Queen's University
Belfast

Development of a rapid low cost fluorescent biosensor for the detection of food contaminants

Published in:
Biosensors and Bioelectronics

Document Version:
Peer reviewed version

Queen's University Belfast - Research Portal:
Link to publication record in Queen's University Belfast Research Portal

Author's Accepted Manuscript

Development of a rapid low cost fluorescent biosensor for the detection of food contaminants

Terry F. McGrath, Karl Andersson, Katrina Campbell, Terry L. Fodey, Christopher T. Elliott
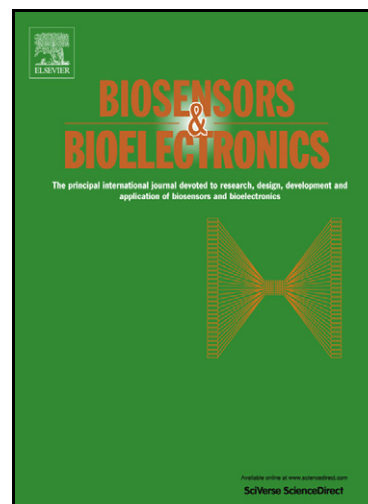
Cite this article as: Terry F. McGrath, Karl Andersson, Katrina Campbell, Terry L. Fodey and Christopher T. Elliott, Development of a rapid low cost fluorescent biosensor for the detection of food contaminants, *Biosensors and Bioelectronics,* http://dx.doi.org/10.1016/j.bios.2012.07.081

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting galley proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Development of a rapid low cost fluorescent biosensor for the detection of food contaminants

Terry F. McGrath[a]*, Karl Andersson[bc], Katrina Campbell[a], Terry L. Fodey[d], Christopher T. Elliott[a]

[a] ASSET Technology Centre, Institute of Agri-Food and Land Use, School of Biological Sciences, Queen's University, Stranmillis Road, Belfast BT9 5AG, Northern Ireland

[b] Ridgeview Instruments AB, Ulleråkersvägen 62, UPPSALA SE75643, Sweden

[c] Dept of Oncology, Radiology, and Radiation Sciences, Uppsala University, Sweden

[d] Agri-food and Biosciences Institute, Stoney Road, Belfast BT4 3SD, Northern Ireland

* Corresponding author. E-mail address t.mcgrath@qub.ac.uk (T. McGrath), Tel +44 2890 976543

Abstract

A prototype fluorescent based biosensor has been developed for the antibody based detection of food related contaminants. Its performance was characterised and showed a typical antibody binding signal of 200-2000 mV, a short term noise of 9.1 mV, and baseline slope of -0.016 mV/s over 4 hours. Bulk signal detection repeatability (n=23) and reproducibility (n=3) were less than 2.4 %CV. The biosensor detection unit was evaluated using two food related model systems proving its ability to monitor both binding using commercial products and inhibition through the development of an assay. This assay development potential was evaluated by observing the biosensor's performance whilst appraising several labelled antibody and glass slide configurations. The molecular interaction between biotin and an anti-biotin antibody was shown to be inhibited by 41% due to the presence of biotin in a sample. A food toxin (domoic acid) calibration curve was produced, with %CVs ranging from 2.7 to 7.8%, and a midpoint of approximately 17 ng/ml with further optimisation possible. The ultimate aim of this study was to demonstrate the working principles of this innovative biosensor as a potential portable tool with the opportunity of interchangeable assays. The biosensor design is applicable for the requirements of routine food contaminant analysis, with respect to performance, functionality and cost.

Keywords

Optical biosensor, domoic acid, food contaminants, prototype fluorescence biosensor, low cost

1 Introduction

Safe food is an essential requirement of modern society, and governmental authorities throughout the world are constantly monitoring the food supply chain in an attempt to ensure an adequate safety level. One well recognised threat is the chemical contamination of food, where compounds such as drug residues, pesticides and natural toxins are unintentionally present in food stuffs.

Biosensor methods can be applied, as rapid screening tools, to detect such contaminants. They can be developed using several types of transducer (Reder-Christ and Bendas, 2011) one of which is based on fluorescence either through quenching (Wang et al., 2011) or labelling of the biological recognition elements, e.g. fluorescently labelled antibody (Taitt et al., 2008). Whilst using labelled

antibodies to detect low molecular weight compounds (<1000 daltons), such as natural toxins or chemical food contaminants, an inhibition based assay is generally needed to reach the required sensitivity (Yu et al., 2005).

There are a limited number of publications showing fluorescence biosensors being used for the detection of low molecular weight food contaminants. Ngundi et al., (2006) and Sapsford et al., (2006) both demonstrate the use of the Naval Research Laboratory (NRL) array biosensor for the detection of mycotoxins. Schultz et al., (2007) show proof-of principle for a portable fluorescence biosensor for the detection of aflatoxin B1 based on quenching. Sun et al., (2011) and Wang et al., (2011) show the use of fluorescence based biosensors for the detection of pesticide residues. Both methods are based on the action of pesticide residues on the activity of acetylcholinesterase and use forms of quenching to indicate enzyme activity thus pesticide concentration.

Commercial biosensors have advanced greatly over the years with improvements in sensitivity, and increased throughput by array format (Malic et al., 2011), multiple simultaneous channels (Roh et al., 2011) or both (Abdiche et al., 2011).These instruments tend to be outside the budgetary restraints of most food contaminant laboratories. A few commercial biosensors have been developed specifically for food analysis. One such example is the surface plasmon resonance (SPR)-based Biacore Q (Ferguson et al., 2005) with others being various adaptations of the NRL array biosensor (Ligler et al., 2007). These biosensors have many attributes highly sought after for routine food contaminant analysis such as ease-of-use, fast assay times, sensitivity, good repeatability and reproducibility (GE Healthcare, 2011) with the prototype NRL array biosensor showing multiplexing capabilities (Taitt et al., 2008). However the substantial cost of the Biacore Q limits its customer base to the larger food companies and government facilities and the applications on the NRL array biosensor have mainly been focused on the detection of bacterial contamination and plant toxins that pose a potential terrorism risk (Constellation Technology, 2009, MBio, 2011, ThomasNet News, 2007).

The aim of the present research was to develop, from first principles, a low cost, potentially portable, fluorescence biosensor capable of detecting low levels of contaminants and compounds of interest in food analysis. We demonstrate that a simple and inexpensive fluorescence biosensor can perform to the detection capabilities required in food monitoring laboratories, and hence prove it is possible to engineer an analytical biosensor which also matches their budgetary requirements~~It was demonstrated that a simple fluorescence biosensor can perform to the detection capabilities required in monitoring laboratories~~. The research presented outlines the performance characterisation of the biosensor, the signal inhibition obtained using two model compounds (biotin and domoic acid, Figure 1) as well as the biosensor detection unit's performance during assay development leading to the construction of calibration curves.

2   Material and methods

2.1   Reagents

HBS-EP buffer was obtained from GE Healthcare, UK. Domoic acid was obtained from Fluorochem, UK. 1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC), 2-(N-morpholino)ethanesulfonic acid (MES), biotin, bovine serum albumin (BSA), ethanolamine, ethylene diamine, fluorescein sodium and N-hydroxysulfosuccinimide (NHS) were obtained from Sigma-Aldrich, UK. The acid surface slide, biotin glass slide and DyLight 488 labelled anti-biotin monoclonal antibody (1.8 mg/ml) were obtained from Stratech Scientific Ltd., UK. DyLight 488 antibody labelling kit, fluorescein isothiocyanate (FITC) antibody labelling kit, goat anti-mouse DyLight 488 labelled antibody (1 mg/ml) and goat anti-rabbit DyLight 488 labelled antibody (1 mg/ml) were obtained from Thermo Scientific, UK. Amino functionalised slides, carboxymethyldextran (CMD) functionalised slides, HC polycarboxylate hydrogel amino derivatised (AHC) slides, HC polycarboxylate hydrogel (HC) slides, HC polycarboxylate hydrogel NHS activated (HCX) slides were obtained from XanTec Bioanalytics GmbH, Germany.

2.2   Instrumentation

From first principles, a prototype biosensor detection unit, L 32 cm x W 24 cm x H 16 cm, was constructed that was composed of an illumination component, a sensor unit, a flow cartridge and a peristaltic pump, as illustrated in Figure 1. The illumination component contained a blue LED, a collecting lens and a 488 nm bandpass (20 nm) optical filter. The sensor unit was based on a fluorescence detector from a LigandTracer Green prototype (Ridgeview Instruments AB, Uppsala, Sweden) containing a Hamamatsu photodiode, a collecting lens and a 535 nm bandpass (20 nm) optical filter. The sensor unit was connected to a PC using a general-purpose LabJack U12 data acquisition device (LabJack Corp, Lakewood, Colorado) which measured the detector output voltage 1-3 times per second. The integrated flow cartridge, see Figure 1, was made of a steel bracket designed to clamp immuno-functionalised glass slides (25 mm × 75 mm × 1 mm) onto a flow cell made of epoxy plastic. The flow cell diameter was approximately 8 mm with a volume of approximately 30 μl and was sealed to the glass slide through an o-ring. The flow cell had one inlet peek tubing and one outlet peek tubing. On the outlet tubing, an aspirating peristaltic pump (Pharmacia Biotech Pump P-1) was attached and set to aspirate at approximately 150 μl/min. The inlet tubing was manually moved from one vial to another to change the aspirating liquid from running buffer, to sample or regeneration solution. When changing liquids, the tubing was kept in air for 20 seconds to make an air bubble. The signal data points, created by the binding of a

fluorescently labelled antibody to the immunospecific surface, were imported into TraceDrawer (v1.3) (Ridgeview Instruments AB, Uppsala, Sweden) for processing of 5 second window report points before evaluation in Microsoft Excel 2007 (Microsoft Corp., USA) or BiaEvaluation software (v4.1) (GE Healthcare, Uppsala, Sweden). All report points were relative to the baseline, measured 10 seconds before the injection of reagents.

[INSERT FIGURE 1]

2.3 Biosensor detection unit performance characterisation

2.3.1 Basic characterisation of the biosensor detection unit

A plain glass slide was clamped within the detection unit and running buffer (HBS-EP plus 0.1% (w/v) BSA) was aspirated over the surface for approximately 4 hours. The baseline signal was continuously observed during this period. Next, a solution of fluorescein sodium (3ng/ml) was prepared in running buffer and used to measure repeatability (n=23) of fluorescein injections (2 minutes long), using a report point at 60 seconds after the start of aspiration. This experiment was repeated on two further days to calculate reproducibility (n=3).

2.4 Model assay 1: Detection of an antibody binding to immobilised biotin

A commercially available pre-immobilised biotin glass slide and DyLight 488 labelled anti-biotin monoclonal antibody (mAb), diluted 1/100 with running buffer, were used to investigate the biosensor's ability to detect and differentiate between binding events. A fresh biotin slide was clamped and allowed to stabilise in running buffer. Unless otherwise noted, each measurement cycle was conducted by aspirating antibody over the surface for 10 minutes followed by running buffer for 2-3 minutes dissociation. The surface was then regenerated for 2-5 minutes with 50 mM sodium hydroxide before stabilisation in running buffer for 3 minutes, in preparation for the next cycle. The binding level was evaluated using a report point taken 30 seconds into the dissociation phase.

Following the initial binding experiment, a further test was performed to determine if the biosensor could detect inhibition of the antibody binding to the surface. A biotin solution (1000 ng/ml) was prepared by serial dilutions in running buffer. Because of the initial high signal obtained for the 1/100 diluted labelled mAb, other dilutions were investigated to see which would still give an acceptable binding level (data not shown). A 1/1200 final antibody dilution was selected. Hence, antibody solution was diluted 1/600 and mixed 1:1 with either running buffer or biotin standard. The binding level was evaluated using a report point taken 30 seconds into the dissociation phase.

Next, fresh 1/1200 diluted antibody was prepared and repeatability of the signal at 30 seconds into the dissociation phase was evaluated over the six cycles.

2.5 Model assay 2: Assay development for domoic acid detection

2.5.1 Production of polyclonal and monoclonal anti domoic acid antibodies

Polyclonal antibodies (pAb) were prepared using domoic acid bovine thyroglobulin protein conjugate in the same manner as that described using domoic acid human serum albumin by Traynor et al., (2006). Monoclonal antibodies using this immunogen were also prepared as described in the fusion procedure by Galfre and Milstein, (1981).

2.5.2 Preparation of FITC and Dylight 488 labelled primary antibodies

The method supplied with the relevant dye labelling kit was followed for the labelling of pAb and mAb with either FITC or DyLight 488. Briefly, the antibody (2mg/ml) was prepared in 50 mM sodium borate pH 8.5 and added (500 μl) to a vial of supplied labelling reagent and incubated for 1 hour at room temperature protected from light. The antibodies were then purified using the purification resin by centrifuging at 1000 × g for 1 minute. The label to antibody ratio was estimated to be 1.4:1 for FITC labelled pAb and 2.2:1 for FITC labelled mAb. The ratios for DyLight 488 labelled pAb and mAb were 3.1:1 and 2.1:1 respectively according to the equation provided, in the labelling kit instructions. Antibody solutions were divided into 50 μl aliquots and stored at -20 °C.

2.5.3 Preparation of domoic acid immobilised glass slides

2.5.3.1 Immobilisation of amino slide

Domoic acid (0.2 mg) was activated by the addition of EDC (0.5 mg) and NHS (0.2 mg) in 100 mM MES buffer pH 5.0. The mixture was allowed to react for 30 minutes at room temperature before the pH was adjusted to approximately 7. This solution was then spread over a small area of the glass slide, corresponding to the flow cell area, and allowed to react overnight at room temperature, protected from light and in a humid environment. The slide was washed with water, clamped into the detection unit and allowed to equilibrate under a flow of running buffer for at least 1 hour before experiments were performed.

2.5.3.2 Immobilisation of CMD slides

The slide was immersed in 1 M sodium chloride, 0.1 M sodium carbonate pH 10 for 5 minutes. EDC (0.67 mg) and NHS (0.27 mg) in 100 μl of 100 mM MES buffer pH 5.0 was then spread over a small area of the glass slide, corresponding to the flow cell, and allowed to react for 30 minutes then removed. Ethylene diamine (100 μl of 0.1 M) in PBS pH 7.4 was placed on the surface for 3 hours then removed. The surface was capped with 100 μl of 1 M ethanolamine pH 8.5 for 30 minutes. Meanwhile, domoic acid was activated via addition of EDC and NHS, as described already, in 100 mM MES buffer pH 5.0 and adjusted to approximately pH 7 after 30 minutes. This solution was then spread over the activated area and allowed to react overnight at room temperature, protected from light and in a humid environment. The slide was washed with water, clamped into the biosensor and allowed to equilibrate under a flow of running buffer for at least 1 hour before experiments were performed.

2.5.3.3   Immobilisation of additional slides

Domoic acid was immobilised on each of the additional slides according to the relevant previously described method (depending on the slide functional chemistry). HC slides were immobilised following the procedure outlined for CMD slides. HCX slides are already NHS activated therefore they were also immobilised in a similar procedure to the CMD slides but starting with the addition of ethylene diamine. AHC slides are terminated in amino functional groups therefore the procedure for immobilising amino slides was followed. The acid slide was immobilised in accordance with the CMD method.

2.5.4   Selection of best performing antibody configuration

Antibody formats were evaluated in order to find the best performing configuration in terms of signal intensity, repeatability and stability. Polyclonal and monoclonal antibodies were used and the following combinations were evaluated using amino and CMD functionalised slides immobilised with domoic acid: FITC labelled primary antibody, DyLight 488 labelled primary antibody and unlabelled primary antibody mixed with DyLight 488 labelled anti-species secondary antibody.

For each of the labelled primary antibody configurations the antibody was diluted 1/100 in running buffer. For the unlabelled primary antibody combined with labelled anti-species secondary antibodies, the primary antibodies were prepared at the same concentration as their labelled counterparts when mixed 1:1 with 1/50 diluted labelled secondary antibody.

For each of the antibody configurations, signal intensity and repeatability was determined by passing five separate identical cycles over the surface of freshly immobilised amino or CMD slides.

The assay parameters for each measurement cycle were as follows: each sample was aspirated over the surface for 10 minutes followed by running buffer for a 2 minute dissociation phase. The surface was then regenerated for 5 minutes with 50 mM sodium hydroxide before stabilisation in running buffer for 2 minutes, in preparation for the next cycle. The binding level was evaluated using several report points taken during the dissociation phase.

2.5.5   Selection of best performing functionalised slide

On selecting the best performing antibody configuration, repeatability experiments using DyLight 488 labelled mAb diluted 1/100 were performed on the additional domoic acid immobilised slides to identify the best performing antibody and slide combination. The assay parameters described above were used throughout. The binding level was evaluated using report point taken several times during the dissociation phase. The drop in signal over time was compared for different antibodies using a two-tailed T-test.

2.5.6   Inhibition studies with domoic acid

Once antibody and slide configuration were established, the biosensor's ability to detect inhibition of antibody binding to the slide surface was evaluated. DyLight 488 labelled mAb was diluted 1/50, 1/100, 1/150, 1/200, 1/250 and 1/300 and mixed 1:1 with either running buffer or domoic acid standard (20 ng/ml). These were then passed over a freshly immobilised AHC slide using the assay

6

parameters previously described. The binding level was evaluated using a report point taken 30 seconds into the dissociation phase.

2.5.7 Development of a domoic acid calibration curve

A domoic acid immobilised AHC slide was clamped into the detection unit. DyLight 488 labelled mAb was diluted 1/300 and mixed 1:1 with 0, 1, 10, 20, 50, 100 and 200 ng/ml domoic acid standards. Samples were analysed in this order using the same assay parameters as previously described. This was repeated to allow a calibration curve to be constructed in duplicate. The binding level was evaluated using a report point taken 30 seconds into the dissociation phase. These report points signals were imported into BiaEvaluation software (v4.1) for the construction of a calibration curve using a 4-parameter fit equation, as is common in other concentration analysis assays such as those based on SPR (Ferguson et al., 2005), ($S = S_{lo} - (S_{lo} - S_{hi}) / (1 + (conc/A_1)^{\wedge}A_2)$), where S is signal, conc is the concentration of standard, A_1 is the half maximal effective concentration (EC_{50}) and A_2 is hill slope. A_1 and A_2 are numbers greater than zero.

3 Results and discussion

3.1 Biosensor detection unit performance characterisation

3.1.1 Basic characterisation of the biosensor detection unit

A total of 15580 data points were collected during the 4 hour measurement and showed an overall signal drift of -253.9 mV (3.1 %CV) with a slope of -0.016 mV/s. Typical short term noise (peak – peak) was 9.1 mV. Repeatability (n=23) at 60 seconds into injection was determined with an average relative signal of 1381.0 mV and 1.0 %CV. When~~ilst~~ comparing average signal from three independent repeatability experiments, reproducibility (n=3) was determined to be 0.6 %CV. The slope measured during the 4 hours was roughly linear. Both repeatability and reproducibility data have low %CVs in this detection unit test. For an exemplary complete assay for determining pantothenic acid levels in food using a Biacore Q, repeatability and reproducibility %CVs ranging from 1.5 % to 7.1 % has been reported (Haughey et al., 2005). ~~and show values similar to those obtained by (Haughey et al., 2005), who describe repeatability and reproducibility %CVs ranging from 1.5 % to 7.1 % in a Biacore Q based method for determining pantothenic acid levels in food.~~ Thus, the detection unit has displayed a~~n~~ better ~~adequate~~ basic performance than what is required for a full assay which leaves space for assay related noise in this ~~for providing a~~ proof-of-principle in food contaminant analysis.

3.2 Model assay 1: Detection of an antibody binding to immobilised biotin

3.2.1 Binding

For the injection of the 1/100 diluted DyLight 488 labelled mAb, the relative signal at 30 seconds into dissociation was 2747.9 mV. This clearly showed that a binding event can be observed using the biosensor detection unit.

3.2.2 Inhibition of antibody binding

Figure 2 shows an overlay of the sensorgrams for negative and positive samples, i.e. samples without and with biotin present. The negative sample produced a signal of 311.4 mV at 30 seconds into dissociation whilst the positive sample produced a signal of 129.4 mV. For the positive sample, the signal has been reduced to approximately 41 % of that of the negative sample. This is due to antibody binding to biotin in the sample and being inhibited from binding to the surface. This proves the instrument can detect inhibition.

[INSERT FIGURE 2]

3.2.3 Repeatability

Repeatability (n=6) was determined by calculating the average relative signal (mV) for a report point at 30 seconds into dissociation. This corresponded to 670.4 mV (4.5 %CV) thus showing that not only can the detection unit provide precise detection of a bulk signal (Section: Basic characterisation of the biosensor detection unit) but is also capable a detecting repeated binding events with a good degree of precision.

3.3 Model assay 2: Assay development for domoic acid detection

3.3.1 Selection of best performing antibody configuration

Table 1 shows the average relative signal (mV) (n=5) and %CV, for various antibody configurations, at several report points during dissociation, on domoic acid immobilised amino and CMD slides. The amino slides consist of a 2D dense monolayer of primary and secondary amino groups whilst the CMD slides consist of a 3D hydrogel brush surface (500 nm thick) composed of carboxymethyldextran with a single COOH group per anhydroglucose unit.

[INSERT TABLE 1]

The mAb ~~appears to~~ has~~ve~~ a slower apparent dissociation rate ~~thus higher affinity for the surface~~ than the pAb in all tested antibody configurations on both glass slides, (~~O~~observed as a lower drop in signal intensity over time (p<0.02). The pAb based assays (Table 1 rows 1-3 & 7-9) show an average drop in signal intensity, by the 90 second report point, to 60 % of the 30 second report point. Whilst the mAb based assays (Table 1 rows 4-6 & 10-12) only show an average drop to 80 %.

The DyLight 488 labelled antibodies consistently provided a more intense signal than the FITC labelled equivalent. This can be observed by comparing the relative signal at the 30 second report point in Table 1 row 1 (FITC labelled pAb, 118.5 mV) against row 2 (Dylight 488 labelled pAb, 445.4 mV), row 4 (FITC labelled mAb, 366.8 mV) against row 5 (Dylight labelled mAb, 532.2 mV) for amino slides. The data for the CMD slides shows the same trend. Compare row 7 (FITC labelled pAb, 216.0 mV) against row 8 (Dylight 488 labelled pAb,363.2 mV), row 10 (FITC labelled mAb, 284.1 mV) against row 11 (Dylight labelled mAb,582.1 mV)

Based on the data in Table 1 and the above analysis, the two antibody configurations showing most promise were the mixed unlabelled primary mAb/DyLight 488 labelled anti species antibody and the DyLight 488 labelled mAb with average signals, at 30 seconds into dissociation, of 1022.8 mV (15.2 %CV) and 853.2 mV (14.3 %CV) on the amino slide and 619.6 mV (6.3 %CV) and 582.1 mV (2.8 %CV) on the CMD slides.

3.3.2   Selection of best performing functionalised slide

The DyLight 488 labelled mAb was chosen to perform the surface selection as there was little difference in signal intensity between it and the more expensive option of mixed unlabelled primary mAb/DyLight 488 labelled anti species antibody, the signal was more stable over time and the %CVs were also lower (Compare Table 1 row 11 and row 12).

A range of other slides available from Xantec Bioanalytics were evaluated: HC, HCX and AHC slides as well as an alternative acid surface slide from Stratech Scientific Ltd. All HC slides are non-saccharide 3D hydrogel based (1000–2000 nm thick) and use a proprietary linear polycarboxylate polymer in a brush conformation. The acid surface slide from Stratech Scientific Ltd. is described as being 2–3 nm thick with a density of 10^{14} per cm^2.

Figure 3 shows the different levels of binding and repeatability (n=5) obtained on the slide showing the highest level of binding (AHC slide) and lowest level of binding (acid surface slide) on repeated injections of the same antibody concentration. Table 2 shows the average signal (n=5) and %CV, at various report points, of repeated injections of Dylight 488 labelled mAb diluted 1/100 on all immobilised slides. The AHC slide (Table 2 row 4) displays superior qualities over all other slides with average signal (n=5) at the 30 second report point being 2115.43 mV (3.7 %CV). All other slides showed signal intensities less than half this value. The signal intensity remained strong with the 90 second report point showing intensity approximately 95 % of the 30 second report point indicating slow dissociation from the surface.

[INSERT FIGURE 3]

[INSERT TABLE 2]

3.3.3   Inhibition studies with domoic acid

Having selected the most appropriate antibody (Dylight 488 labelled mAb) and surface (domoic acid immobilised to an AHC slide) inhibition was performed. The % binding obtained for the positive compared to the negative for 1/50, 1/100, 1/150, 1/200, 1/250 and 1/300 antibody dilutions were 76.8 %, 64.2 %, 56.7 %, 53.9 %, 54.0 % and 54.0 % respectively. The signal obtained for negative and positive samples decreased with increasing dilution as expected (data not shown). The signal, for the

positive, dropped from 76.8 % for the 1/50 dilution to approximately 54 % by the 1/200 dilution then did not alter significantly between that and the 1/300 dilution.

3.3.4 Development of a domoic acid calibration curve

A calibration curve was constructed using 1/300 diluted DyLight 488 labelled mAb. Report points were taken 30 seconds into the dissociation phase. These report point signals were then plotted against concentration of domoic acid, using a 4-paramater fit equation, to produce a calibration curve (Figure 4). The range (between highest and lowest standard) and midpoints, in parentheses, was 289.7 mV (17.9 ng/ml). The %CVs for the replicates ranged from 2.7 to 7.8 (n=2). The %CVs for the calibration curve replicates are again similar to those for fluorescein bulk signals, even though this assay contains more steps than the initial basic characterisation of the detection unit. Since the range in %CV obtained for the seven calibrants is acceptable, we conclude that the assay has acceptable repeatability.

[INSERT FIGURE 4]

The sensitivity of the calibration curve compares favourably against other published biosensor data on domoic acid. The curve midpoint value is low (17.9 ng/ml) and similar to those produced by Le Berre and Kane, (2006), Micheli et al., (2004), Stevens et al., (2007) and Yu et al., 2005) who showed midpoints of approximately 20 ng/ml using either optical or electrochemical biosensors. The curve is more sensitive than those produced by Kreuzer et al., (2002) (midpoint 42 ng/ml) and Lotierzo et al., (2004) (midpoint 58 ng/ml), however it is not as sensitive as that produced by Campbell et al., (2011) (midpoint 2.6ng/ml) using a fully automated prototype multichannel array surface plasmon resonance biosensor. Traynor et al., (2006) approached development from a different angle and desensitised their test to allow for the detection of domoic acid with its action limit of 20 µg/g (Traynor et al., 2006) close to the midpoint of their assay (18 µg/g). This guaranteed maximum sensitivity to concentration variations at and around this level by placing the action limit concentration in the middle of their linear range. This is another approach used to set an assay range at the action limit rather than employing a large sample dilution step that would be used in the other assays.

4 Conclusion

A prototype fluorescent biosensor detection unit has been successfully developed that exhibits highly acceptable baseline stability, and in its current form, i.e. a manual system, shows excellent signal repeatability. This unit has been tested using two models and has proven to be capable of detecting binding and inhibition with both compounds with a good degree of repeatability. Furthermore the unit has shown that it can be used as an assay development tool that can result in the production of calibration curves with excellent %CVs. The assay developed for domoic acid had a very promising sensitivity which could be improved by further optimisation of assay variables as well as improvements to the biosensor detection unit. The fact that the biosensor can be rapidly configured to analyse a different analyte by simply changing the slide and using the appropriate labelled recognition element increases its applicability in food testing laboratories were many different analytes often require detection. Also the unit's inexpensive design and compact size

makes it potentially portable and with a dedicated power source it could become a field based instrument which matches the performance and budgetary requirements of the typical food quality control laboratory.

Acknowledgements

References

Abdiche, Y.N., Lindquist, K.C., Pinkerton, A., Pons, J., Rajpal, A., 2011. Anal. Biochem. 411, 139-151

Campbell, K., McGrath, T., Sjölander, S., Hanson, T., Tidare, M., Jansson, Ö., Moberg, A., Mooney, M., Elliott, C., Buijs, J., 2011. Biosens. Bioelectron. 26, 3029-3036

Constellation Technology (2009) ABS. Available via http://www.contech.com/Technology_Biological.htm Accessed February 1 2012

Ferguson, J., Baxter, A., Young, P., Kennedy, G., Elliott, C., Weigel, S., Gatermann, R., Ashwind, H., Stead, S., Sharman, M., 2005. Anal. Chim. Acta. 529, 109-113

Galfre, G., Milstein, C., 1981. Methods Enzymol. 73, 3-46

GE Healthcare (2011) Biacore Q. Available via http://www.biacore.com/food/index.html?setsession=5c442a06a2ccec7b39e07adc96beabbe Accessed May 02 2011

Haughey, S.A., O'Kane, A.A., Baxter, G.A., Kalman, A., Trisconi, M.J., Indyk, H.E., Watene, G.A., 2005. J. AOAC Int. 88, 1008-1014

Kreuzer, M., Pravda, M., O'Sullivan, C., Guilbault, G., 2002. Toxicon. 40, 1267-1274

Le Berre, M., Kane, M., 2006. Anal. Lett. 39, 1587-1598

Ligler, F.S., Sapsford, K.E., Golden, J.P., Shriver-Lake, L.C., Taitt, C.R., Dyer, M.A., Barone, S., Myatt, C.J., 2007. Anal. Sci. 23, 5-10

Lotierzo, M., Henry, O., Piletsky, S., Tothill, I., Cullen, D., Kania, M., Hock, B., Turner, A., 2004. Biosens. Bioelectron. 20, 145-152

Malic, L., Sandros, M.G., Tabrizian, M., 2011. Anal. Chem. 83, 5222-5229

MBio (2011) About us. Available via http://www.mbiodx.com/company/ Accessed February 01 2012

Micheli, L., Radoi, A., Guarrina, R., Massaud, R., Bala, C., Moscone, D., Palleschi, G., 2004. Biosens. Bioelectron. 20, 190-196

Ngundi, M.M., Shriver-Lake, L.C., Moore, M.H., Ligler, F.S., Taitt, C.R., 2006. J. Food Prot. 69, 3047-3051

Reder-Christ, K., Bendas, G., 2011. Sensors. 11, 9450-9466

Roh, C., Kim, S.E., Jo, S., 2011. Sensors. 11, 6685-6696

Sapsford, K.E., Ngundi, M.M., Moore, M.H., Lassman, M.E., Shriver-Lake, L.C., Taitt, C.R., Ligler, F.S., 2006. Sens. Actuator B-Chem. 113, 599-607

Schultz, E, Perraut, F., Flahaut, T., Planat-Chretien, A., Galland, R., Lesbre, F., Hoang, A., Boutal, H., Volland, H., 2007. A robust fluorescence multiplex immunoassay biosensor designed for field applications

Stevens, R.C., Soelberg, S.D., Eberhart, B.L., Spencer, S., Wekell, J.C., Chinowsky, T.M., Trainer, V.L., Furlong, C.E., 2007. Harmful Algae. 6, 166-174

Sun, X., Liu, B., Xia, K., 2011. Luminescence. 26, 616-621

Taitt, C.R., Shriver-Lake, L.C., Ngundi, M.M., Ligler, F.S., 2008. Sensors. 8, 8361-8377

ThomasNet News (2007) Hanson Technologies, Inc. Introduces UltraRapid(TM) Food and Beverage Safety Solutions at Food Safety and Security Summit Expo & Conference. Available via http://news.thomasnet.com/companystory/Hanson-Technologies-Inc-Introduces-UltraRapid-TM-Food-and-Beverage-Safety-Solutions-at-Food-Safety-and-Security-Summit-Expo-and-Conference-511480 Accessed February 01 2012

Traynor, I., Plumpton, L., Fodey, T., Higgins, C., Elliott, C., 2006. J. AOAC Int. 89, 868-872

Wang, K., Wang, L., Jiang, W., Hu, J., 2011. Talanta. 84, 400-405

Yu, Q., Chen, S., Taylor, A., Homola, J., Hock, B., Jiang, S., 2005. Sens. Actuator B-Chem. 107, 193-201

Captions

Table 1: Selection of best performing antibody configuration

Table 2: Selection of best performing slide functionality

Figure 1a: Chemical structure of biotin.

Figure 1b: Chemical structure of domoic acid.

Figure 1c: Diagram of the biosensor detection unit showing light source, sensor unit, flow cartridge and glass slide integrated within a light-proof enclosure (L 32 cm x W 24 cm x H 16 cm) and connected to a PC via a labjack U12 data acquisition device.

Figure 1d: Photograph showing clamping of glass slide within the biosensor.

Figure 2: Biotin sensorgram overlay showing inhibition between a negative and 1000ng/ml standard. Signal (mV) is plotted against time for the two cycles. Baseline, sample injection and association phase, dissociation and regeneration phases are evident. Difference in signal is clearly observed between the negative and positive samples during the dissociation phase.

Figure 3: Sensorgram overlay showing repeatability (n=5) and signal intensity for DyLight 488 labelled anti-domoic acid mAb on domoic acid immobilised AHC and acid surface slides. Signal (mV) is plotted against time over the sample cycles which have been overlaid. During the dissociation phase, there is a clear difference in obtained binding level (relative to baseline) between the AHC slides and the acid surface slides. The AHC slides obtain a much higher signal due to an increased level of binding of the fluorescently labelled antibody.

Figure 4: Calibration curve of signal against domoic acid concentration. Error bars represent %CV (n=2) for replicates. The calibration curve was constructed, from report points taken during the dissociation phase of each calibrant cycle, using a 4-parameter fit equation.

Tables

| Row	Antibody format	Slide		30s	End 45
1	FITC labelled pAb	Amino slide	Average signal (mV) (n=5)	147.7	118
			%CV	3.7	8.1
2	DyLight 488 labelled pAb	Amino slide	Average signal (mV) (n=5)	445.4	357
			%CV	7.7	8.3
3	Mixed unlabelled primary pAb/DyLight 488 labelled anti species	Amino slide	Average signal (mV) (n=5)	795.1	670
			%CV	6.6	5.2
4	FITC labelled mAb	Amino slide	Average signal (mV) (n=5)	366.8	345
			%CV	7.9	7.6
5	DyLight 488 labelled mAb	Amino slide	Average signal (mV) (n=5)	8532.2	822
			%CV	14.3	14.
6	Mixed unlabelled primary mAb/DyLight 488 labelled anti species	Amino slide	Average signal (mV) (n=5)	1022.8	886
			%CV	15.2	18.
7	FITC labelled pAb	CMD slide	Average signal (mV) (n=5)	216.0	181
			%CV	11.0	13.
8	DyLight 488 labelled pAb	CMD slide	Average signal (mV) (n=5)	363.2	298
			%CV	8.5	5.8
9	Mixed unlabelled primary pAb/DyLight 488 labelled anti species	CMD slide	Average signal (mV) (n=5)	458.6	343
			%CV	8.4	7.8

13

| 10 | FITC labelled mAb | CMD slide | Average signal (mV) (n=5) | 284.1 | 269 |
			%CV	7.1	9.4
11	DyLight 488 labelled mAb	CMD slide	Average signal (mV) (n=5)	582.1	564
			%CV	2.8	2.4
12	Mixed unlabelled primary mAb/DyLight 488 labelled anti species	CMD slide	Average signal (mV) (n=5)	619.6	527
			%CV	6.3	7.1

Table 1:

Row	Slide		End report point			
			30s	45s	60s	90s
1	Amino slide	Average signal (mV) (n=5)	853.2	822.6	804.5	777.8
		%CV	14.3	14.9	15.3	16.1
2	CMD slide	Average signal (mV) (n=5)	582.4	564.1	533.2	491.6
		%CV	2.8	2.4	2.8	3.2
3	HCX slide	Average signal (mV) (n=5)	678.5	621.3	584.8	531.2
		%CV	6.0	4.5	4.6	4.0
4	AHC slide	Average signal (mV) (n=5)	2115.4	2076.8	2050.0	2008.4
		%CV	3.7	3.7	4.0	3.9
5	HC slide	Average signal (mV) (n=5)	762.2	716.0	683.6	631.5
		%CV	7.5	7.1	6.3	6.3
6	Acid surface slide	Average signal (mV) (n=5)	351.2	296.0	267.1	224.0
		%CV	1.5	4.4	5.6	6.8

Table 2:

Figures

a

b

c   d
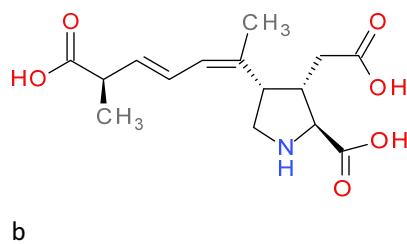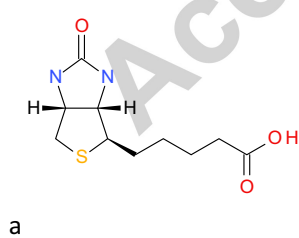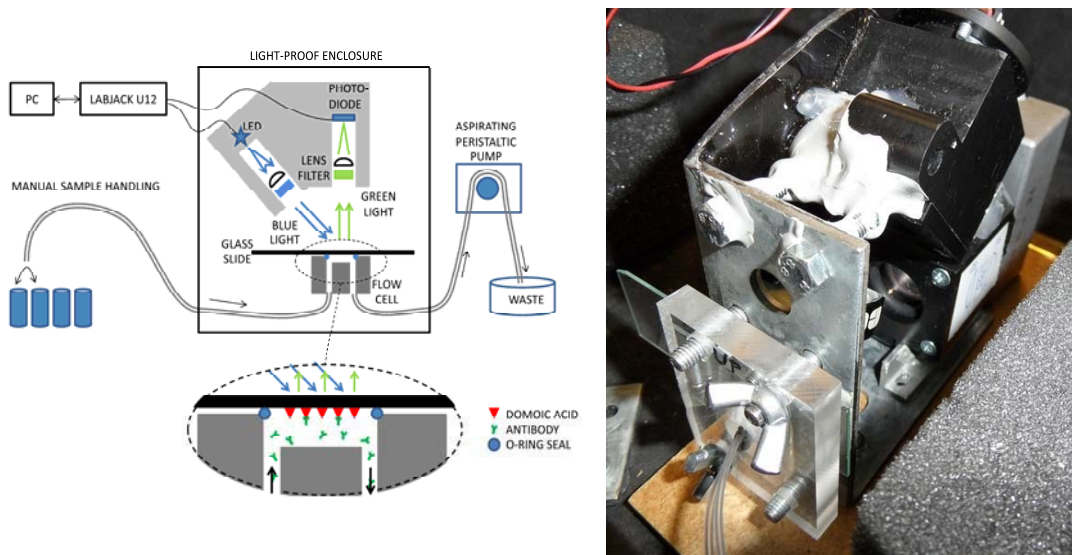
Figure 1:

Figure 2:

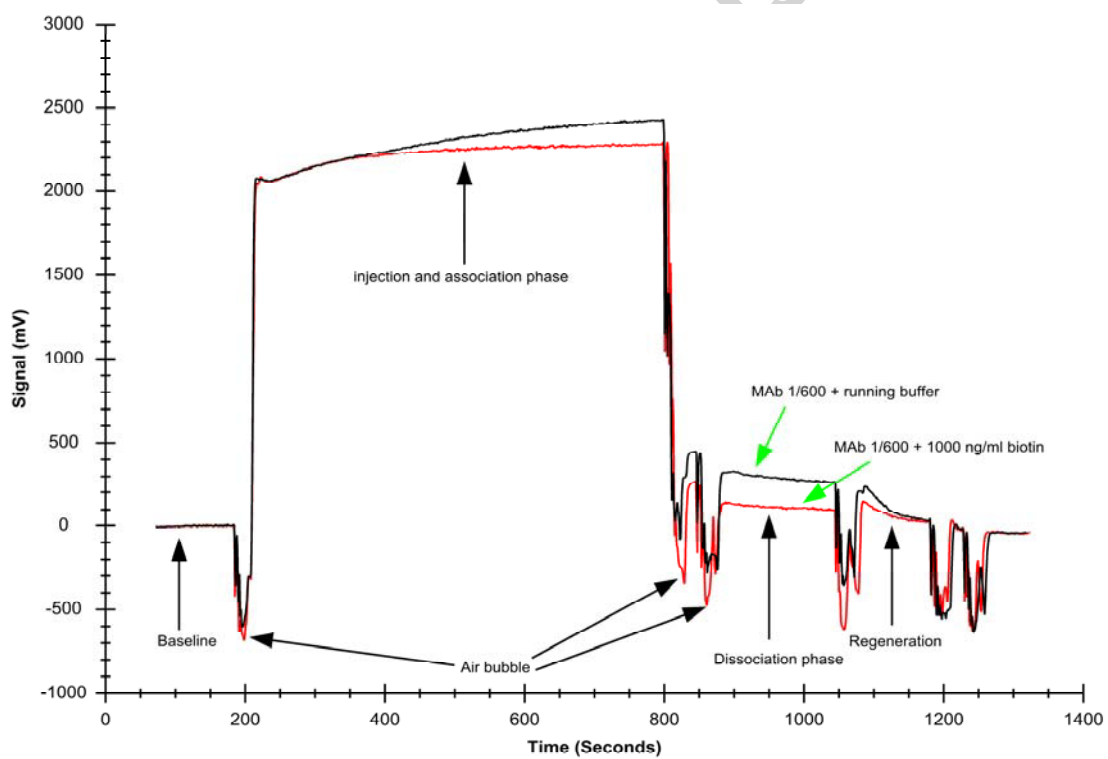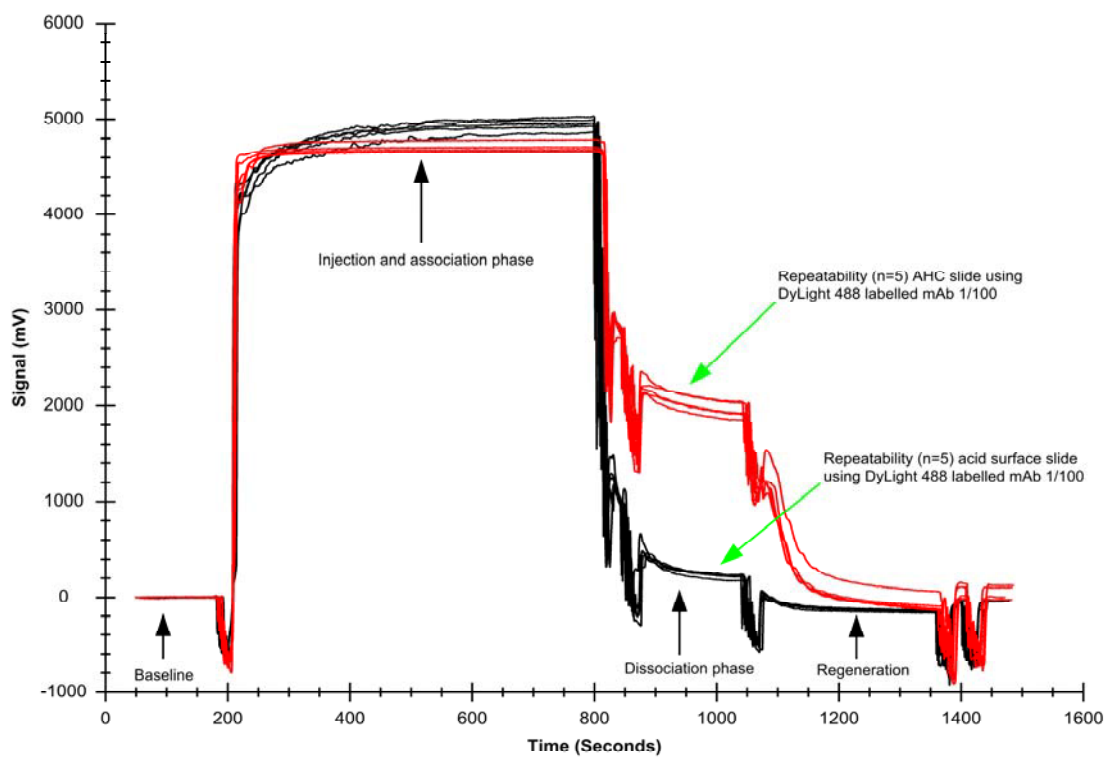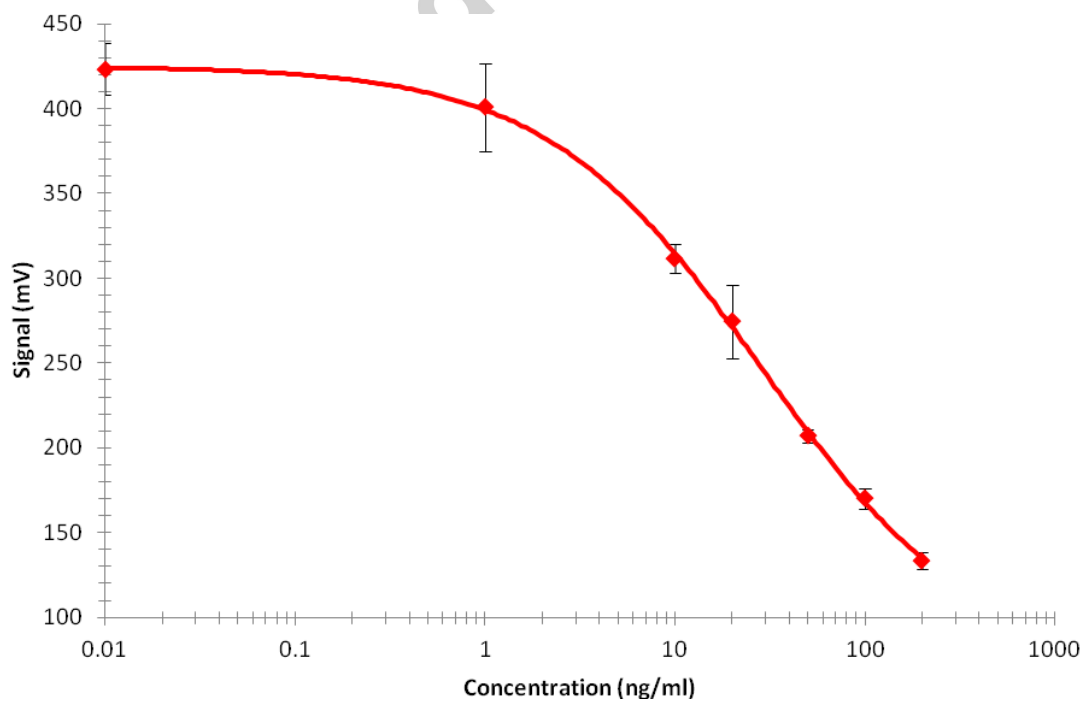**Figure 3:**

Figure 4:

We developed a prototype low cost biosensor targeted towards food contaminant analysis

Excellent repeatability and reproducibility matching more expensive contemporaries

Showed proof-of-principle for two food related compounds

Investigated assay development characteristics

Produced calibration curves matching rival biosensor sensitivities.