 mg (4 × 10^9 beads)	0.15 mg (1 × 10^7 beads)	400	0.99
Human IL-3 Antibody (RnD, MAB603R-01M)	1299.00	1000 µg	1.0 µg	1000	1.30
Mouse IgG1 Isotype Control (RnD, MAB002)	175.00	500 µg	1.0 µg	500	0.35
Human IL-3 Biotinylated Antibody (RnD, BAF203)	420.00	50 µg	0.05 µg	1000	0.42
Streptavidin-HRP (Thermo, 21130)	202.98	0.5 mL	0.05 µL	10000	0.02
TMB Substrate Solution (Thermo, 34028)	142.88	250 mL	40 uL	6250	0.02
8-tube strip	162.97	400	2	200	0.81
Disposable tip/cover for Magnetic pipette (ThermoFisher, 4358293)	95.00	125	1	125	0.76
				Total	**4.67**