bio-stream DIAGNOSTICS INC

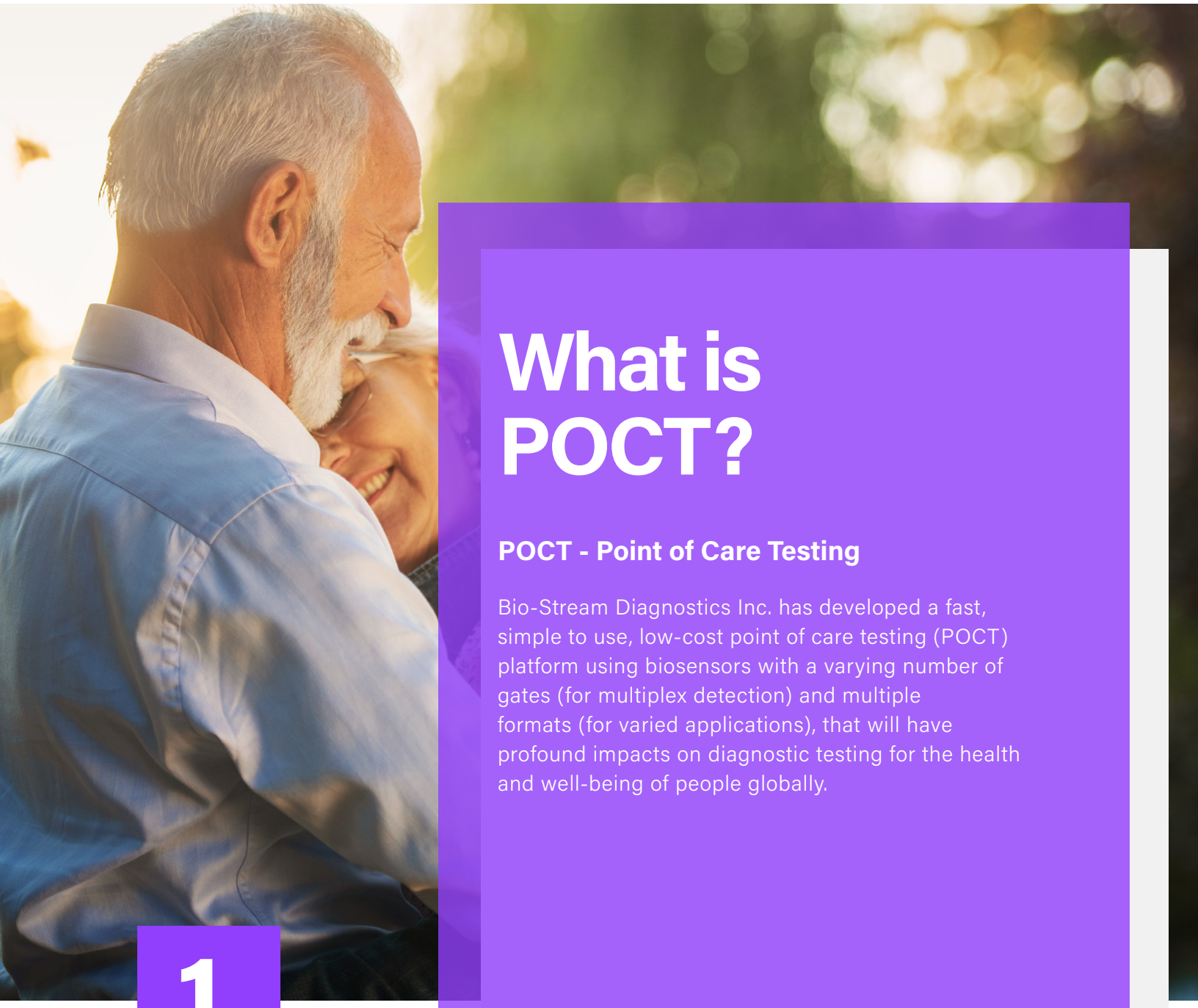
trax™

A Sensor Development and Commercialization Platform

About Us

Bio-Stream Diagnostics Inc. is a privately owned medical device manufacturing and software development company headquartered in Edmonton, Alberta, Canada. Bio-Stream's senior management team has cumulative experience in start-up and exits of over 160 years.

The Board of Directors is chaired by Alfred Berkeley, former President of NASDAQ. The company also boasts a world-class scientific and product development team with expertise in immunology, cancer, genetics, medical diagnostics and electrical engineering.



What is POCT?

POCT - Point of Care Testing

Bio-Stream Diagnostics Inc. has developed a fast, simple to use, low-cost point of care testing (POCT) platform using biosensors with a varying number of gates (for multiplex detection) and multiple formats (for varied applications), that will have profound impacts on diagnostic testing for the health and well-being of people globally.

Bio-Stream's trax™ Platform

The core pieces of the trax platform includes disposable sensors, a reusable reader, and the related software. The platform is based on an organic electrochemical transistor (OECT) that consists of a proprietary three dimensional semi-conductor for signal amplification. The technology works by detecting the electrical signal produced when a bait (such as an antibody or aptamer), that has been functionalized onto the gold gate surface of a sensor, is exposed to its corresponding target, be it a virus or biomarker protein. Cations are attracted to the transistor and anions to the sample gate. Like a lock and key, a bait/target combination will generate a localized change in the electronic charge on the gate that is relayed to the transistor. This electronic change is detected by the small inexpensive, universal traxReader to produce a positive/negative or quantitative result.

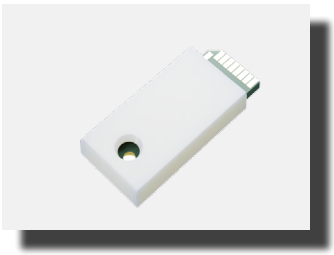


Figure 1

The universal reusable traxReader is uniquely developed to accept Bio-Stream traxSensor strips as well as those made by many other companies.

Researchers can use the traxReader in combination with software called traxInsight to capture, view and analyze a sensors response. The traxReader can be easily customized to perform numerous measurement types and adapted to perform diagnostic tests for a range of common health and non-health applications. Other measurement types include but not limited to: Cyclic Voltammetry (CV) measurement, Square Wave Voltammetry, Differential Pulse Voltammetry, Normal Pulse Voltammetry measurement, Zero Resistance Amperometry, MultiStep Amperometry, Pulsed Amperometric Detection and Multiple-Pulse Amperometric Detection measurement and Open Circuit Potentiometry measurement.

Figure 2

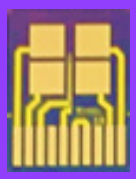
Developing a test on the trax platfrom

In order to speed the development of new biosensors, we have developed a workflow process that includes sourcing valid bait/capture biomolecules, optimize their performance to enable a successful OECT analysis. This workflow also includes preliminary analysis to select the most optimal drain and gate voltages to obtain accurate readings of drain current, threshold voltage and transconductance that will be utilized in establishing target detection. Validation data is provided below for a proof-of-concept test for the biological target C-Reactive Protein (CRP), a clinical biomarker for inflammation. Data is also shown for pathogen detection, detection of ions with no bait on gate, and for non-biological targets (Streptavidin).

The trax platform - for Developers

The primary elements of the trax platform used to develop a test would include traxSensors, the traxReader and development software called traxInsight.

Figure 3



traxSensors may contain either one or multiple gates. These gates are functionalized by the test developer, attaching a “bait” which will bind to the target of interest when the test is used.

Figure 4

traxReader is used as the detection device, and ‘reads’ the sensor. During the development of the test, traxReader would typically be connected to a laptop running traxInsight.

Figure 5

traxInsight is the development software used to capture, view and analyze the data provided by traxReader to establish and optimize settings for a new test and provide a yes/no or quantitative answer for presence of target.

The trax platform - for the end user

A trax test is very simple for the end user.
The traxReader is plugged into a smartphone, and the tests’ sensor is inserted into the traxReader. The mytrax app running on the phone detects which test is being preformed, and displays the appropriate instructions for use.

The user simply applies a sample to the sensor and results are displayed on the phone and stored in the users account.

Schematic of bait/target detection of the OECT platform.

Bait (such as antibody or aptamer) capture of a biomarker will result in a binding event to promote a voltage change, amplified by the OECT and detected by our reader. Several measurement tactics can be utilized to quantify this binding event including linear sweep voltammetry (LSV) or chronoamperometry [CAP], to result in the production of curves to give a positive/negative or quantitative difference between target and no target additions that represents binding events between bait and target.

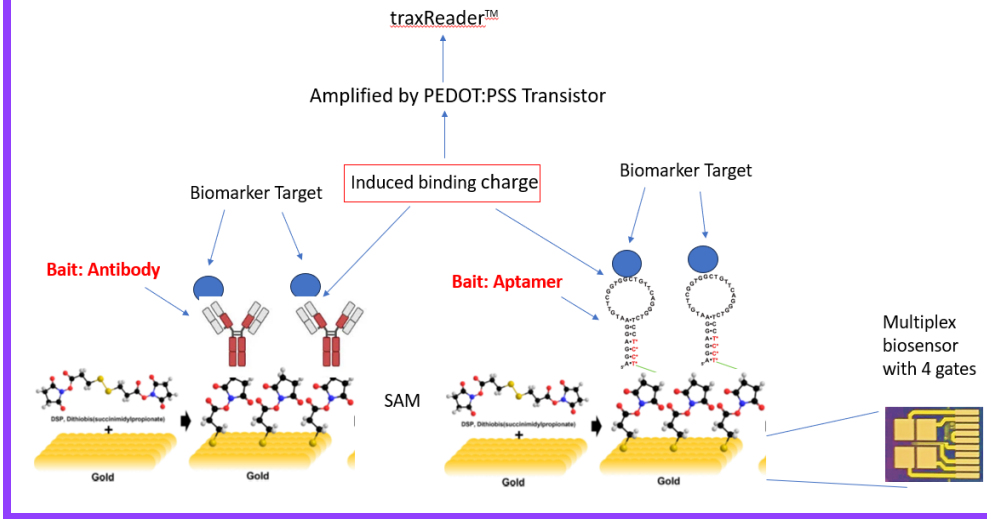


Figure 6

Workflow process of an OECT Biosensor development

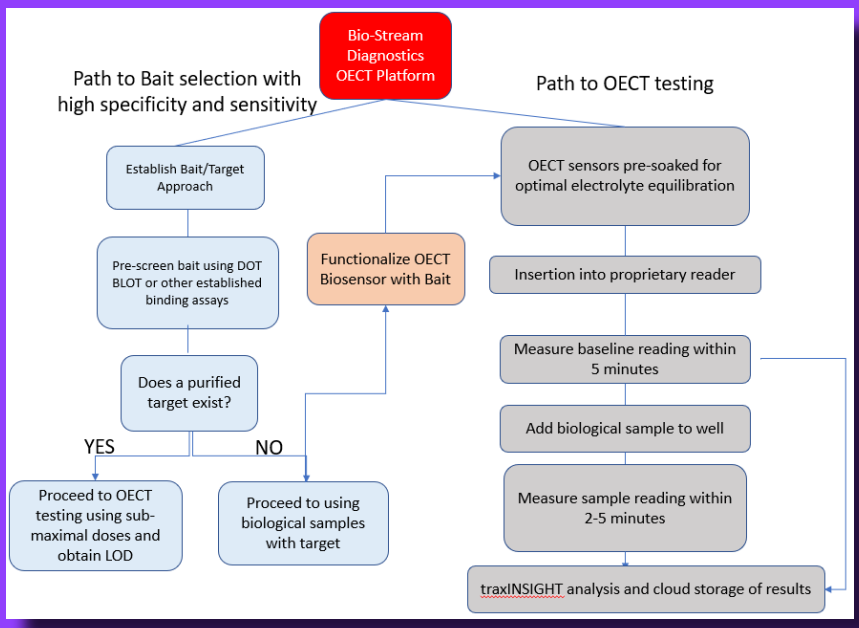


Figure 7

Bio-Stream's OECT workflow process is outlined in this flowchart. "Bait/Target Selection" is a process that will allow for rationale selection of the appropriate antibody for antibody-based baits. This selection will be based on knowledge of the binding kinetics of bait/target and pI of bait and target.

Bio-Stream's workflow involves utilization of traditional dot blot immunoassays or other biophysical techniques to establish the best bait to be utilized on the OECT platform. Once pre-screening is completed the bait selected will be functionalized (covalently bound) to the gate surface using NHS-coupling chemistry. After functionalization, Bio-Stream's OECT analysis will be carried out via LSV or CAP. Results will be analyzed via traxInsight. Analytics will produce a "POSITIVE" or "NEGATIVE" result or can be quantitated, and these results will be stored in our proprietary cloud server.

The combination of unique bait selection and our workflow testing protocol will produce results with high sensitivity and specificity.

Validation data for inflammation biomarker detection (C-reactive protein [CRP] Proof of concept 1).

LSV will produce transfer curves (Fig. 8 left panel) that illustrates a change in the electrochemical environment between the anti-CRP antibody bait and a buffer or sample containing CRP. This is converted into two quantitative parameters (two right panels) to illustrate how a yes/no or quantitative result can be obtained. Results are obtained in under 10 minutes.

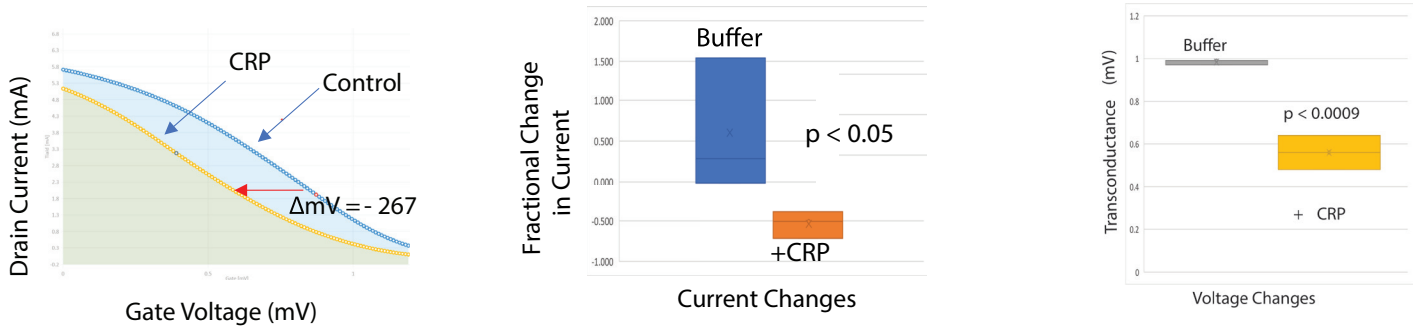


Figure 8 Panel A, B, C

Analysis of CRP binding via CAP is shown in Fig. 9. Left two panels show detection of buffer and CRP as a downward curves. Far right panel shows quantitation of downward curves on independent sensors to illustrate specificity of signal for CRP. The green line represents the actual current changes, the orange line a best fit line to compare changes after target was added. Fractional change in current was calculated based on the difference between the current change of the green line (where specific binding is observed) to the current reading on the orange line (the trajectory if no binding was observed). p values between -/+ CRP are < 0.0001.

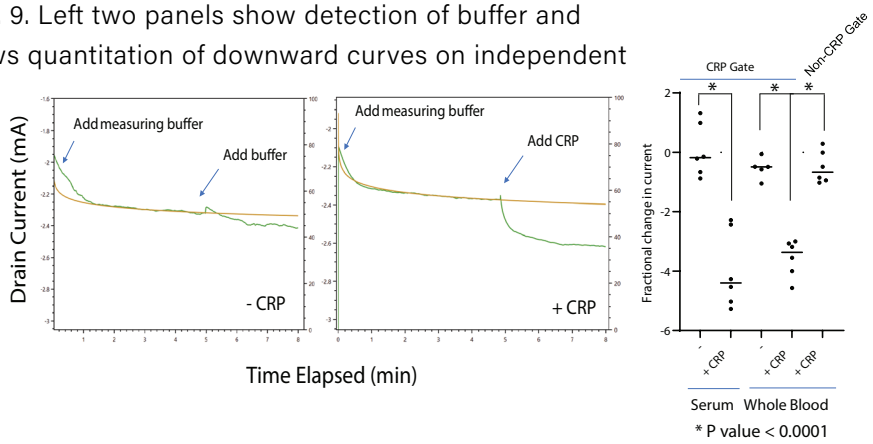


Figure 9 Panel A, B, C

Validation Data for a pathogen detection (Proof of concept 2).

Left panel: LSV will produce transfer curves (COVID-19 positive) that illustrates a change in the electrochemical environment between the anti-Spike protein antibody bait and a buffer or sample containing SARS-CoV2 virus (spike protein on virus). A plot of 18-20 positive and negative patient samples reveals a ΔmV shift of above 55 as a possible cut off between positive and negative samples.

We have estimated that the specificity (accuracy of detecting negative samples) and sensitivity (accuracy of detecting positive samples) as shown. Furthermore, we also estimate detection at day 2 viral loads from infected patients or about 300-400 viral particles/µl (Ct value of 35-38).

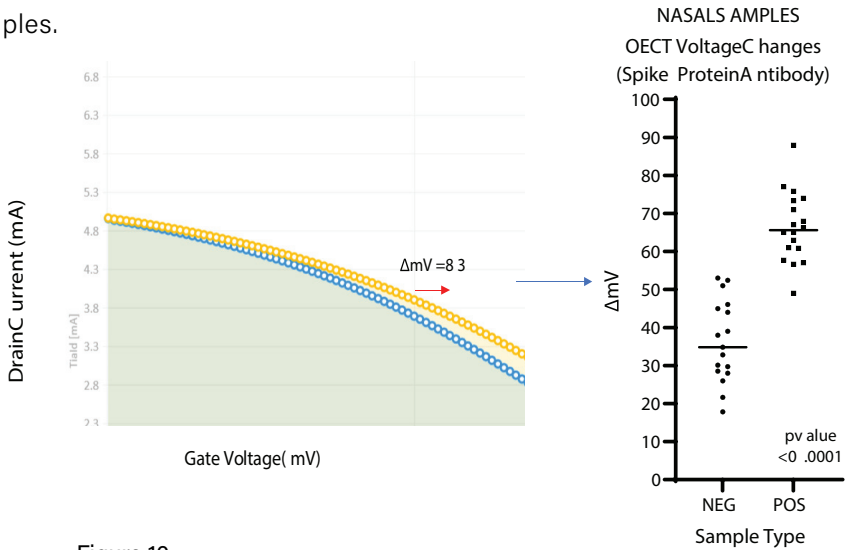


Figure 10

Validation data for detection of ionic changes on bare sensors (Proof of concept 3)

The trax platform can also detect non-biological targets such as seawater. Shown is the detection of seawater in a coolant solution (far left panel), showing increasing amounts of seawater with a rough limit of detection at < 950 ppm (middle panel). Our platform is robustly sensitive to changing ionic capacities as observed by very small changes in buffer concentrations from deionized water to 6 mM PBS (far right). These observations were carried out using a bare sensor (with no bait/capture molecule).

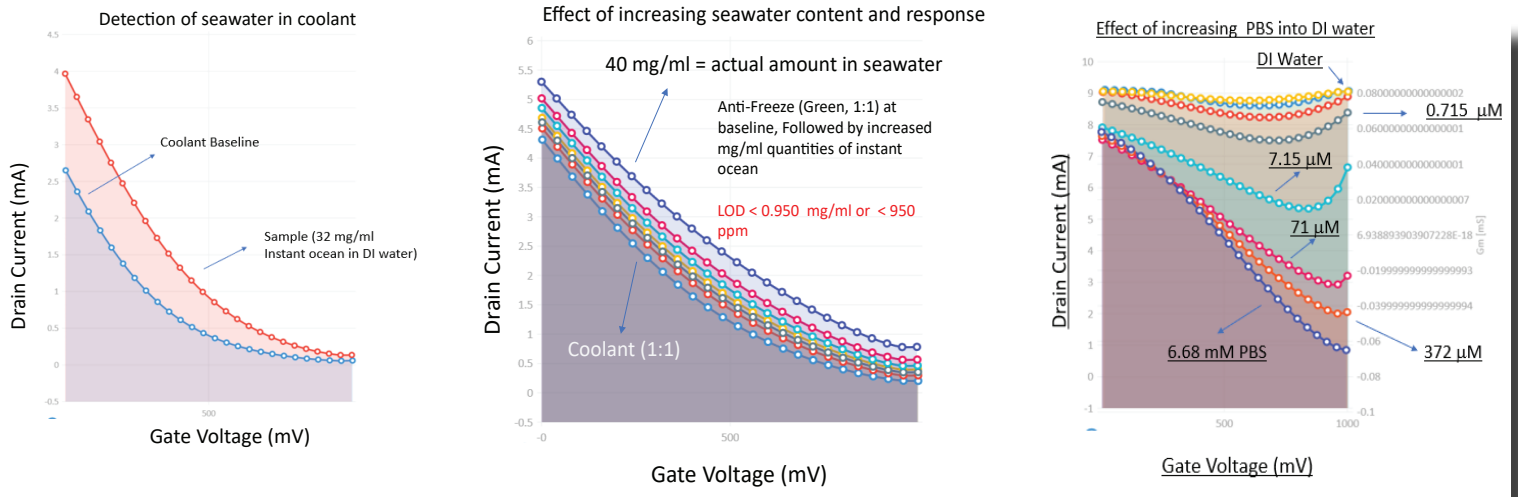


Figure 11 Panel A, B, C

Validation data for detection of streptavidin binding to a D-biotin gate (Proof of concept 4)

Analysis of streptavidin (SA) binding via CAP to a D-biotin gate is shown. Left panel show detection of buffer or streptavidin as a upward curve. Far right panel shows quantitation of the upward curves on independent sensors to illustrate specificity of signal for streptavidin. Data is shown for binding in PBS or serum to illustrate no matrix effects for detection Biotin/SA binding. The green line represents the actual current changes, the orange line a best fit line to compare changes after target was added. Fractional change in current was calculated based on the difference between the current change of the green line (where specific binding is observed) to the current reading on the orange line (the trajectory if no binding was observed).
p values between -/+ SA are < 0.0001.

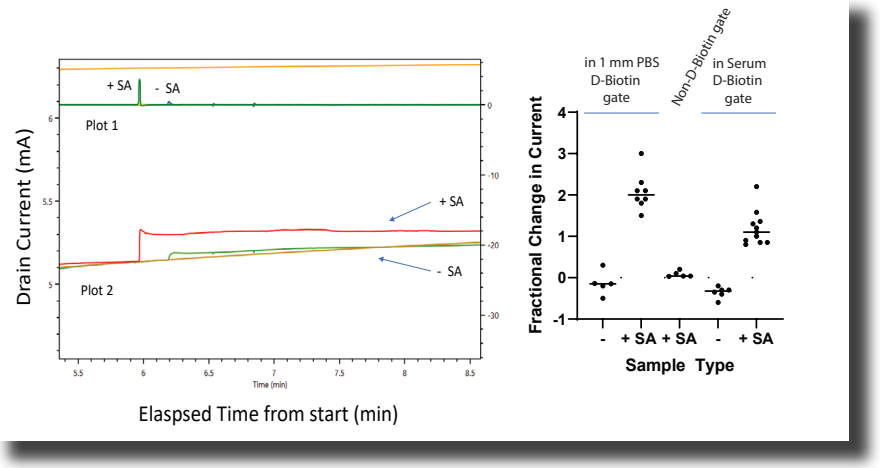


Figure 12

These proof of concepts demonstrate the trax platform can both capture numerous targets and be utilized as an ion sensor.

Analysis of ELISA vs OECT results and workflow are show in tables 1 and 2 below. In Table 1, insulin detection has not been optimized yet, but does perform well on trax sensors. These analyses were all carried out on contrived samples in serum. Table 3 compares traxSensors with the commercially available field effect transistor (FET) based market.

Table 1: Comparative features and limit of detection (LOD) of three biomarkers on our OECT platform.

Test Name	Manufacturer (FDA approved)	Protocol Method	Sample Type	Lab Test LOD	OECT LOD
C-reactive Protein	Cobas CRP Test	Antibody based	Blood/serum	~ 0.3 mg/dL	~ 0.5 - 1 mg/dL
Cystatin C	Gentian Immunoassay on Beckman Coulter® AU	Antibody based	Serum (but data exist for blood)	~ 0.50 - 1 mg/L	~ 0.10 - 0.5 mg/L
Insulin	Cobas Insulin Test	Antibody based	Serum (but data exists for blood)	0.2 µU/mL (1.39 pmol/L)	< 0.7 µU/mL (<3 pmols/L)

Table 2: Process speeds of ELISA vs OECT. Comparisons are made based on time to process 96 samples using ELISA, 3-5 minutes to process each sample on our OECT and 15 minutes to process samples using lateral flow platforms.

Test Name	Protocol Method	Sample Type	Number of tests	Data Storage
ELISA	Antibody based	Limited to serum or plasma	48 samples in duplicate in 6 hr (8 samples/hr)	No
OECT	Versatile to include antibody capture	Versatile to include whole blood	48 samples in duplicate 5 hr (9.6 samples/hr)	Bluetooth enable reader, YES
Lateral Flow	Antibody based	Versatile to include whole blood	48 samples in duplicate in 16 hr (3 samples/hr)	No

Table 3: Comparison OECT vs FET.Below are performance comparisons between our OECT and state of the field effect transistors (FET). Table below is adapted from reference #2.

OECT	FET
Volumetric Capacitance	Area Capacitance
Ions can enter the semiconducting material	Ions can only collect on the surface
3 orders of magnitude higher gate-channel capacitance than FET	3 orders of magnitude less gate-channel capacitance than OECT
Low-Cost Manufacturing is possible	High Cost is the only option
Stable Performance in aqueous solutions	Aqueous environment strongly limits the FET organic materials to work properly
Solid and Flexible substrates	Solid Substrates
Semiconductor is made from a polymer and can be customized for specific requirements	Semiconductor is commonly made from silicon

The Bio-Stream Diagnostics trax platform is versatile, easy to use, with a universal reader and sensors that can provide accurate point of care tests for biomarker targets. Biological medium tested to date includes swabs, serum, whole blood, and urine samples. Results are comparable to ELISA based results and, in some cases are comparable to PCR. Information presented here is designed to provide an indication of suitability for other applications.

For more information contact info@bio-stream.ca

Bio-Stream Diagnostics Inc.
F200 2011-94 St. NW
Edmonton, Alberta
T6N 1H1
Phone: (780) 800-1900

References:

1. Ji X, Lin X and Rivnay J. Organic electrochemical transistors as on-site signal amplifiers for electrochemical aptamer-based sensing. Nature Communications 14 (1), 2023.
2. Friedlein JT, McLeod RR and Rivnay J. Device physics of organic electrochemical transistors Organic Electronics 63: 398 - 414, 2018.
3. Sophocleous M. et al. Organic electrochemical transistors as an emerging platform for bio-sensing applications: A review. IEEE Sensors Journal 21(4): 3977 – 4066, 2021.

Printed in Canada v1.1