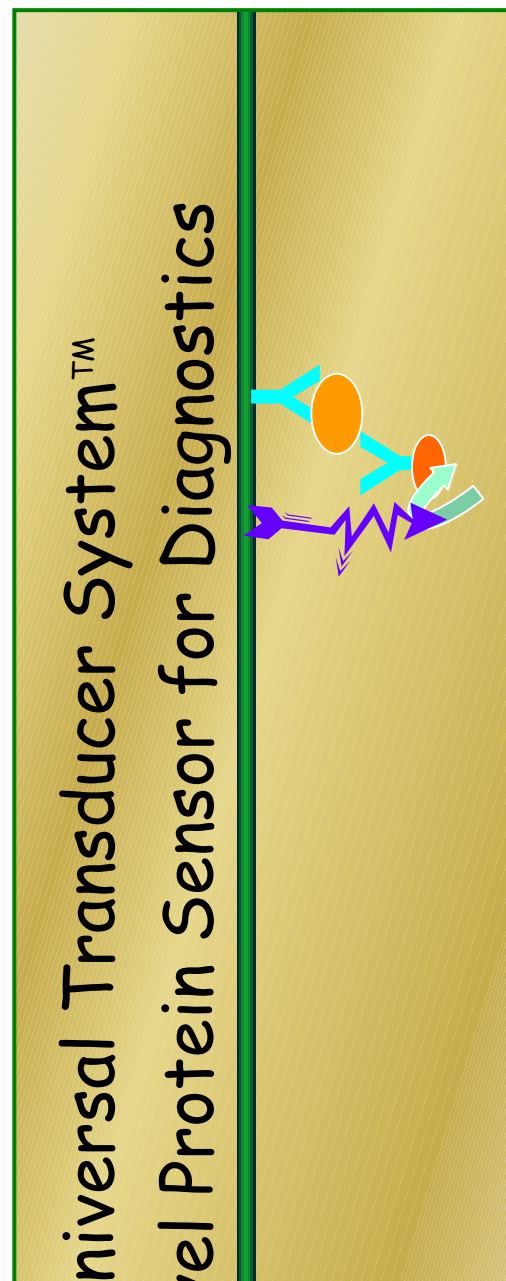


Acidification Assays

Biomonitoring



### History and Vision

**Sensortec Limited** was founded 1996 to develop and commercialise the proprietary research carried out in the 1990's at the Vernadsky Institute of Geochemistry and Analytical Chemistry in Moscow, Russia. Research focussed on conductive polymers to derive prototypes of the Universal Transducer System (UTS) hardware and software, and to address a growing market for in-vitro diagnostics and biosensor applications in environmental monitoring and food quality assurance.

Substantial private and institutional investment has now been made. Namely by Generics Asset Management starting in 1999 and the Interkea Technology Fund (investment is ongoing), further private investment has been achieved from Norcay Holdings Limited, a Hong Kong based fund.

The current round of funding enabled the purchase of an analytical testing laboratory (NRM), to help with sample preparation and validation and enable market access, and the formation of an onshore company, Universal Sensors, detailed with the commercialization of the Sensortec IP. Initially SENSORTEC LIMITED will be focusing on the environmental monitoring and food quality assurance sectors.

Two international filings covering the platform technology have been granted in Russia. National and International patents have also been filed. As a result of work developed further patent applications have been filed.

### The Technology

The patented UTS™ technology provides a universally applicable proprietary platform suitable for a whole range of diagnostic tests and other quantitative assays requiring high analytical sensitivity. Utilising simple novel potentiometric measurements UTS™ has already shown it can achieve higher sensitivity levels than those generally achieved by amperometric and optical biosensors. UTS™ technology has the advantage of simplicity and sensitivity (<50fM), at a low cost, utilising tried and tested technologies in a novel arrangement.

### The UTS™ Protein Sensor

The sensor comprises an immobilised bioreceptor at the surface of an inexpensive polypyrrole coated screen-printed gold or carbon electrodes with an integrated Ag/AgCl reference electrode on a plastic support. Polypyrrole is a conductive polymer and is laid down by electrochemical polymerisation.

The detection range and sensitivity of the UTS™ Technology can be influenced by changing the properties of the polymer layer at the polymerisation step to enable measurement of a wide range of concentrations. Sensortec Limited has elucidated and patented the main factors responsible for imparting the required metallic redox properties to the polymer film.

The sensors are used with established ELISA techniques, to produce rapid (<15mins), sensitive (<50ppt) and reproducible biosensors which work in complex fluids such as milk, homogenates, serum, blood and urine. The sensors can be monitored at point-of-use with a handheld potentiometer or a desktop PC.

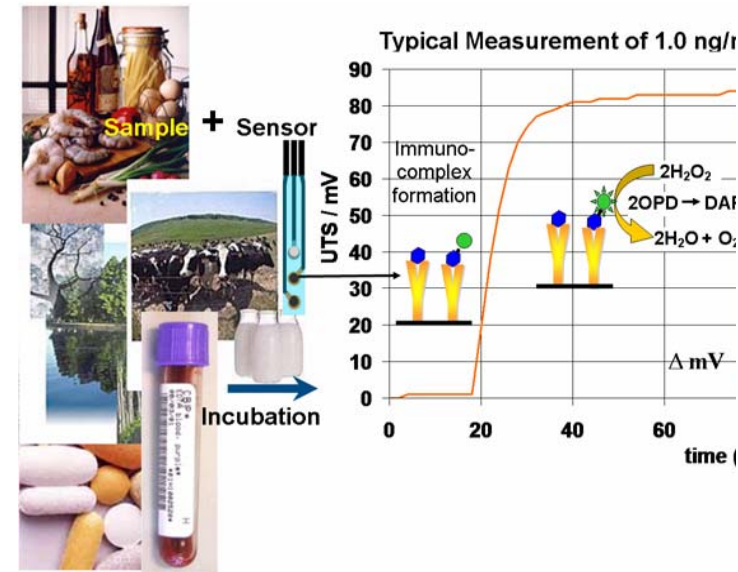


Figure 1. Basic Measurement Protocol including the Charge-Step Procedure

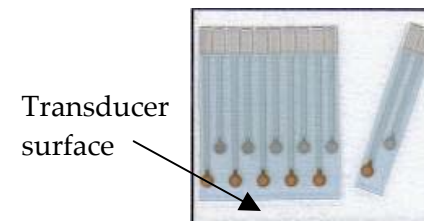


Figure 2. The Current UTS™ Sensor (to scale)

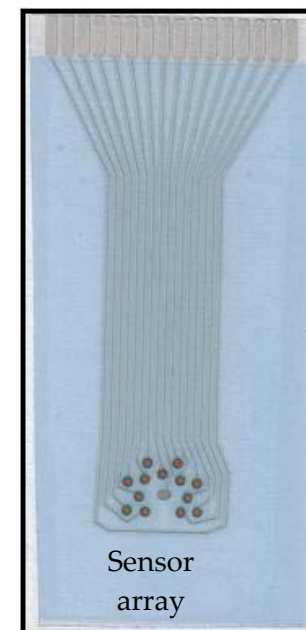


Figure 3. A UTS™ Multianalyte Sensor

### How does it work?

Bound antibody-target-label complexes formed at the surface of the immunosensor are detected potentiometrically, using a "charge-step" procedure (Fig 1).

One of the components of the complex is conjugated with horseradish-peroxidase (HRP), an electrochemically active label. After the immunocomplex has formed the UTS™ Sensor is transferred to the measuring device where it is washed with UTS™ Basic Solution, and the first potentiometric measurement is taken. The UTS™ Basic Solution is then replaced by UTS™ Enhancer Solution and a second potentiometric measurement is taken. Both measurements are taken against a Ag/AgCl reference electrode. A shift in potential is measured at the sensor surface due to local changes in redox state and pH caused by the activity of the HRP label. The change in potential is related to the concentration of the formed receptor-target complex. Fig 1

Specific bioreceptors are physically adsorbed directly to the UTS™ Sensor (Fig 2) transducer surface. Alternatively they can be prepared as a universally applicable solid phase by either covalent or physical attachment of streptavidin after the polymerisation process. Other bridging systems such as Anti-FITC/FITC, Lectin/carbohydrates, protein G/antibodies and protein A/antibodies can be employed.

### Feasibility

This ultrasensitive potentiometric technology has been demonstrated in clinical assays for hepatitis B surface antigen, Troponin I, and Digoxin, as well as food quality assurance (antibiotics and mycotoxins) and environmental (PCB) assays. These represent a large range of molecular weights and assay formats, all with a requirement for high analytical sensitivity and precision.

### Future

A proprietary improved substrate/reagent system is being developed to improve sensitivity, increase measuring range and simplify reagent requirements.

UTS™ technology lends itself not only to single test strips but also multi-analyte panel testing (Fig 3). The preferred array size would be less than fifty, although, arrays of a hundred or more are conceivable, subject to more complicated electronics and tracking. The sensor sensors can be screen-printed or engineered onto an electronic PCB board. The shape and layout of the sensors can have bespoke design. For automation and POC applications the sensors can be incorporated into a plastic moulded enclosure containing the fluidics required to apply the sample and perform the assay steps.

## Accelerated Stability Studies

Bare polypyrrole coated sensors were shown to be stable at 37°C over a 4 month study. Using our HRP-Biotin QC assay Improvements in stability can be made by adopting well-known optimised stabilisation procedures currently used in the immunoassay community.

The graphical illustration below show that the sensors are stable over this time period, any deterioration would be indicated by changes in the shape of the calibration curve.

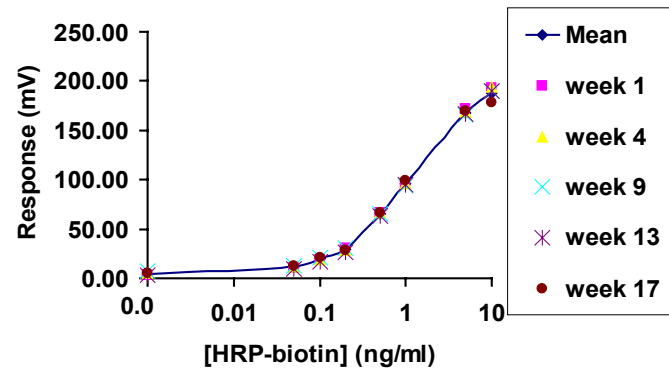


Figure 5. Polypyrrole stable after 4 months at 37°C

## Assay Transfer

Many assays have been transferred to this platform technology to show proof-of-principle. These assays have not been fully optimised and require little or no sample preparation, but they still show significant improvement in sensitivity and time to result over the standard ELISA technique. These assays include several different formats of sandwich and competitive immunoassays, a wide range molecular weight and different market sectors. The results of which can be seen in the table below.

Typical results after initial assay transfer				
Diagnostic Use	Target	Molecular Weight	Dynamic Range	Sensitivity
Diseases	HbsAg	300 -1000 Da	<0.05ng/ml - 10µg/ml	50fM
Markers	Troponin I	23 Da	<0.05ng/ml - 100ng/ml	~2pM
	hCG	36.7kDa	0.5ng/ml - 100ng/ml	~13pM
	hTNF	21-28kDa	<0.008ng/ml - 1ng/ml	~0.3pM
Drugs	Digoxin	780 Da	0.5ng/ml - >2ng/ml	Therapeutic window
Toxins	Aflatoxin M1	328 Da	< 250ppt - >2000ppt	< 0.7nM
Contaminants	Penicillin G	372.5 Da	<1ppb - >10ppb	~2.7nM
	Chloramphenicol	323 Da	<50ppt - >1.2ppb	~0.15nM

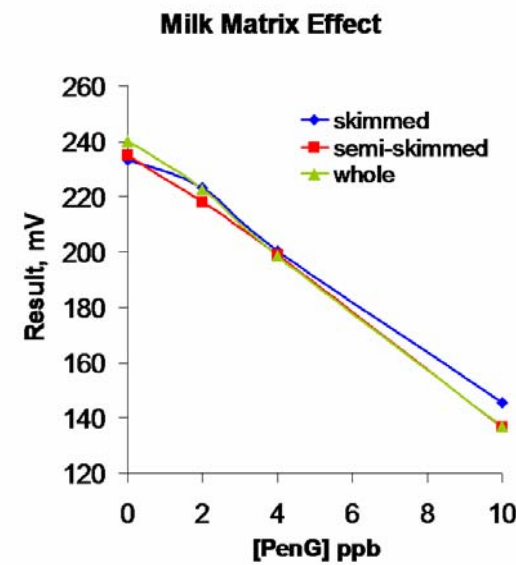
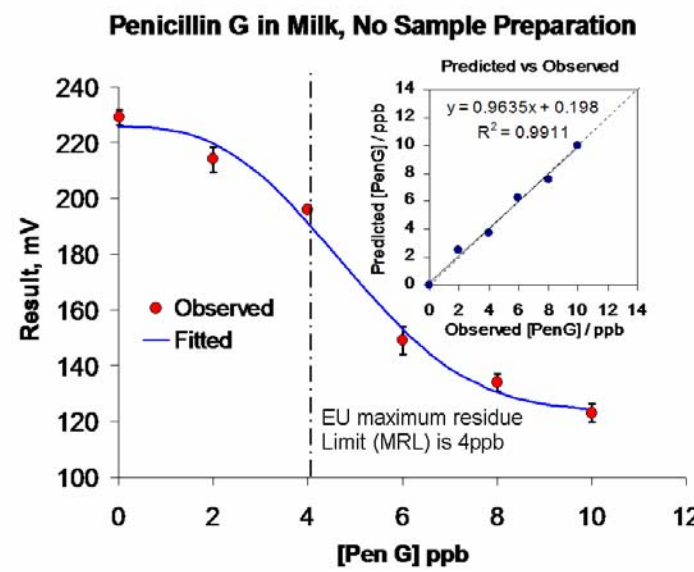
## Assay Transfer Example: Penicillin G Assay

This is an example of a typical assay transfer using well characterised bioreagents.

A 1 ml milk sample was incubated for 10 minutes in a vial containing 25ul of a stabilised solution of a penicillin-binding-protein-/horseradish-peroxidase complex (R39-/HRP). A b-lactam-conjugate coated biosensor was then applied to the sample mixture and incubated for a further 2 minutes. The biosensors were then rinsed and measured. The total time to perform this test is 15 minutes.

A standard five parameter log-logistic model was used to curve fit the data. Each point represents 40 samples, measured over a period of 8 days, using 5 different sensor batches. Error bars = ± 1 x standard deviation. In addition, experiments were performed using milk with different fat content to gauge the matrix effect, the results of which are also illustrated below. Similar results are obtained at RT (~18-24°C). Validated results can be seen in the table on the right. These were results from blind sample testing. The spiked samples were provided by an independent company.

Future systems based on semi-automated processes will take 5 - 10 minutes depending on required sensitivity. Fully automated systems based on active cartridge fluidics will take ~2 minutes.



## Instrumentation

Handheld instruments are being developed for the point-of-use testing environment, based on a PDA system with an integrated CF DataQ card (C-cubed) and a Uniscan PG580



## Validated Results

Blind Sample Testing: No Sample Preparation				
Independently supplied reconstituted freeze dried milk samples				
Sample	Penicillin G (ng/ml, ppb)		Chloramphenicol (ng/ml, ppb)	
	Given	Measured	Given	Measured
1	0	0	0	0.05
2	2	2	0	0
3	4	4	0	0
4	6	5	0	0
5	8	7	0	0
6	10	8	0	0
7	0	0	0	0
8	0	0	0.05	0.05
9	0	0	0.1	0.05
10	0	0	0.2	0.1
11	0	0	0.3	0.2
12	0	0	0.5	0.4
13	0	0	1.0	1.0
14	0	0	0	0
15	4	4	0.3	0.2
16	8	7	0.5	0.3

- Precise quantitative measurements in complex sample matrices
- Little or no sample preparation
- Rapid and simple testing regime
- Well established assays can be directly transferred to the technology
- Universal and flexible (proteins, nucleic acids, toxins, drugs, contaminants and cells)
- Accurate and reproducible
- Long term stability
- Low cost and suitable for automation