

# An Ultrasensitive and Stable Potentiometric Immunosensor: UTS™

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Fast, <15 minutes  
No Sample Preparation  
Sensitive (<50fM)

Keywords: potentiometric, biosensor, polypyrrole, ultrasensitive, reproducible, stable, immunoassay.

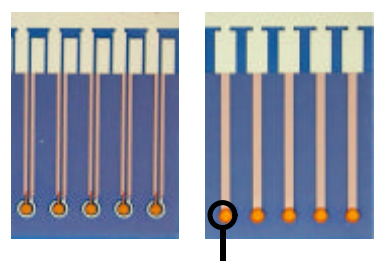
## Abstract

We present a novel quantitative potentiometric method called the Universal Transducer System (UTS™), which provides broad-spectrum assay capability. The technology is rapid (<15mins), ultrasensitive (<50fM), reproducible (CV <5% at 0.1ng/ml), has a wide dynamic range (4-5 orders of magnitude), and is scalable for multi-analyte determination.

The universality of the technology has been demonstrated in assays for hepatitis B surface antigen (HBsAg), Troponin I, Digoxin and Tumour Necrosis Factor (hTNF-alpha). These model targets were chosen to represent analytes of a range of molecular weights, and because of their requirement for assays of high analytical sensitivity and precision

UTS™ detects bound bioreceptor-target complexes formed at the surface of a polypyrrole coated, screenprinted gold electrode. Detection is mediated by a secondary reaction that produces charged products (a 'charge-step' procedure). A shift in potential is measured at the sensor surface, caused by local changes in charge, redox state or pH. The magnitude of the difference in potential is related to the concentration of the formed receptor-target complex.

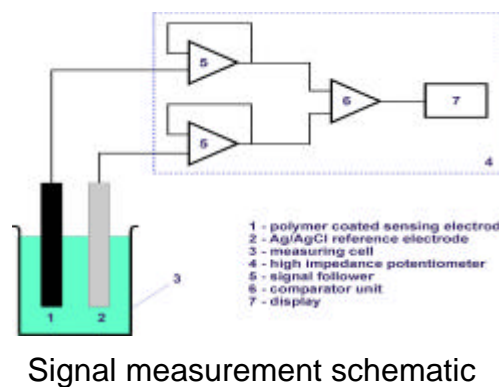
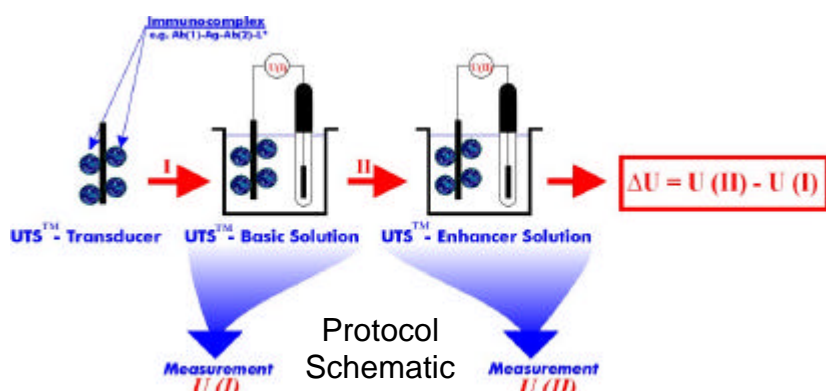
- Assays have been conducted using complex solutions e.g. serum and whole blood .
- The polypyrrole-coated sensors are shown to be stable at 37°C for 4 months.
- Patented Technology



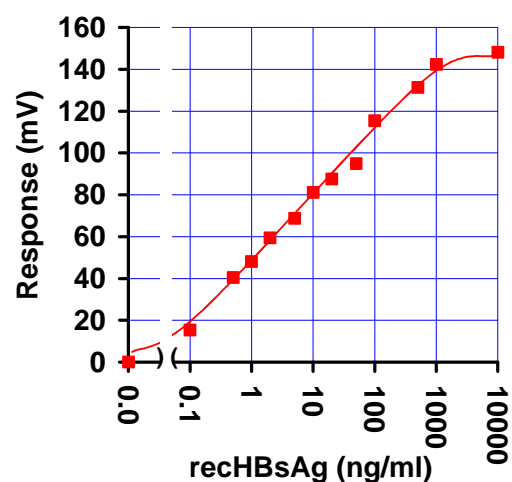
Screen-printed sensor (1 mm<sup>2</sup>) working area



Laboratory Measuring Device



The data is collected and results presented using a PC with an analogue to digital converter (ADC) card and bespoke software.



**Sensor preparation:** A screenprinted gold electrode on a 175µm thick PET (polyethylene terephthalate) substrate is coated with polypyrrole and specific bioreagents. A strip of up to 50 sensors are immersed in the polymerization solution and a cyclic voltage between -0.2 to +1.9V, scan rate 0.05V/sec is applied (mAulab, type II, EcoChemie); the amount of electricity passed through each sensor is 0.3mC (mCoulomb). The sensors are washed several times with deionised water, dried, spot coated with bioreceptors solution (e.g. streptavidin, antibodies, antigens, etc.) and dried again at 37°C.

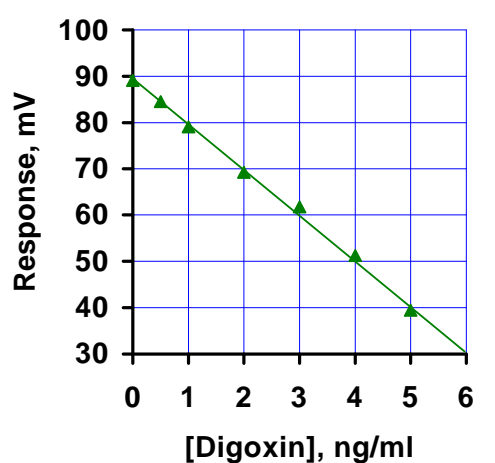
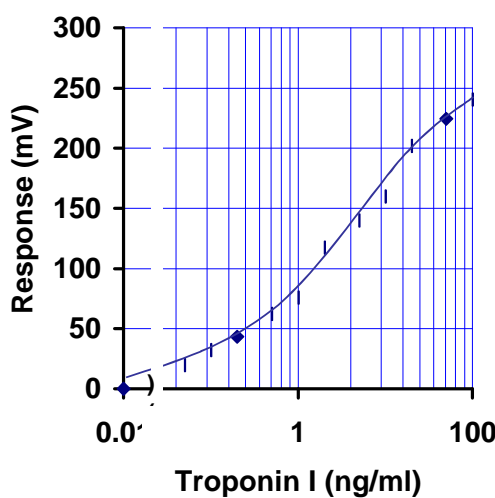
**Solutions:**  
Polymerization solution: 0.001 - 0.02M pyrrole and 0.0003 - 0.001M sodium dodecyl sulphate (SDS).  
Coating solution: 0.05M potassium phosphate buffer, pH 7.8. (can be used for sample dilution)  
Wash solution (Basic): 0.1mg/ml OPD in 0.05M sodium citrate buffer, pH 5.0.  
Active substrate solution (Enhancer): wash solution plus 0.014% hydrogen peroxide.

## RESULTS

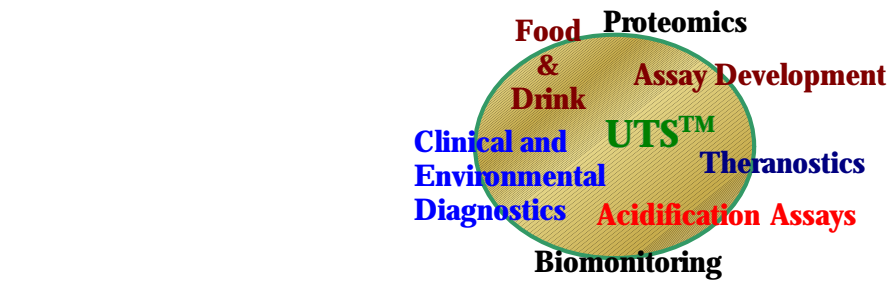
Using ELISA protocols, this technology is rapid (<15mins), ultrasensitive and precise, and has been demonstrated for:

- Hepatitis B surface antigen;** (HbsAg), 0.05IU/ml, 50pg/ml - 1µg/ml, CV's of 2% -5% at the lowest concentrations. (functional sensitivity limit ~50fM)
- Troponin I** complex in Troponin I Free Serum, 10pg/ml, - 100ng/ml (functional sensitivity limit ~0.4pM)
- Tumor Necrosis Factor-alpha;** (TNF-alpha), 8pg/ml - 1ng/ml (functional sensitivity limit ~0.3pM)
- Digoxin;** using a two-step competitive assay or sequential saturation assay, the concentration of the active components was designed to give good discrimination in the clinical therapeutic window of digoxin (0.5 - 2ng/ml).

As a small molecule, digoxin also serves as a model for food, drug and environmental monitoring assays.



Typical Calibration Curves

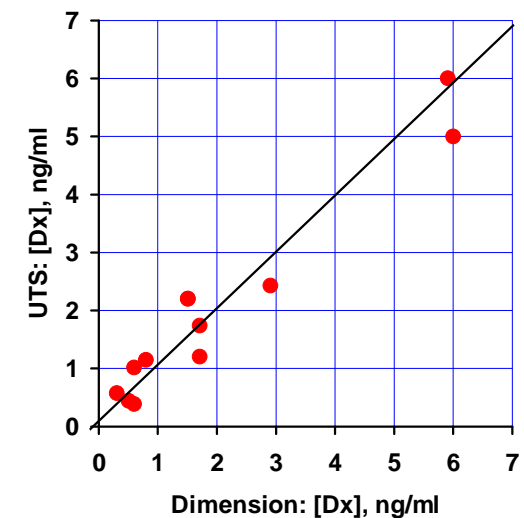
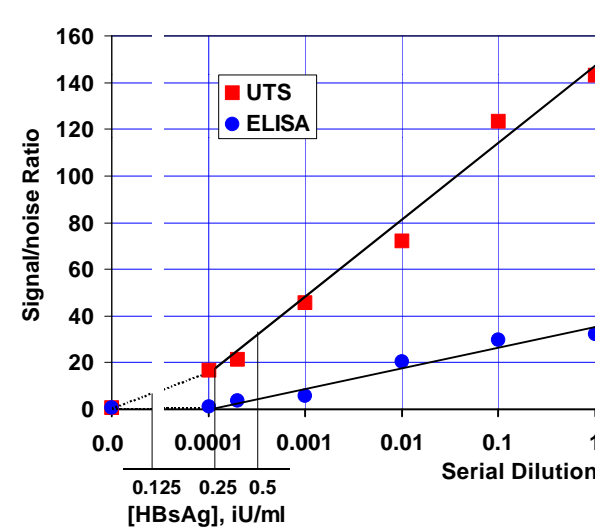


## Comparison with Commercial Systems

Comparative studies have been carried out against a commercially available ELISA (Biokit) and State-of-the-Art Dade Behring instruments

Troponin I	Assay Range (ng/ml)	Sensitivity (ng/ml)	CV's at ~0.5 ng/ml	CV's at 5 ng/ml
UTS™	0-100	<0.05	4%	2%
Stratus® CS	0-50	0.03	>5%	>3.4%

Troponin I assay comparison: UTS™ Vs Stratus™CS

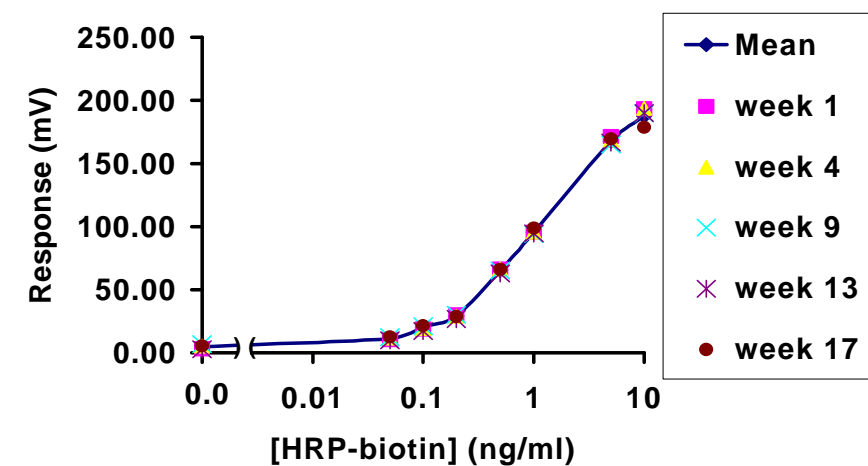


## HbSag assay comparison: UTS™ Vs ELISA (Biokit)

Using serial dilution of a highly positive sample it was demonstrated that the working range of UTS™ Technology is significantly larger than that of the reference ELISA. Using dilutions of the 2nd British Standard (0.5 IU/ml) and the 2nd NIBSC/UKBTS Monitor Sample (0.125 IU/ml), it was found that discriminatory power at low concentrations was clearly superior in the case of the UTS™ assay.

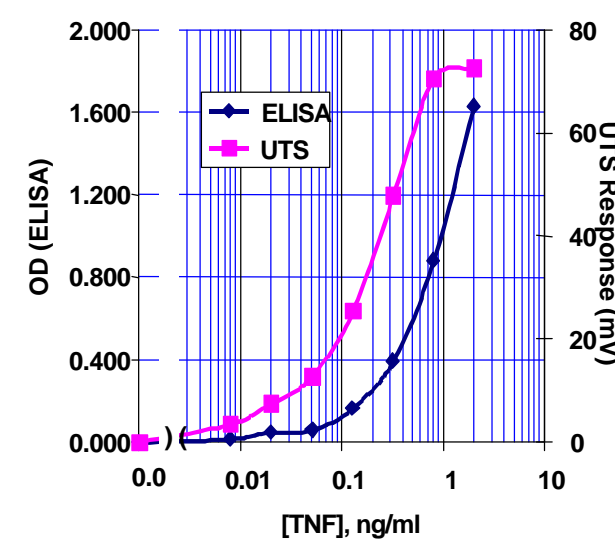
And

Digoxin assay correlation curve: UTS™ Vs Dimension™ (Dade Behring)



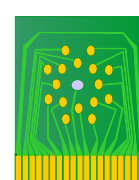
## Accelerated Stability Studies

Polypyrrole-coated sensors with no further treatment were shown to be stable at 37°C over a 4-month period. Polypyrrole sensors coated with streptavidin and treated with a simple sucrose stabilisation layer were also stable at 37 °C for 4 months. A calibration curve for a quality control (QC) assay based on the capture of biotinylated-HRP was performed once a week for first seven weeks, and then once every two weeks for the next ten weeks. Any deterioration would be indicated by changes in the shape of the calibration curve over time.



**TNF-alpha assay directly transferred from commercial ELISA kit to UTS™.** Comparative calibration curves and assay parameters are shown.

Assay	ELISA	UTS™
Sample Buffer	0.1% BSA in PBS	50% serum in PBS
Time	5 hrs	45 min
Sensitivity	50pg/ml	8pg/ml



## The Future

The use of proprietary substrate/reagent systems are being developed to enhance the sensitivity, increase the measuring range and simplify reagent requirements. Multi-array Chip formats can be envisaged.

## Conclusions

A stable, charge sensitive polypyrrole layer has produced a quantitative potentiometric immunosensor, which has ultrahigh sensitivity and good precision.

Applicable to high, medium and low molecular weight analytes, it therefore has the ability to perform a wide range of immunoassays currently required in routine and special clinical laboratories.

Potential in the pharmaceutical, veterinary, food and environmental industry sectors. The technology is adaptable to multianalyte assays, point-of-care (use) testing, and can be easily automated.

Assays have been conducted using both diluted and non-diluted human samples: serum, plasma, urine and whole blood.