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Research paper

Low cost, disposable biosensors allow detection of antibodies with results equivalent to ELISA in 15 min

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ABSTRACT

An assay for detection of antibodies to bovine herpesvirus-1 (BoHV-1) was developed by combining a commercial low cost, disposable biosensor system (Vantix™) and reagents from an established Enzyme-Linked Immunosorbent Assay (ELISA). The biosensor assay produced equivalent results to ELISA within 15 min when testing 194 bovine serum and 50 bulk milk samples submitted for routine testing. The biosensor assay can provide quantitative analysis demonstrated by measuring the level of antibody in milk samples. The results of this study suggest that Vantix™ is a promising platform for routine immunological testing. The technology may be particularly useful for low to medium throughput tests where rapid results are required. The biosensors could also form the basis of a future point-of-care test platform.

1. Introduction

Biosensors are analytical devices that allow a biochemical event to be directly converted into a measureable signal via a transducer (Gruhl et al., 2012). The use of biosensors for monitoring and diagnosis of disease offers the prospect of methods which are rapid, real-time, sensitive, and have the potential to be used as point-of-care (POC) tests and devices (Ince and McNally, 2009; Gruhl et al., 2012; Holford et al., 2012).

This study explored the feasibility of transferring an indirect ELISA which detects antibodies to bovine herpes virus 1 (BoHV-1) to the commercially available Vantix™ biosensor system (Vantix™ Ltd, Cambridge, UK). The Vantix™ system (Purvis et al., 2003) comprises a low cost reader unit and disposable potentiometric biosensors, a type of electrochemical biosensor (Pohanka and Skládal, 2008). An electrochemical biosensor has been defined as a self-contained integrated device, which is capable of providing specific quantitative or semi-quantitative analytical information using a biological recognition element (biochemical receptor) which is retained in direct spatial contact with an electrochemical transduction element (Thévenot et al., 1999).

Vantix™ biosensors consist of a working and reference electrode coated in the conductive polymer polypyrrole and covered in a protective plastic film. The result is a flexible, semi-transparent sensor, which can be handled easily. The electrodes are connected by electrical tract (circuitry) to an electrical connector end of the biosensor (Fig. 1) which is used to connect to a reader unit. Biochemical or enzymatic activity taking place on the surface of the working electrode as a result of immunocomplexes built up on the electrode, causes electrochemical changes in the conductive polymer layer on and around the working electrode. This generates a measurable change in electrical potential (measured in millivolts; mV) relative to the reference electrode (Purvis et al., 2003). The Vantix™ system has been used to develop immunoassays for a variety of analytical targets such as antigens, biochemicals and drugs. This has included successful testing of complex samples such as milk and feed samples (Purvis et al., 2003; Stead et al., 2011).

Infectious bovine rhinotracheitis (IBR), caused by BoHV-1, is a highly contagious disease of cattle that is endemic in the UK and many other parts of the world (Beer, 2010). ELISA is commonly used in the detection and control of this disease

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(Hebert et al., 1985; Ackermann et al., 1990; Beer, 2010). To assist in the diagnosis of this disease in the UK, the Animal Health and Veterinary Laboratories Agency (AHVLA) uses an in-house indirect ELISA (Anderson et al., 2011). During the test, BoHV-1 antigens, bound to the surface of a microtitre plate, capture BoHV-1 specific antibodies present in test samples. Bound antibodies are then detected using a horse-radish peroxidase (HRP) labelled anti-bovine immunoglobulin and subsequent HRP catalysed oxidation of the chromogen substrate 3,3′,5,5′-tetramethylbenzidine (TMB) produces a colour change. The ELISA has a total assay time of over 3 h which is typical of ELISA tests.

In this work, we investigated if a Vantix™ biosensor assay, based on the same reagents as the current ELISA, was capable of producing results equivalent to the ELISA in a shorter assay time. A panel of serum and bulk milk samples, submitted for routine BoHV-1 antibody ELISA testing, was tested in parallel using both methods to evaluate the performance of the Vantix™ biosensor assay.

2. Materials and methods

2.1. Serum and bulk milk samples

The 194 serum and 50 bulk milk samples used for this study had been received at the AHVLA for routine diagnostic testing. Serum samples were submitted on suspicion of BHV-1 infection. Bulk milk samples were tested as part of programmes to monitor BHV-1 infection. Serum samples were randomly selected from routine submissions and included samples with a variety of breeds and ages. Blood samples collected in vacutainers were processed to obtain serum for testing. The clot was removed from the vacutainer using a wooden applicator stick and the remaining liquid spun at 250–300 ×*g* at room temperature for 10 min. Serum was then transferred to a clean storage tube and stored at 4 °C until testing. Bulk milk samples were processed prior to testing by addition of a preservative and removal of the cream as follows. 150 μL of Bronopol was added to 25 mL of milk at the time of collection, or upon receipt of samples at the laboratory. All samples were then centrifuged at 1000 ×*g* for 10 min at room temperature to separate the cream from the milk. Skimmed milk was then separated from the cream layer and stored at 4 °C until testing.

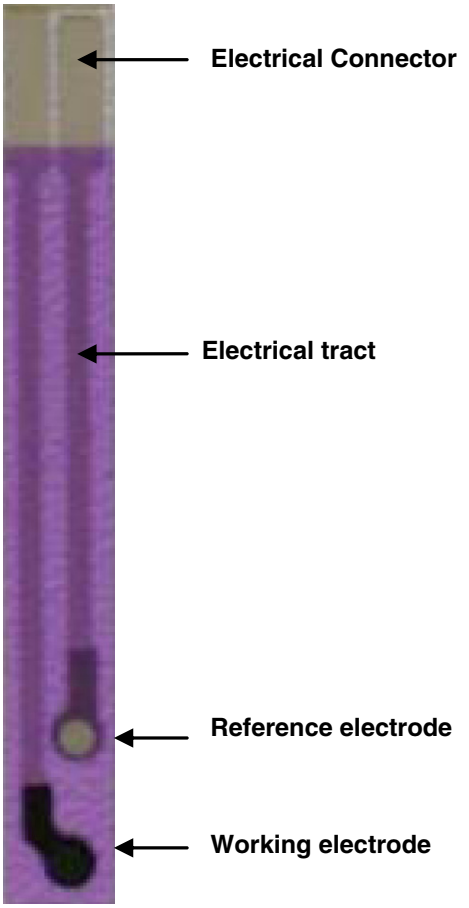


Fig. 1. Structure of the Vantix™ biosensors. Size is approximately 4 cm.

2.2. Control serum and bulk milk samples

Positive (strong and weak) and negative control serum and bulk milk samples were used as controls in the ELISA and Vantix™ assays. These were produced in house and were prepared from well defined and previously tested samples which were aliquoted and stored for use as subsequent controls. Strong BHV-1 antibody positive control serum were obtained from an experimentally infected animal - a colostrum deprived calf, reared in isolation and given multiple inoculations of BHV1 subtype 1 (ED1) and subtype 2 (Oxford) intratracheally and intraveneously. Negative control serum was a sample taken from an animal previously tested and confirmed as BHV-1 antibody negative. Weak positive control serum was obtained by diluting the strong positive control sera with negative material. Strong BHV-1 antibody positive milk control was obtained from a confirmed naturally infected and previously tested animal. Milk negative control samples were from milk that had tested negative for BHV-1 antibodies in previous tests. Weak positive milk control samples were prepared by diluting strong positive control milk sample with milk negative control material. The test results from these control samples were used in the calculation of the assay results for the unknown samples.

2.3. BoHV-1 antigen production

The BoHV-1 antigen used in both ELISA and Vantix™ biosensor assays was a relatively crude whole viral antigen preparation that is well established for use in ELISA (Anderson et al., 2011). Briefly, BoHV-1 (Oxford ED6 strain) was cultured in calf kidney cells using viral seedstocks with $TCID_{50}$ values of 10^{-4} or above. When viral cytopathic effect had reached 80-90%, cells were pelleted at 1500 ×*g* and resuspended in 0.01 M phosphate buffered saline (PBS) buffer (pH 7.2) containing 0.5% Nonidet P-40 (Sigma, Poole, UK) for 1 h at 4 °C. The cell debris was then removed by centrifugation and the supernatant stored in aliquots. This preparation was used as positive antigen for the subsequent test. Control negative antigen was prepared in parallel using non-infected cells to allow determination of non-specific interactions in the ELISA assay.

2.4. ELISA test

ELISA testing was carried out as described in Anderson et al. (2011). Briefly, microtitre plates (Polyvinyl chloride 96-well; BD Falcon, Oxford, UK) were coated with BHV-1 positive and negative control antigens by adding 60 μL of antigen diluted at 1/1500 in coating buffer (0.05 M bicarbonate buffer pH 9.6) to individual microtitre plate wells. Plates were incubated overnight at 4 °C, after which the plates were washed three times with wash buffer (0.1 M phosphate buffer, 0.15 M NaCl and 0.05% v/v Tween 20, pH 7.2).

To perform the ELISA, serum samples and controls (strong positive, weak positive and negative control) were diluted 1/100 in serum/conjugate diluent (0.1 M Phosphate buffer, 0.5 M NaCl, 1 mM EDTA, 1% w/v Polyvinyl pyrolidone 40 and 0.05% v/v Tween 20, pH 7.2). Milk samples were diluted 1/2 in serum/conjugate diluent. 50 μL of the diluted samples were added to the wells of the microtitre plate. All samples and control sera were tested in duplicate. The control weak positive, strong positive and negative control sera were included on every plate. Plates were covered and incubated in a moist chamber for 2 h at room temperature (18–25 °C), after which they were washed five times with wash buffer. Rabbit anti-bovine horseradish peroxidase conjugated secondary antibody (Dako, Cambridge, UK) was diluted 1/20,000 in serum/conjugate diluent and 50 μL added to each well. The test plates were incubated for 1 h at room temperature (18–25 °C), after which the plates were washed five times with wash buffer. 3,3′,5,5′-Tetramethylbenzidine (TMB) substrate (50 μL) was added to all wells and the plates incubated at 37 °C in the dark. TMB substrate made by adding 1 part 4.16 mM TMB in absolute ethanol, to 9 parts 25 mM citric acid, 65 mM NA_2HPO_4 and 0.025% v/v hydrogen peroxide (pH 5.0) immediately before use. Following visual inspection (typically after 5–10 min) 50 μL of stopping solution (2 M sulphuric acid) was transferred to the wells to stop the reaction. The results were obtained by measuring optical density (OD) using an Anthos HT2 microplate reader (450 nm absorbance filter and a 620 nm reference filter). Test results were calculated by first subtracting the mean negative antigen OD (mean of the duplicate reactions carried out for each sample) from the mean positive antigen OD signal for each sample. This value was then divided by the mean strong positive serum control OD value. Serum samples with a resulting OD ratio value of 0.24 and above were considered positive for BoHV-1 antibody. For bulk milk samples, samples were considered negative if the OD ratio was less than 0.10. Low positive samples had OD ratios greater than, or equal to, 0.10 and less than or equal to 0.40. Mid positive samples had OD ratios greater than 0.40 and less than, or equal to 0.70. High positive samples had an OD ration greater than 0.70.

2.5. Coating Vantix™ biosensors with BoHV-1 antigen

Vantix™ biosensor probes (Vantix, Cambridge, UK) were supplied in strips of 40 sensors. Biosensors were prepared for use by spotting 3 μL of BoHV-1 antigen (diluted 1/10 in 0.05 M bicarbonate buffer, pH 9.5) onto the working electrode of the Vantix™ biosensors and incubating in a moist chamber at 37 °C for 30 min. Unbound antigen was removed by rinsing once in 0.1 M PBS (pH 7.2). The working electrode of the biosensors was then immersed in StabilGuard® (Surmodics, Minnesota, US) for 10 min prior to drying at 37 °C. Probes to be used immediately were dried for 30 min whereas those to be used at a later date were dried for 2 h prior to being sealed in bags and stored at +4 °C. Before use, individual Vantix™ biosensors were cut from the strip with sharp scissors.

2.6. Vantix™ biosensor assay

Serum samples were diluted 1/10 in PBS (pH 7.2) containing 2.5% gelatin from cold water fish skin (Sigma Aldrich, Poole, UK) immediately before testing, while milk samples were tested undiluted. The weak positive serum control and negative serum control were tested alongside each batch of serum samples tested. The strong milk positive control and negative milk control were tested alongside each batch of milk samples tested. Because the Vantix™ reader has space for 12 sensors, unknown test samples were tested in batches of 10 (plus 2 controls).

The steps involved in carrying out the Vantix™ biosensor assay, compared to the ELISA, are summarised in Table 1. The antigen coated Vantix™ biosensors were incubated in a microtitre plate well containing 300 μL of test sample for 5 min at room temperature. Biosensors were washed by swirling for 2 s in a trough of PBS pH 7.2 (serum samples) or PBS + 0.05% Tween 20 pH 7.2 (milk samples) and then blotted on a paper towel to remove excess liquid. Individual biosensors were handled using a simple slotted cork strip into which the electrical connector of up to 12 biosensors was inserted, allowing simultaneous washing of several biosensors. The wash/blot step was performed four times and then repeated a further four times in a fresh trough of wash buffer. The biosensors were then incubated for 5 min in a microtitre plate well containing rabbit anti-bovine horseradish peroxidase conjugated secondary antibody (same as ELISA) diluted 1/20,000 in PBS (pH 7.2). Unbound conjugate was then removed by washing as described above. The electrical connector end of the biosensors was inserted into the Vantix™ Version 1 Research Reader, prior to placing the electrodes into TMB substrate

Table 1
Comparison of methodology and protocol of the Vantix™ biosensor assay and ELISA. Biosensors and ELISA plates are pre-coated with BoHV-1 antigen and stored ready for use.

Vantix™ biosensor assay		Indirect ELISA	
Step	Time	Step	Time
Incubation with diluted sample	5 min	Incubation with diluted sample	2 h
8 washes no soak	0.5 min	5× washes 10 second soak	5 min
Incubation with diluted Rabbit anti-bovine HRP conjugate	5 min	Incubation with diluted Rabbit anti-bovine HRP conjugate	1 h
8 washes no soak	0.5 min	5× washes 10 second soak	5 min
Probes added to TMB substrate and read immediately. Potential difference reading.	4 min	Plate incubated with TMB substrate. Addition of stop Colorimetric read	5–20 min 1 min 1 min
Total assay time	~15 min	Total assay time	>3.25 h

solution (as used for the ELISA). The reader was attached to a PC via a connector cable. The resulting electrical potential differences generated by the biosensors were recorded using the NUTS software (Vantix, Cambridge, UK) following the manufacturer's recommendations. An initial so called "potential step" (used to stabilize the sensor and recommended by the manufacturer) was carried out which consisted of subjecting the sensor to three 10 s periods of −60 mV voltage, inter-dispersed by two 10 s periods where no voltage was applied. This potential step (which was also conducted with the biosensors inserted into the reader and the electrodes of the sensor were immersed in TMB solution) was immediately followed by the main signal measurement stage. The signal measurement stage involved measuring the mV signal generated by the sensor for 180 s. The final mV reading, following completion of the signal measurement stage, was taken as the result for each sample.

2.7. Calculation of Vantix™ biosensor results

The result from the Vantix™ biosensor assay was a reading in mV. This value was used to calculate the result for each sample as a percentage of the positive control (weak positive control used for serum samples, strong positive control for milk samples) run alongside each batch of unknown samples using the formula:

$$\%\ \text{of positive control} = \frac{\text{Test sample result (mV)} + 100}{\text{Positive control result (mV)} + 100} \times 100.$$

The use of +100 in the formula was adopted to cope with data where mV readings typically decrease in numerical value up to a signal value of zero (as antibody concentration increases) but then increase once signals are above zero (see typical results in discussion). By adding 100 to all samples they all became positive but in a manner that maintains their relative value and allows straightforward calculations.

Using the calculated values for the biosensor assay, scatter plot and receiver operating characteristic (ROC) curve analysis (Greiner et al., 2000) was performed using Prism 4 software (GraphPad Inc., CA, US). ROC curve analysis was used to determine the optimal cut-off value for the biosensor assay which gave the best balance of sensitivity and specificity relative to the ELISA. These optimal cut-offs were used to categorize the samples as positive or negative and these results were then used to generate 2×2 boxes and calculate the diagnostic sensitivity and specificity of the Vantix™ assay compared to the ELISA.

2.8. Test repeatability

To explore the repeatability of tests carried out on the Vantix™ platform, five serum samples were tested on five separate occasions using the same reagent batches by the same operator using the test method described above. Mean, standard deviation and coefficient of variation were calculated with Microsoft Excel.

3. Results

Results generated by the Vantix™ biosensor consist of a graph of mV reading against time (Fig. 2). Typically, positive samples produced a rapid initial increase in potential difference (following placement of biosensors in TMB) that reached a plateau at a stable mV reading. It was this value that is taken as the mV result for each sample. Negative or weak samples result in flat graphs or weaker signals (Fig. 2). Typical results ranged from −60 mV to +70 mV.

Following initial assay development and optimisation, the Vantix™ biosensor assay was used to test a panel of 194 individual serum and 50 bulk milk samples submitted from farms around the United Kingdom.

The results from testing serum samples (Fig. 3a, b, c) demonstrate that the biosensor assay produces test results that closely matched those obtained with the parent ELISA. A scatter plot of the Vantix™ results (Fig. 3a) shows that they matched the distribution of the ELISA results and allowed positive and negative samples to be distinguished. ROC curve analysis (Fig. 3c), using the serum ELISA as the reference test, resulted in an area under the curve (AUC) value of 0.9938, indicative of excellent test agreement between the biosensor assay and ELISA (Greiner et al., 2000). Using the optimal cut-off value (105.5% of serum positive control; derived from the ROC analysis) allowed construction of a 2×2 table (Fig. 3b) which showed the biosensor assay had a sensitivity of 95.6% (95% CI 89.0–98.78) and a specificity of 98.1% (95% CI 93.3 -99.8) when compared to the ELISA, with only 6 of 194 samples resulting in a different categorisation (positive or negative) compared with those originally obtained using the ELISA.

The Vantix™ biosensor assay also demonstrated good agreement with ELISA when testing bulk milk samples (Fig. 3d, e, f). A scatter plot analysis (Fig. 3d) shows the biosensor was capable of assigning the samples into the same result categories (negative, low positive, mid positive or high positive) as the ELISA (Fig. 3d), demonstrating the capability of the Vantix™ system to provide quantitative test data. When analysing the bulk milk testing data in a way comparable to the serum data (where samples were classified as simply positive or negative) ROC curve analysis (Fig 3f), using the bulk milk ELISA as the reference test, resulted in an area under the curve

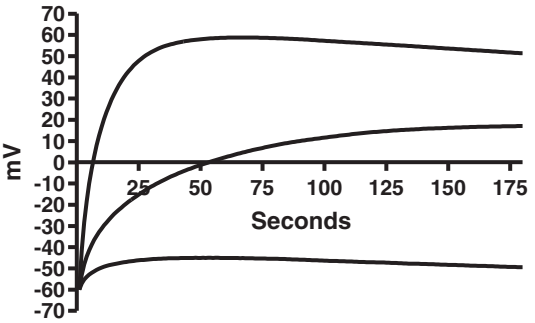
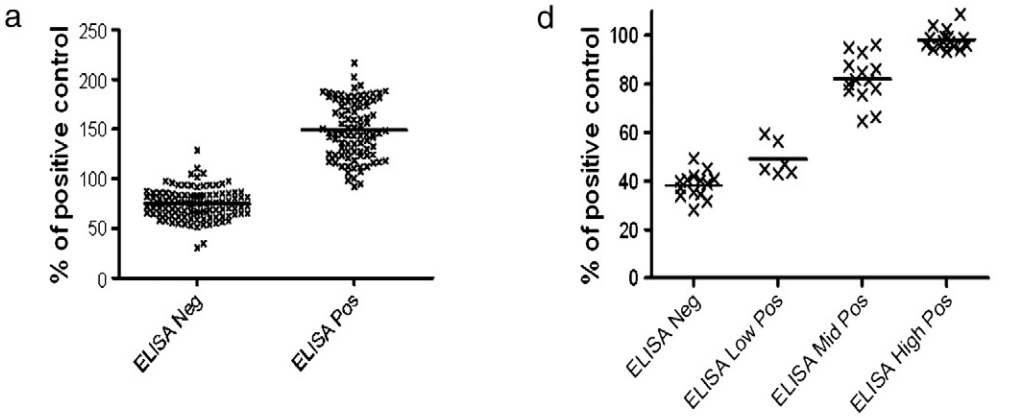


Fig. 2. Typical results from the Vantix™ biosensor assay. The figure shows the results recorded from analysis of 3 separate samples using the NUTS software, where the potential difference on the biosensor surface is measured in the Vantix™ reader. Positive samples result in an increase in potential difference (mV) over the period of read. The graph shows typical results for negative (bottom trace), positive (middle trace) and strong positive samples (top trace).



b

2 x 2 box analysis Serum Samples		Biosensor assay		
		Positive	Negative	Totals
ELISA	Positive	86	4	90
	Negative	2	102	104
	Totals	88	106	194

e

2 x 2 box analysis Milk Samples		Biosensor assay		
		Positive	Negative	Totals
ELISA	Positive	32	4	36
	Negative	0	14	14
	Totals	32	18	50

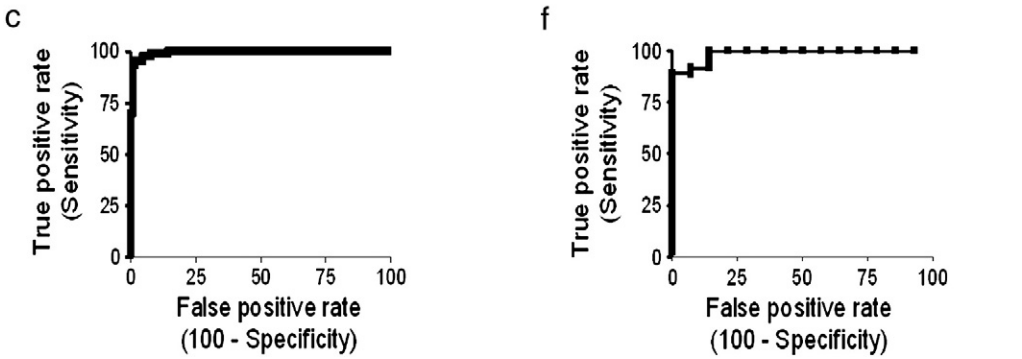


Fig. 3. The Vantix™ biosensor assay produces equivalent results to ELISA when testing bovine serum and bulk milk samples for BoHV-1 antibodies. (a) Scatter plot of biosensor results obtained for ELISA positive and negative serum samples. (b) 2×2 table comparing biosensor and ELISA results for serum samples. (c) ROC curve analysis of results obtained for panel of serum samples. (d) Scatter plot of biosensor results obtained for ELISA positive and negative bulk milk samples. (e) 2×2 table comparing biosensor and ELISA results for bulk milk samples. (f) ROC curve analysis of results obtained for panel of bulk milk samples.

(AUC) value of 0.9861 indicative of excellent test agreement. Using an optimal cut-off of 52.9% of the milk positive control, the resulting 2×2 table (Fig. 3b) showed the biosensor assay had a diagnostic sensitivity of 88.9% (95% CI 73.9–96.9) and a diagnostic specificity of 100% (95% CI 76.8 -100) when compared to the ELISA.

Analysis of the repeatability of assays carried out on the Vantix platform (Table 2) showed that 4 of the 5 results were had similar repeatability to that typically expected for ELISA testing, with a coefficient of variation (CV) ranging from 5 to 15%. One of the five samples had a higher CV (33%) than might be expected for ELISA.

4. Discussion

The results of this study suggest that the Vantix™ Biosensor system is a promising platform for routine immunological testing — the promise of being able to convert an existing established ELISA assay to a more rapid test format was achieved. Importantly, a similar sensitivity and specificity to that of the parent ELISA could be achieved with the biosensor assay. It is also encouraging that the biosensor worked using sample types that are often submitted for testing including serum and undiluted milk samples which suggests the system is robust and will be suitable for “real world” testing.

The biosensor assay allowed use of more concentrated samples than used in the ELISA (1/10 dilution of serum and undiluted milk). It should be noted that gelatine had to be added to the sample dilution buffer to prevent non-specific signal generation with the serum samples at this dilution; however, overall this strategy produced the best results.

Following ELISA testing, bulk milk samples are classified into different classes depending on the level of antibodies present within the sample. Testing these milk samples

Table 2
Repeatability of the Vantix™ biosensor assay. Five serum samples were tested on 5 separate occasions by the same operator. SD is standard deviation. CV is coefficient of variation. Results for repeats 1 to 5 are the % of positive control results calculated as described.

		% of positive control results							
		Repeat 1	Repeat 2	Repeat 3	Repeat 4	Repeat 5	Mean	SD	CV
Sample	1	33.14	80.12	97.77	96.58	86.78	78.88	26.58	33.70
	2	94.03	97.44	94.39	95.96	105.55	97.47	4.71	4.84
	3	105.37	92.91	118.72	109.62	122.81	109.89	11.77	10.71
	4	31.19	23.71	32.26	28.33	34.87	30.07	4.26	14.16
	5	110.73	123.06	138.09	120.5	134.86	125.45	11.13	8.87

therefore provided an opportunity to test the ability of the biosensor assay to provide quantitative data beyond simply classifying samples as positive or negative for BoHV-1 antibodies. The results (Fig. 3d) show that the Vantix™ biosensor system allows the level of antibodies present within a bulk milk sample to be determined, in a manner analogous to the ELISA. In the case of the BoHV-1 biosensor assay, this means that the assay could be suitable for use as part of a testing programme which determines subsequent on-farm action, as bulk milk samples with no or low levels of antibody are likely to come from herds where active infection is unlikely whereas samples with high levels of antibodies are more likely to indicate active infection and virus excreting animals (Pritchard, 2001). The use of the Vantix™ biosensors may be applicable to other animal or human health situations where a quantitative result is necessary to allow the correct downstream action, such as treatment, to be applied.

Although the results of this study were promising, with both the serum and milk samples, there was some overlap in the results obtained using the biosensors for samples which had been classified as weak positive or negative using the indirect ELISA (visualised by Fig. 3a and d). It is likely that the performance of the Vantix™ biosensor assay could be improved by further optimisation such as use of alternative antigens (this work utilised a relatively crude whole viral antigen) and further optimisation of assay conditions. It is encouraging that antigens could be simply adsorbed onto the biosensor surface in a manner analogous to adsorption of antigen onto ELISA plates. This lack of a requirement for any chemical conjugation again suggests that conversion of existing ELISAs is more likely to be straightforward. In the current study, the biosensor assay used seven and half times more antigen than the ELISA assay which could be problematic with expensive antigens. However, it is possible that further optimisation could reduce the amount required.

Non-specific signals are sometimes experienced with the ELISA which necessitates the use of a negative control antigen (and subsequent subtracting the signal from the negative antigen coated wells from the positive antigen coated wells). We did not include the use of a negative control antigen in the Vantix™ biosensor assay. This was because during initial evaluation and optimisation work, non-specific reactions appeared to be less of a problem than in ELISA. This may be because the reduced reaction and incubation times that can be used in the biosensor assay (while still maintaining adequate test performance) lead to less non-specific interactions (and hence signals) developing.

An analysis of the results obtained on a small panel of serum samples tested on five separate occasions showed that in general the Vantix system was able to produce results with a repeatability similar to that of typically seen with ELISA (CV ~ 10%). Testing and analysis of one sample (sample 1 in Table 2) resulted in a significantly higher CV. This was caused by one of the five readings (reading 1) being significantly different from the other four. This was probably the result of a laboratory mistake and is analogous to results that can occur with ELISA. More work will be needed to build on this preliminary work and demonstrate that the Vantix biosensor system is robust and suitable for routine analysis. It is likely that the repeatability of the assays could be improved by optimisation of the testing process and further analysis to understand which parts of the testing procedure is producing the most variation; for example, in this study, coating of the antigen onto the sensor was carried out by hand pipetting small volume of antigen (3 μL) which will be prone to error. Automated production and robotic pipetting could possibly reduce test variation resulting from differences in antigen loading.

Importantly, the biosensors are produced using established screen printing technology which offers the prospect of cheap mass production. Current list prices of 1–2 Euro per sensor (for research scale use) could probably be reduced for high throughput routine use. The reader cost is also reasonable (approximately 4000 Euro) and certainly much lower in cost than some better known biosensor system instrumentation such as surface plasmon resonance.

In the Vantix™ assay the enzymatic turnover of the TMB substrate in the final step of the protocol results in electrochemical changes on the biosensor electrodes which is measured by the reader. This differs from measuring the colour change that this reaction also produces as measured in the ELISA. The ability to use exactly the ELISA reagents and basic assay principles to carry out a Vantix™ biosensor assay might be an important advantage when it comes to potential acceptance and adoption of this technology. Vantix™ appears to be a pragmatic and practical “ready now” technology which could be adopted at reasonable cost and without specialist knowledge of biosensors.

In our opinion, some changes to the reader, software and biosensor handling would make the Vantix™ system more user-friendly and make the technology better suited to routine laboratory application. For example, use of combs of sensors which have spacing compatible with 96 well plates would allow for easier sensor handling and laboratory processing and

prevent the need for handling individual biosensors, or use of the cork strips, which can be awkward. As far as we understand, these developments are in hand at Vantix and an updated reader (Vantix 2), software and combs of biosensors are now available.

When fully developed the Vantix™ platform might be particularly suitable for low to mid volume assays that require a rapid turnaround in a diagnostic laboratory by allowing small numbers of samples to be tested quickly and efficiently. This approach may offer advantages in meeting the increased demand for same day testing. In practical terms, this may reduce the need to open full ELISA kits and reduce the need to batch up samples and perform tests on a limited number of days which can be a common occurrence in routine laboratories. For large numbers of samples it seems unlikely that use of the Vantix™ platform would be as efficient as ELISA, although the use of combs of sensors (mentioned above) could provide the basis for practically testing larger numbers of samples.

The relative simplicity and speed of the Vantix platform and method could make it amenable to so called "point-of-care" or "on-site" testing – or at least testing in simple, low resource laboratories or settings. Clearly, further development and validation would be required for such applications – for example, in this work sample pre-treatments were used (centrifugation of milk) that would be difficult to reproduce in such non-laboratory settings. It would need to be demonstrated that the technology worked on samples that were realistic to take in such on-site testing situations. In terms of on-site testing, another potentially important advantage is that the Vantix™ biosensors produce an electronic, numerical result which would be suitable for transmission via mobile networks to a skilled operator for interpretation and decision-making regarding downstream disease control/treatment options. In addition, Vantix™ are currently developing a microfluidic controlled hand held device which will incorporate the same biosensors but offers the potential for more rapid (<5 min) automated testing. This could form the basis of true point-of-care testing capability.

This study has shown, using detection of BoHV-1 antibodies as a model, that the Vantix™ potentiometric biosensors provide a promising tool for antibody detection in frequently used biological sample types. The characteristics of Vantix™ biosensors allow results to be available within a short timeframe using simple testing protocols. Results obtained are comparable to those from well-accepted ELISAs. Given the widespread use of antibody detection in diagnostic testing, the results obtained in this study suggest that this approach could be useful for a wide variety of diagnostic testing requirements. Further work could also include investigation of the possibility of carrying out antigen detection assays; preliminary work in our hands has shown promise for this application.

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