bio-stream DIAGNOSTICS INC

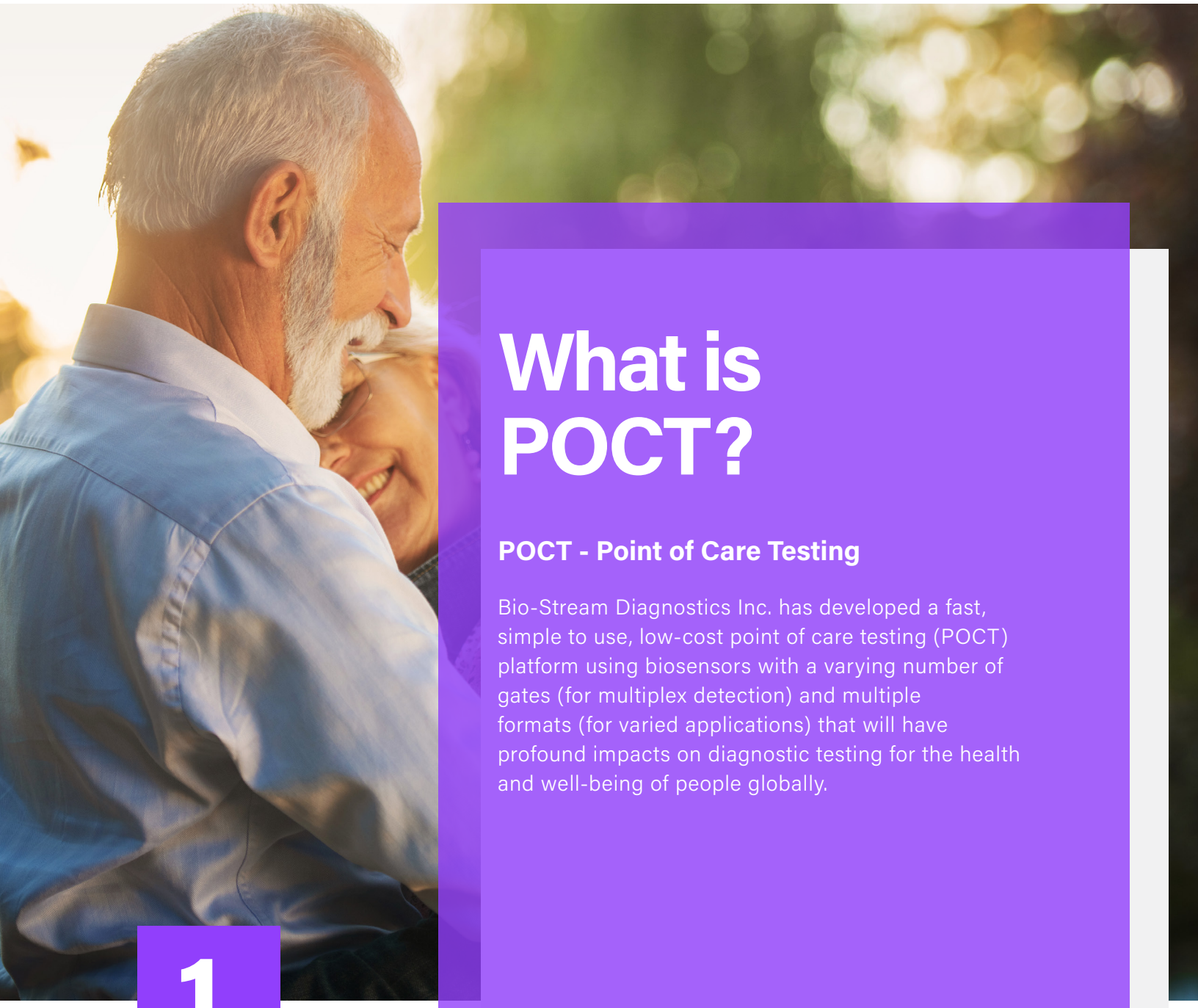
trax™

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 are 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 semiconductor 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. When a voltage is applied to the gate, cations are attracted to the transistor and anions to the sample gate. The 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 and universal traxReader to produce a positive/negative or quantitative result.

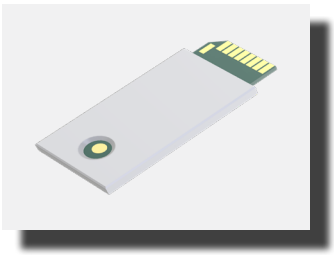


Figure 1

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 sensor's response. The traxReader can be easily customized to perform numerous measurement types and diagnostic tests for a range of common health and non-health applications. Other measurement types include but are not limited to: cyclic voltammetry (CV), square wave voltammetry, differential pulse voltammetry, normal pulse voltammetry, zero resistance amperometry, multiStep amperometry, pulsed amperometric detection and multiple-pulse amperometric detection and open circuit potentiometry.

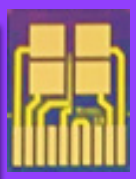
Figure 2

Developing a test on the trax platform

In order to speed the development of new biosensors, Bio-Stream has developed a workflow process that includes obtaining valid bait/capture biomolecules and optimizing their performance to enable a successful OECT analysis. This workflow also includes preliminary analysis to select the optimal measurement protocol to be utilized in establishing target detection. Validation data is provided below for proof-of-concept tests for detection of biomarkers of inflammation, pathogens, antibiotics, ions with no bait on gate, human cells, and non-biological targets.

The trax platform - for developers

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



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 3

Figure 4

traxReader is used as the detection device, that reads the sensor. During the development of the test, traxReader is typically connected to a laptop running traxInsight.

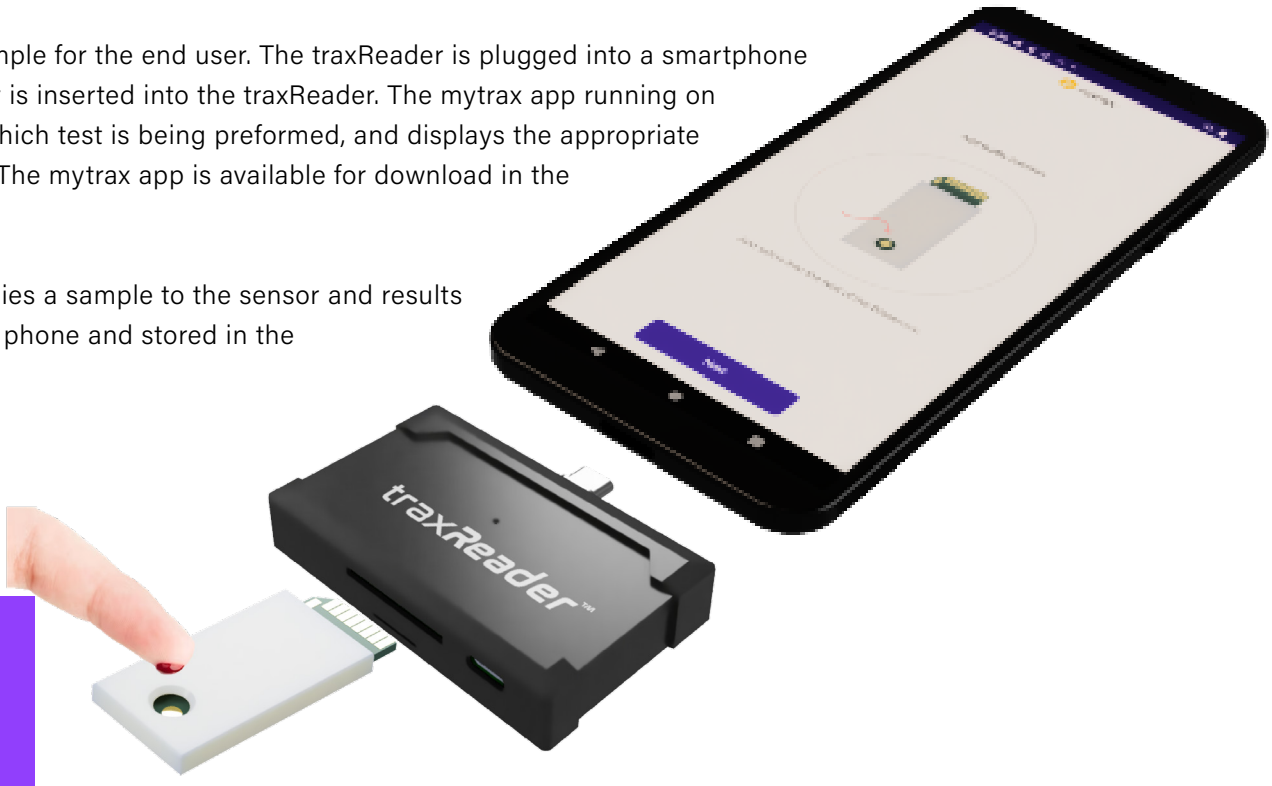
Figure 5

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 the 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 test's 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 mytrax app is available for download in the Google Play Store.

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



How it works

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 the trax reader. Several measurement tactics can be utilized to quantify this binding event including linear sweep voltammetry (LSV), square wave voltammetry (SWV) 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.

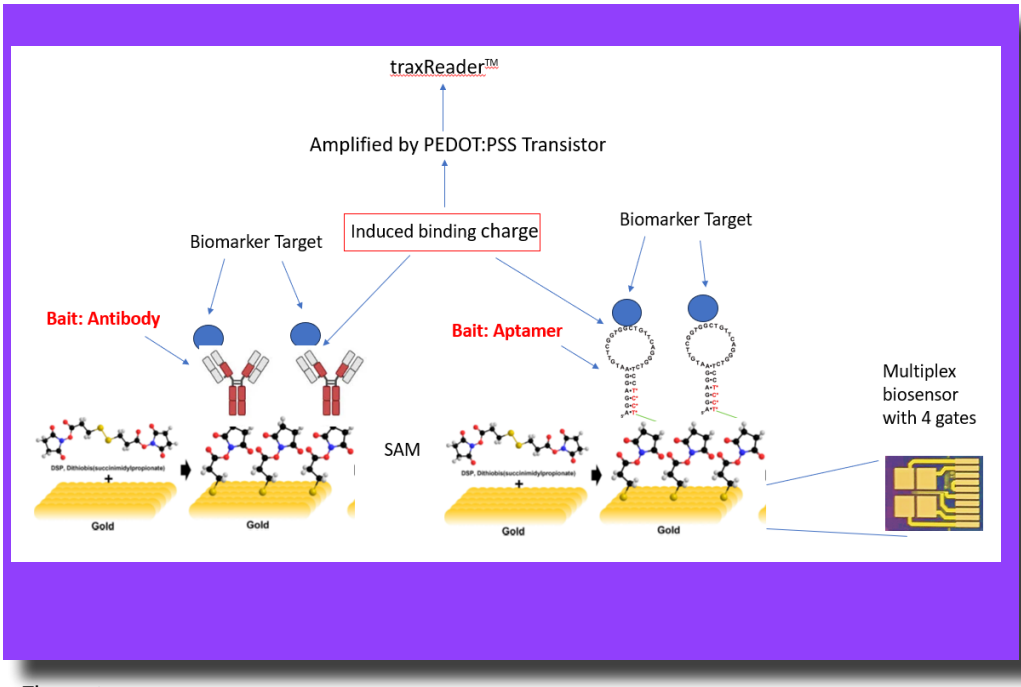


Figure 6

Workflow process of an OECT biosensor development

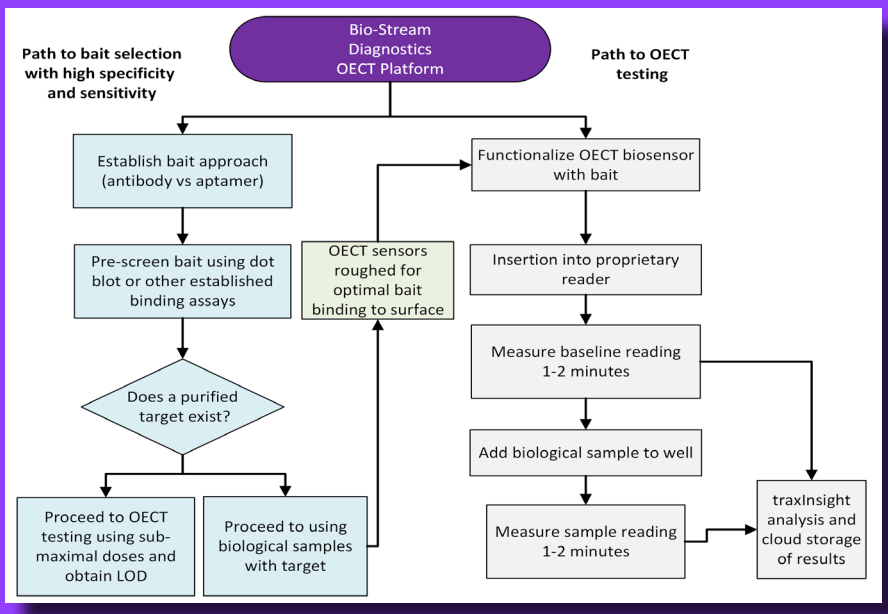


Figure 7

Bio-Stream's OECT workflow process is outlined in this flowchart. "Establish Bait Approach (Antibody vs Aptamer)" is a process that will allow for selection of the appropriate bait molecule to capture the target. This selection will be based on knowledge of the binding kinetics of bait/target, pI of bait and target or conformational change of bait upon target binding. Bio-Stream's workflow involves utilization of traditional dot blot immunoassays or other biophysical techniques to establish the best antibody bait to be utilized on the OECT platform. For aptamers, the workflow utilizes conformational change analysis using circular dichroism to select the optimal aptamer as bait.

Once pre-screening is completed the bait selected will be functionalized (covalently bound) to the gate surface using NHS-coupling chemistry for antibodies and thiol linkage for aptamers. After functionalization, Bio-Stream's OECT analysis is carried out via LSV, CAP or SWV. Results are analyzed via traxInsight.

Analytics will produce a "POSITIVE" or "NEGATIVE" result or can be quantitated, and these results will be stored on Bio-Stream's secure cloud servers.

Pre-screening validation

Examples of pre-screening workflow for antibody and aptamer selection. (A) Dot blot test of sensitivity comparison between two antibodies - the Abcam antibody with a limit of detection (LOD) of < 0.2 ng/µL whereas the ThermoFisher antibody has an LOD of > 0.2 ng/µL; clinically, CRP detection is at > 10 mg/dL or 0.1 ng/µL for infections. Generally, when an antibody is applied to the trax OECT platform, the LOD is 1,000x more sensitive than observed by dot blotting.

(B) Circular dichroism analysis of two target/bait combinations. Panel B shows the immunosuppressant drug, FK506, binding to an FK506 aptamer to induce a conformational change in aptamer shape. Panel C shows C-reactive protein (CRP), a marker of inflammation, binding to a CRP aptamer to also induce a conformational change in its aptamer shape. The frequency monitors vibration of the chemical structures within the aptamer and > 40% change can be observed in chemical vibrations between 200 and 320 nm for both aptamers. The aim is to have a substantial change upon target binding so when a redox tracer is attached to the 3' end of the aptamer, the conformational change will displace the redox tracer and induce an electron positional change. These events will trigger an electrical charge change to be picked up by the trax-Reader on the trax OECT platform (an example is shown in Figure 13, panel A).

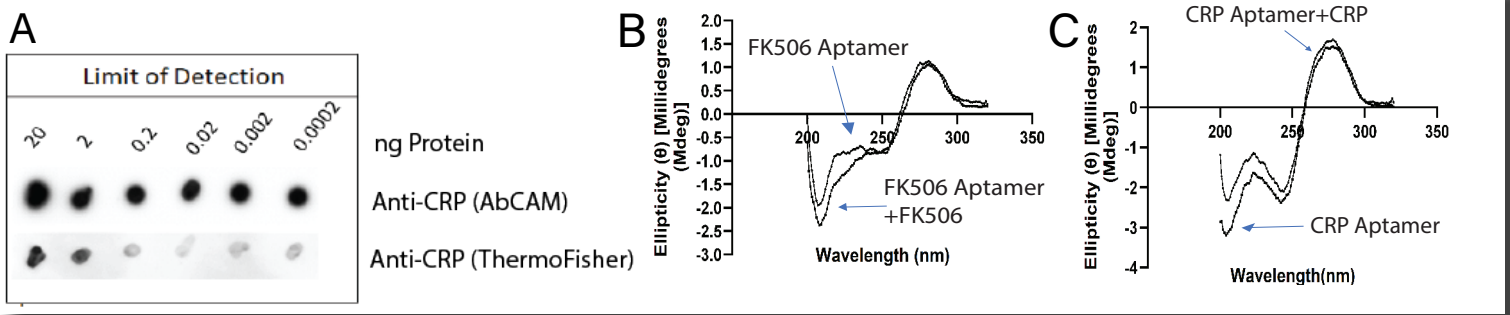


Figure 8 Panel A, B, C

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

LSV will produce transfer curves (Fig. 9, panel A) that illustrate a change in the electrochemical environment between the anti-CRP antibody bait and a sample containing CRP at 50 mg/dL. These curves are converted into two quantitative parameters (panels B and C). Results are obtained in < 5 minutes. P-value < 0.001 for CRP dose vs buffer control (n=11-28 for buffer control and CRP 50 mg/dL dose). Normal levels of CRP are < 0.1 mg/dL and > 10 mg/dL during an infection.

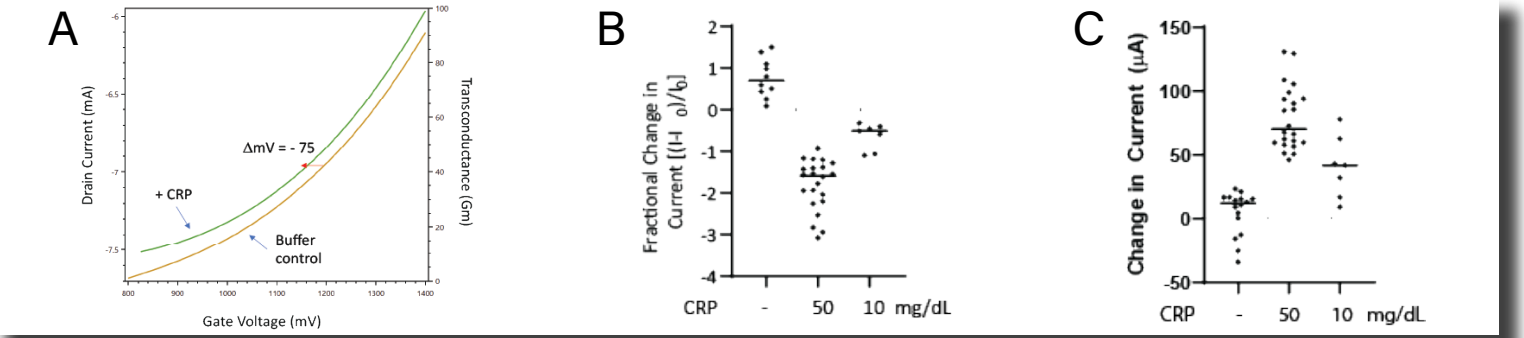


Figure 9 Panel A, B, C

Concentration of CRP	Average µA change	Sensitivity (%)	Specificity (%)	P-value (target vs buffer)	N-value
50 mg/dL	75	93	90	< 0.0001	28
10 mg/dL	42	100	90	< 0.002	16

Assay outcomes for CRP detection using LSV.

Analysis of CRP binding via CAP is shown in Fig. 10. The two left panels show detection of buffer and CRP as downward curves. The far right panel shows quantitation of the downward curves on independent sensors to illustrate the specificity of the signal for CRP.

The green line represents the actual current changes and 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.

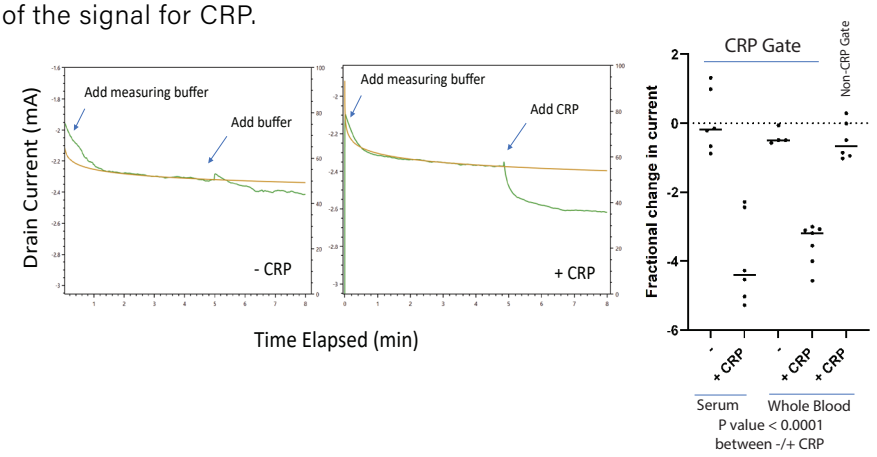


Figure 10

Validation data for biomarker detection for cardiac health (cardiac troponin I [cTnI] proof of concept 2)

LSV will produce transfer curves (Fig. 11, panel A) that illustrate a change in the electrochemical environment between the anti-cardiac troponin I (cTnI) antibody bait and a sample containing cTnI at 0.5 ng/ml. The curves are converted into two quantitative parameters (Figure 11, panels B and C). Results are obtained in less than 5 minutes. P-value < 0.0001 for cTnI dose vs buffer control (n=10-21). Normal levels of cTnI are < 0.04 ng/mL and > 4 ng/mL during a heart attack. According to the American Heart Association, high sensitivity assays for cTn today fall within the range of < 0.1 ng/mL which Bio-Stream assays meet and exceed with detection at 0.05 ng/mL. Assays that can detect high sensitivity of cTn would be more beneficial to identifying an acute myocardial infarction (heart attack).

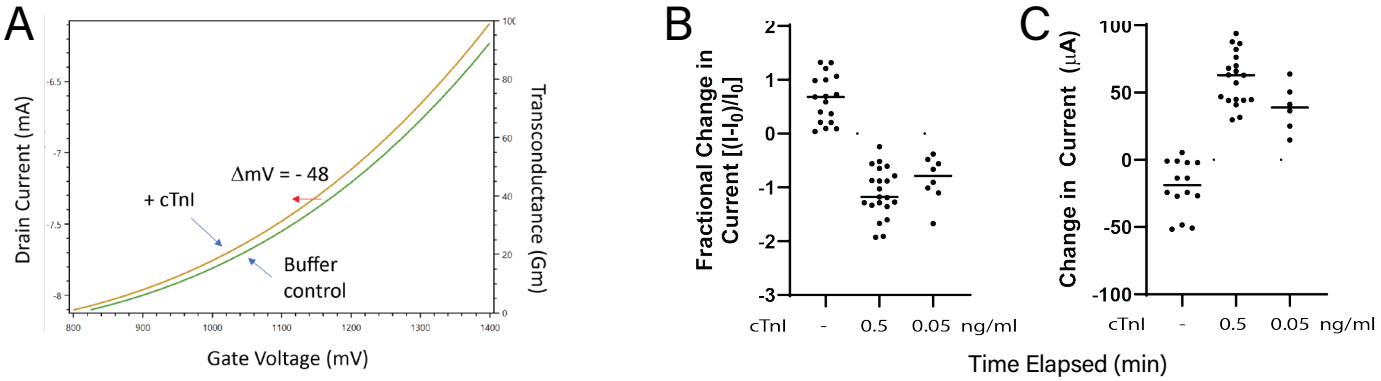


Fig 11 Panel A, B, C

Concentration of cTnI	Average μA change	Sensitivity	Specificity	P-value (target vs buffer)	N-value
0.5 ng/mL	65	92	95	< 0.0001	21
0.05 ng/mL	36	87	95	< 0.0001	10

Assay outcomes for cTnI detection using LSV.

Validation data for a pathogen detection (proof of concept 3)

Left panel: LSV will produce transfer curves (COVID-19 positive) that illustrate 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).

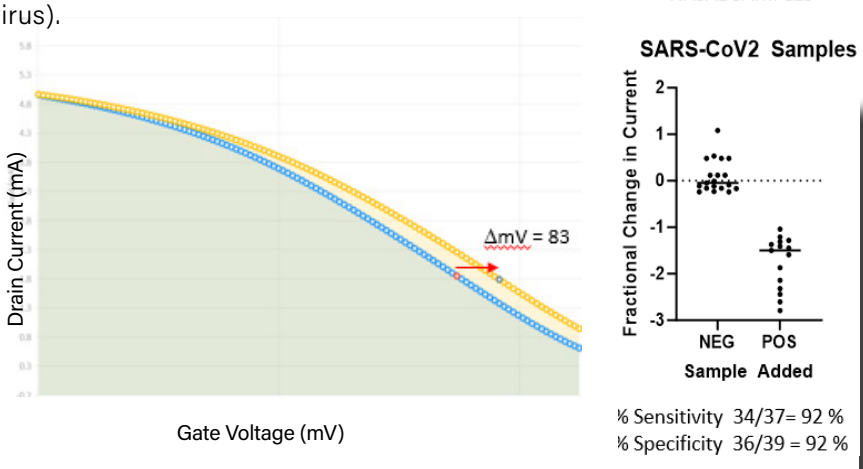


Figure 12

The use of an electrochemical based aptamer biosensor (EAB) to detect vancomycin using a redox- sensor-tagged aptamer (proof of concept 4)

Electrochemical detection of vancomycin binding (+target) to its aptamer using the trax platform (Figure 13, panel A). In addition, at various concentrations of vancomycin in 1XPBS and in serum (Figure 13, panels B and C), we can observe stronger current responses (reflected in the signal gain %), confirming the sensor's sensitivity to vancomycin at clinically relevant concentrations (the red rectangle marks the therapeutic concentration range for vancomycin).
Interestingly, we can observe an enhancement of this binding in the presence of serum (from over 40% to over 70% signal gain at maximum vancomycin concentration). Furthermore, the detection of vancomycin binding to its aptamer is specific to the vancomycin EAB (Figure 13, panel D).

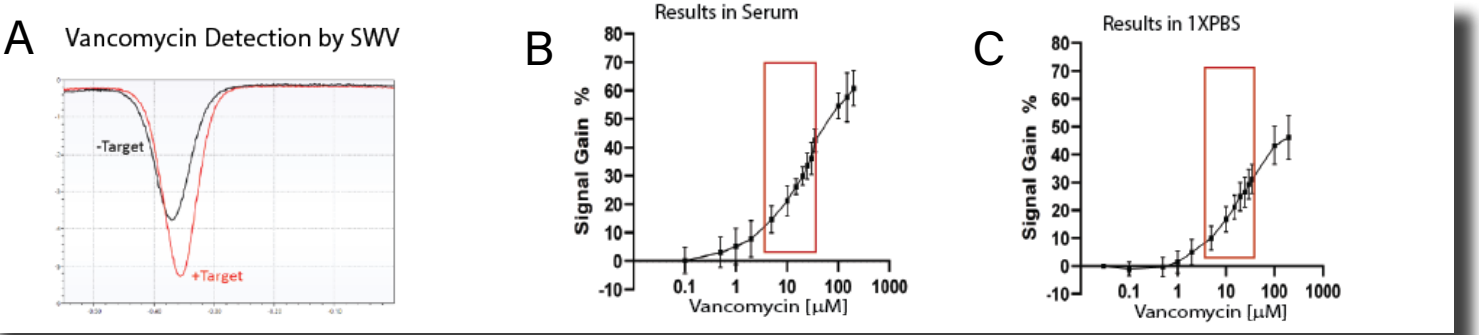


Figure 13 Panel A, B, C

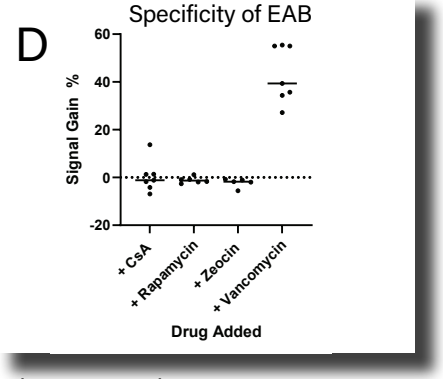


Figure 13 Panel D

The signal gain % in response to non-vancomycin drugs is plotted in Figure 13D (208 nM for CsA, 16.4 nM for Rapa, 58.3 µM for Zeocin are the highest clinical concentrations of these drugs). This response is compared to a vancomycin concentration of 25 µM (an amount in the middle of the clinical range for vancomycin detection).

Concentration of Vancomycin	Average Signal Gain %	Sensitivity (%)	Specificity (%)	P-value (target vs buffer)	N-value
25 µM (1X PBS)	25.6 ± 5.2	94	100	< 0.0001	18
25 µM (Serum mimic buffer)	39 ± 4.4	100	100	< 0.0001	5
25 µM (in 100% serum)	33.5 ± 4.7	100	100	< 0.0001	5

Assay outcomes for vancomycin detection using SWV.

Validation data for detection of ionic changes on bare sensors (proof of concept 5)

The trax platform can also detect non-biological targets such as ions from seawater. Panel A: Detection of seawater in a coolant solution (containing > 900 ppm chloride). Panel B: The trax platform is robustly sensitive to changing ionic capacities as observed by very small changes in buffer concentrations from deionized water to 6 mM PBS. Panel C: The detection of chloride ions in distilled water using the balanced redox reaction:

$$CaCl2\ (aq) + 2AgNO3\ (aq) \rightarrow 2AgCl\ (s) + Ca(NO3)2\ (aq)$$

The insoluble precipitate, AgCl, is formed and has no charge. Thus, the reaction goes from an electron-rich composition to a less electron-rich composition and the release of electrons is picked up by the trax reader and translated into defined electrical changes depending on the chloride ion content of the sample on the gate. An almost linear relationship between gate current changes and chloride ppm content was observed. These observations were carried out using a bare sensor (with no bait/capture molecule).

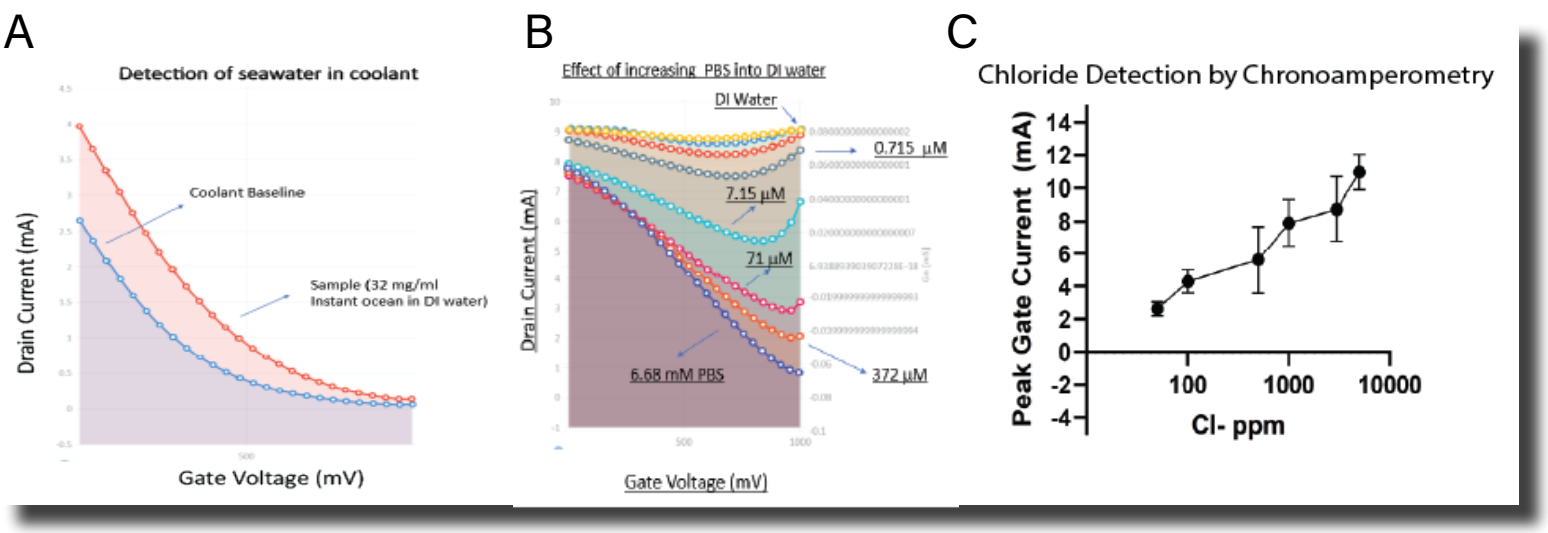


Figure 14 Panel A, B, C

Validation data for detection of streptavidin binding to a D-biotin gate (proof of concept 6)

Analysis of streptavidin (SA) binding via CAP to a D-biotin gate is shown. The left panel shows detection of buffer or streptavidin as an upward curve. The 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 of Biotin/SA binding. The green line represents the actual current changes, and the orange line is 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.

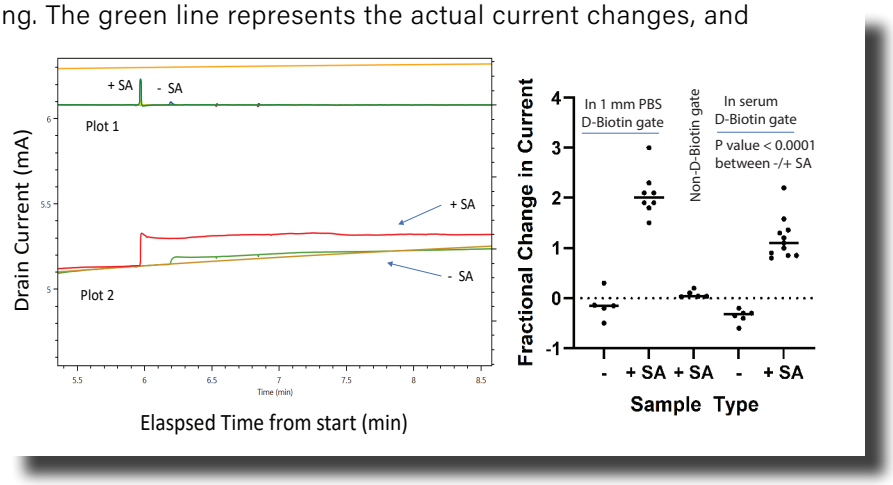


Figure 15

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

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 commercially available field effect transistors (FET).

Table 1: Comparative features and limit of detection (LOD) of three biomarkers on the trax 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 exists 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, less than 5 minutes to process each sample on trax OECT sensors and 15 minutes to process samples using lateral flow platforms.

Test Name	Protocol Method	Sample Type	Number of Tests	Data Link to Patient	TIme for Results
ELISA	Antibody based	Limited to serum or plasma	48 samples in duplicate in 6 hrs (8 samples/hr)	No	4-6 hours
Bio-Stream trax OECT	Versatile to include antibody capture	Versatile to include whole blood	75-80 samples in duplicate in 5 hrs (30 samples/hr)	Bluetooth enabled reader, YES	<5 minutes
Lateral Flow	Antibody based	Versatile to include whole blood	48 samples in duplicate in 16 hr (3 samples/hr)	No	15-20 minutes
Flow Cytometry	Antibody based	Various including blood	>90 samples within 15-20 minutes	No	15-20 minutes
Mass Spectrometry	Elemental analysis of sample	Various including blood	Several hours for 100 samples	No	1-3 hours

Table 3: Comparison OECT vs FET. Below are performance comparisons between trax OECT sensors and state-of-the-art 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 mediums 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.
2011-94 St. NW
Edmonton, Alberta
T6N 1H1
Phone: (780) 800-1900

References:

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Printed in Canada v7.11