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Fast track validation of pond-side test kits for use on farms and in regions



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Abbreviations

| Abbreviation | Definition |
|----------------|---|
| AAHL | CSIRO Australian Animal Health Laboratory, Geelong |
| C _T | Cycle threshold of a qPCR reaction: the number of cycles for the fluorescent signal to be detected above the background level. |
| DSe | Diagnostic specificity: likelihood of a test returning a detection where the target is present in a sample |
| DSp | Diagnostic specificity: likelihood that a positive test result is correct (i.e. not a false positive due to cross-reaction with non-target DNA) |
| NEC | Negative extraction control |
| OIE | World Organisation for Animal Health |
| OsHV-1 | Ostreid herpes virus 1 |
| POMS | Pacific oyster mortality syndrome: disease of Pacific Oysters caused by ostreid herpes virus 1 |
| qPCR | Quantitative polymerase chain reaction |
| SARDI | South Australian Research and Development Institute |
| SAOGA | South Australian Oyster Growers Association |

Executive Summary

Following a review of potential rapid and portable testing options, researchers from SARDI Aquatic Sciences selected and investigated the sensitivity and specificity of the Biomeme Franklin platform for rapid detection of ostreid herpes virus 1 (OsHV-1) in Pacific Oyster (*Crassostrea gigas*) samples. OsHV-1 is the virus responsible for Pacific Oyster Mortality Syndrome (POMS), a disease characterised by acute high mortality in Pacific Oysters. The accepted approach in Australia to detecting the virus is by using the Martenot B region and Jenkins C region qPCR tests, with oysters or oyster tissue samples collected in the field, sent to a veterinary diagnostic laboratory and tested following conventional nucleic acid extraction on a bench-top qPCR instrument. Results are available within ~24 hours but transport delays, workload in labs and other factors can slow delivery of results. Delays affect the capacity to make decisions about responding to POMS outbreaks, therefore the need for a test that can deliver results in hours rather than days, at low-cost, with ease of use and field applicability was identified as a priority. This project investigated and delivered such a test.

A custom assay for the Biomeme Franklin and its chemistry was designed based on the Martenot B region and Jenkins C region qPCR assays duplexed into a single reaction. The Biomeme Franklin device, which runs a custom OsHV-1 assay through a smartphone pre-installed with the Biomeme GoApp, is small, portable, does not require an external power source. This system also has fast assay run times (~45 minutes/run), with total preparation time from extraction to result of ~55 minutes. It also has the benefit of being able to sync results from the Franklin to the Biomeme Cloud Portal, allowing results to be viewed in real-time as the assay is being performed in the field. The chemistry and device are inexpensive and easy to use, with consumables and reagents required to run the assay provided in the kits.

Two hundred and fifty seven feral Pacific Oyster samples were collected between June 2018 and January 2019 from four sites in the Port River, South Australia. All oyster samples were assessed using the Biomeme chemistry and platform, and results compared to those obtained from tissue samples from the same oysters tested using the laboratory-based bench-top qPCR approach. Results were analysed by a Bayesian latent class model, which demonstrated that diagnostic performance was similar for both tests and platforms. All tests achieved high specificity (≥ 0.94) and good sensitivity (mean posterior estimate: 0.64 – 0.75). Applying both the Jenkins and Martenot tests and a case definition to maximize sensitivity (i.e. considering a sample infected if either test returns a positive), provides near identical sensitivity for either platform: 0.77 (95% credible interval 0.64 – 0.88) for Biomeme, compared with 0.79 (0.67 – 0.92) for laboratory-based testing.

The Franklin device and Biomeme chemistry are suitable for rapid detection of OsHV-1. We recommend that detections need to be confirmed using the C2/C6 conventional PCR and sequencing to meet the Australian case definition for a confirmed case of OsHV-1 microvariant infection. Rapid detection using the Biomeme system, however, could facilitate more effective emergency responses to POMS outbreaks by providing rapid field diagnostic capability and improve disease management by expediting batch testing in farming areas to inform livestock translocations.

Keywords

Pacific Oyster (*Crassostrea gigas*), POMS, OsHV-1, qPCR, rapid pond-side test, Biomeme platform, Franklin device, diagnostic performance, disease surveillance.

Introduction

Ostreid herpes virus 1 microvariant (OsHV-1) is associated with mass mortalities in a range of aquatic bivalve species from Japan, USA and Europe, with outbreaks typically occurring during the summer months (Pepin et al., 2008; Sauvage et al., 2009). In 2010, OsHV-1 was first detected in Australia associated with high mortalities of farmed and wild Pacific Oysters (*Crassostrea gigas*) in the Georges River, New South Wales (Paul-Pont et al., 2013; NSW DPI, 2016). The disease, termed Pacific Oyster Mortality Syndrome (POMS) was detected in the Hawkesbury River NSW in 2013 and in Tasmania in 2016 (Ugalde et al., 2018). South Australia (SA) remained free of OsHV-1 until a detection in feral Pacific Oysters in Port Adelaide in February 2018 (Deveney et al., 2019; Roberts et al., 2019). All life stages of Pacific Oyster are susceptible to POMS, although highest mortalities occur in juvenile oysters (NSW DPI 2016).

Management of POMS mainly involves surveillance, rigorous biosecurity protocols to contain the disease, and a breeding program to produce disease-resistant animals (Paul-Pont et al., 2013). Although several diagnostic approaches, including transmission electron microscopy, *in situ* hybridisation (ISH), immunohistochemistry, antigen-based tests and polymerase chain reaction (PCR), have been applied as diagnostic tools to detect OsHV-1 infection (Martenot et al. 2011; Schikorski et al., 2011), bench-top real-time quantitative PCR (qPCR) remains the principal tool used for OsHV-1 monitoring and disease diagnosis (Department of Agriculture, 2015).

Numerous qPCR assays are available for detection of OsHV-1 in Pacific Oysters (e.g. Martenot et al. 2011; Jenkins et al. 2013; Lynch et al. 2013), however for diagnostic purposes, the Australian case definition requires a positive Martenot qPCR test, a positive with the C2/C6 conventional PCR test (Segarra et al., 2010) and sequence analysis to confirm the systematic deletion of 12–15 base pairs in ORF 4 of the genome (encompassed by the C2/C6 primers) in comparison with OsHV-1 (GenBank # AY509253; Department of Agriculture, 2015). These protocols are costly and time consuming, and delays can be experienced between field sample collection, diagnosis and implementation of emergency response measures. These delays can aid the spread of disease. A need was therefore identified for a low-cost, field applicable, easy to use test assay, with results provided in hours rather than days. This would facilitate much earlier implementation of response measures to prevent spread of the disease to unaffected areas.

This project aimed to identify a platform for a rapid OsHV-1 pond-side test and assess its diagnostic sensitivity (DSe, likelihood of detection where a sample is infected) and specificity (DSp, likelihood that a test negative is correct) for detection of OsHV-1 in Pacific Oyster tissue samples. We aimed to compare the DSe and DSp of the rapid test with that of the laboratory-based qPCR approach. Following a search of the literature, Biomeme was identified as having the specific chemistry and portable qPCR device (Franklin) required to achieve rapid detection of viral DNA from tissue samples. The Franklin also provides throughput of up to nine samples per run with up to three targets per sample with fast assay run times (~45 minutes/run) and total test times (~55 minutes from extraction to results), field applicability with no external power source required, a low overall sample preparation and run cost and the benefit of assay results being automatically uploaded to the Biomeme Cloud Portal, allowing results to be viewed in real-time as the assay is being performed in the field.

We tested 257 Pacific Oyster samples collected from four sites in the Port River, South Australia, each of which had been tested using the laboratory-based qPCR approach. Samples were assessed on the Franklin using a custom assay developed by Biomeme that duplexes the Martenot B region and Jenkins C region assays. We applied latent class modelling to the results to assess the diagnostic performance of the Biomeme Franklin compared to the laboratory-based qPCR approach. This data provides evidence about the suitability of the Biomeme chemistry and Franklin device for OsHV-1 rapid surveillance and disease diagnosis.

Objectives

The objectives of this project were to:

1. Identify a platform that will allow a rapid diagnostic test for OsHV-1;
2. Implement qPCR OsHV-1 tests on the chosen rapid platform and test samples;
3. Assess DSe and DSp of the OsHV-1 tests on the chosen rapid platform;
4. Provide guidelines and instructions for use.

Method

Identifying a suitable platform

A review of the literature was undertaken to search for suitable testing platforms for the rapid OsHV-1 diagnostic assay, taking into consideration sample preparation protocol, ease of application, throughput, ability to use the device in the field/remotely and total cost of the platform and consumables (Table 1). The Franklin device developed by Biomeme was selected as the optimum testing platform, with a rapid sample preparation time that includes an extraction step (~10 minutes/sample), no additional consumables/reagents required alongside those included in the kit, high throughput of up to nine samples per run by a maximum of three targets with fast assay run times (~45 minutes/run), field applicability with no external power source required to run assays on the device, an overall low cost that fitted within the project budget and the added benefit of assay run results being automatically uploaded to the Biomeme Cloud Portal, allowing results to be viewed in real-time as the assay is being performed in the field.

Table 1. Comparison between the most relevant available platforms and their suitability for the rapid diagnostic assay of OsHV-1.

| Device | Company | qPCR | Additional extraction kit required | Throughput | Field applicable (no external power source required) | Cost within budget |
|------------------|--------------------|------|------------------------------------|------------|--|--------------------|
| Mini16 | Harvard University | N | Y | medium | N | Y |
| Liberty16 | Ubiquitome | Y | Y | medium | Y | N |
| Accutus Hydrogel | Aquila | Y | Y | medium | N | Y |
| Franklin | Biomeme | Y | N | high | Y | Y |

Custom assay design – laboratory-based OsHV-1 qPCR established on the Biomeme Franklin device

A custom assay design of two qPCR tests routinely used for the detection of OsHV-1 were duplexed into a single reaction/well of the Biomeme Go-Strips. Specifically, the B and C regions of the OsHV-1 genome were targeted, using the primer and probe sequences in Martenot et al. (2010) and Jenkins et al. (2013) respectively, with a final primer concentration of 200 nM and probe concentration of 100 nM. Custom Go-Strips were provided as three well low-profile plastic strips containing lyophilised master mix, probe and primer sequences, which were stable at room temperature and only required the addition of extracted nucleic acid for testing. To allow for target duplexing, the B region was visualised using the green (FAM) channel and the C region using the red (ATTO647N) channel on the Biomeme Franklin device.

Testing of samples on the Biomeme Franklin device

Samples collected and tested

A total of 257 feral oyster samples were collected from four sites in the Port River, SA, namely Inner Harbour, North Arm, Lipson Reach and Upper Port (Figure 1). Seven sets of samples were collected at the first three locations between June 2018 and January 2019, with Upper Port sampled in the December 2018 and January 2019 sets. Sampling dates and numbers of oysters sampled are shown in Table 2. Oysters were kept cool ($\leq 4^{\circ}\text{C}$) after collection and processed with 24 hours. After shucking, two approximately 5 x 5 mm samples of mantle and gill were taken from each oyster, with one preserved in 70% ethanol and stored at room temperature and the other frozen and stored at -20°C .

Laboratory-based approach to qPCR

Subsamples (approximately 25 mg each) from the ethanol preserved oyster tissue samples were tested for OsHV-1 at the Australian Animal Health Laboratory (AAHL). DNA was extracted from the tissue sample of each oyster with the QIAamp Mini kit, following the manufacturer's protocol. Extracted DNA quality and concentration was assessed using a Nanodrop[®] ND-2000 spectrophotometer (Thermo Fisher Scientific). Nucleic acid extracts were tested with Martenot et al. (2010) and Jenkins et al. (2013) OsHV-1 qPCR assays in duplicate. Plasmid positive controls and no template negative controls were included in each PCR plate and all performed as expected. All wells with qPCR amplification curves that crossed a 0.1 fluorescence threshold were considered positive and a cycle threshold (C_T) was recorded. Samples with duplicate positive wells by qPCR were reported as positive and those with one well positive and one well negative were reported as indeterminate. Assay details are provided in Table 3.

Biomeme trial assay

As an initial trial to confirm that the Biomeme sample preparation protocol, custom assay design and Franklin device were working as desired, three of the ethanol preserved and associated frozen tissue feral oyster samples (55, 77 and 187) were tested following Biomeme's homogenisation (release of nucleic acid), sample preparation (extraction and purification of nucleic acid) and test run instructions. Positive plasmid controls for the B and C regions (NQC-1 and NQC-3, respectively) and a negative extraction control (NEC) were also included in the Biomeme trial run.

Following the Biomeme trial assay, all remaining ethanol preserved feral oyster tissue samples ($n = 254$) were tested neat and in singlicate using the Biomeme chemistry and Franklin device across both duplexed tests (Martenot B region and Jenkins C region). These samples had previously been assessed neat and in duplicate by AAHL using laboratory-based qPCR for the Martenot and Jenkins tests in singleplex.

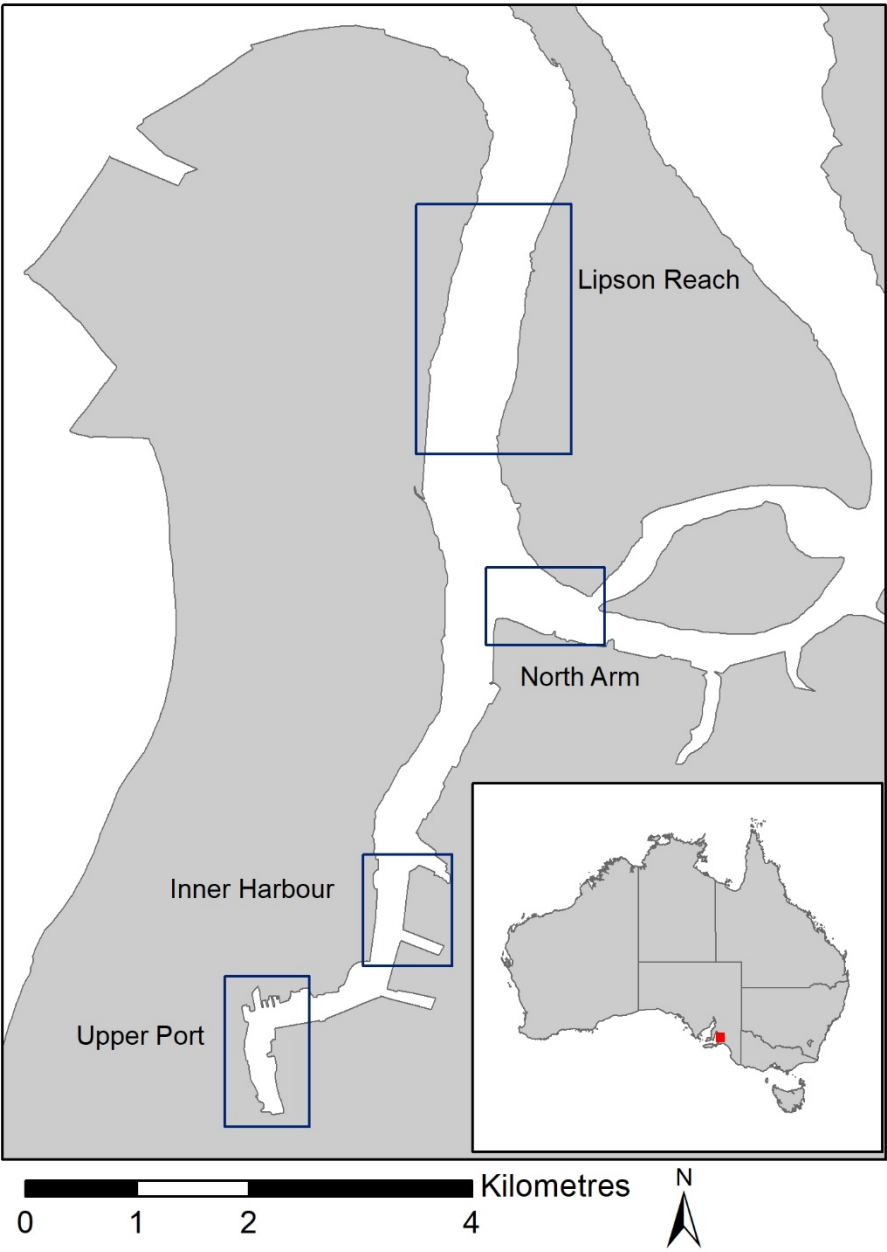


Figure 1. Sampling location in South Australia.

Table 2. Sampling locations, dates and number of feral oyster samples collected for OsHV-1 testing in South Australia. See Figure 1 for locations.

| Sampling location | Sampling date | No. of samples collected | Total |
|--------------------------|----------------------|---------------------------------|--------------|
| Inner Harbour | 26-Jun-18 | 10 | 95 |
| | 14-Aug-18 | 16 | |
| | 12-Sept-18 | 14 | |
| | 23-Oct-18 | 21 | |
| | 26-Nov-18 | 11 | |
| | 20-Dec-18 | 12 | |
| | 22-Jan-19 | 11 | |
| North Arm | 26-Jun-18 | 10 | 82 |
| | 14-Aug-18 | 8 | |
| | 12-Sept-18 | 10 | |
| | 23-Oct-18 | 18 | |
| | 26-Nov-18 | 11 | |
| | 20-Dec-18 | 11 | |
| | 22-Jan-19 | 14 | |
| Lipson Reach | 26-Jun-18 | 11 | 68 |
| | 14-Aug-18 | 8 | |
| | 12-Sept-18 | 13 | |
| | 23-Oct-18 | 7 | |
| | 26-Nov-18 | 10 | |
| | 20-Dec-18 | 8 | |
| | 22-Jan-19 | 11 | |
| Upper Port | 20-Dec-18 | 8 | 12 |
| | 22-Jan-19 | 4 | |

Table 3. Primer and probe sequences, cycling conditions and amplicon sizes for Biomeme field-based and AAHL laboratory-based OsHV-1 qPCR assays. Fluorescent signal molecules (5') and quenchers (3') are bold.

| qPCR name | Device | Primer/ probe name | Sequence (5'-3') | Cycling | Reaction | Amplicon | Reference |
|----------------------------|--|------------------------|---|--|----------------------|----------|---------------------------|
| Biomeme Martenot B region | Franklin | F R Probe B | GTC GCA TCT TTG GAT TTA ACA A ACT GGG ATC CGA CTG ACA AC FAM TGC CCC TGT CAT CTT GAG GTA TAG ACA ATC 3IABkFQ | 1 cycle 95 °C 60 s, 45 cycles 95 °C 1 s, 60 °C 20 s | 20 µl eluted DNA | 102 bp | This study |
| AAHL OIE Martenot B region | 7500 Fast or QuantiStudio real-time PCR systems | BF B4 Probe B | GTC GCA TCT TTG GAT TTA ACA A ACT GGG ATC CGA CTG ACA AC 6FAM TGC CCC TGT CAT CTT GAG GTA TAG ACA ATC TAMRA | 1 cycle 95 °C 20 s, 45 cycles 95 °C 3 s, 60 °C 30 s | 2 µl DNA 23 µl MM | 102 bp | Martenot et al. (2010) |
| Biomeme Jenkins C region | Franklin | F R Probe C | CGT TTT ATC CAC CAC GAT TTT TAT T TAC ATC AAA CCC ACT TTT CCT ATG AT ATTO647N CAC TCA TGA AAA CAC CGC TAA GAT CAC TGC 3IAbrQSp | 1 cycle 95 °C 60 s, 45 cycles 95 °C 1 s, 60 °C 20 s | 20 µl eluted DNA | 88 bp | This study |
| AAHL EMAI Jenkins C region | 7500 Fast or QuantiStudio real-time PCR systems | CRF CRR CR probe | CGT TTT ATC CAC CAC GAT TTT TAT T TAC ATC AAA CCC ACT TTT CCT ATG AT 6FAM CAC TCA TGA AAA CAC CGC TAA GAT CAC TGC TAMRA | 1 cycle 95 °C 20 s, 45 cycles 95 °C 3 s, 60 °C 30 s | 2 µl DNA 23 µl MM | 88 bp | Jenkins et al. (2013) |

Abbreviations: B4, B region reverse primer; BF, B region forward primer; CRF, C region forward primer, CRR, C region reverse; MM, TaqMan Fast Universal PCR master mix; OIE, World Organisation for Animal Health.

Biomeme sample preparation including extraction of nucleic acids

Up to 25 mg of ethanol preserved feral oyster tissue was subsampled from the original collected material and added to a 5 mL screw cap tube that contained a 1/2" stainless steel ball. Using a 1 mL transfer pipette, 2 mL of homogenisation buffer was added, and the tube was vigorously shaken for 30 – 40 s to mechanically disrupt the sample (Figure 2A). If large particulates were still present after shaking, the tube was let to sit for ~ 5mins and re-shaken. For the NEC, 2 mL of homogenisation BLB buffer was added to the 5 mL blue screw cap tube containing the stainless steel ball and the tube was vigorously shaken for 30 – 40 s.

The Sample Prep Column (filter) was then secured onto the end of a single-use 1 mL syringe and two holes were punctured in the red section (labelled 'start') of the M1 Sample Prep Cartridge for DNA (Figure 2B). One millilitre of the tissue/NEC homogenate was added into the red section of the cartridge using a transfer pipette. For the lysis and binding step, the syringe with the attached Sample Prep Column was placed into the red foil section of the cartridge and the fluid was drawn slowly all the way up the syringe and pumped all the way back out. This was repeated for a total of 10 pumps. All the fluid in the syringe was pushed out before a hole was made in the red-orange foil section of the cartridge (protein wash) and the fluid was drawn in and out for a total two pumps. A hole was then made in the orange foil section (salt wash) with the fluid drawn in and out once, followed by a hole in the yellow foil section (drying wash) with the fluid drawn in and out once. To air dry the column, a hole was made in the blue foil section and 30-40 fast pumps were performed before moving to the green foil section of the cartridge (elution) and slowly pumping for a total of 5 pumps (Figure 2B). The eluted DNA (~800 µl) was then drawn all the way up the syringe and transferred to a 1.5 mL tube while the Sample Prep Cartridge, Sample Prep Column and syringe were discarded. The eluted DNA was stored at 4 °C before being loaded into the Biomeme custom Go-Strips (Figure 2C) and run on the Franklin device (Figure 2D), or stored long-term at -20 °C. The bench area was wiped down with 70% ethanol between each sample preparation, and sub-sampling equipment (forceps and scalpel blades) were sterilised with LookOut DNAErase and 70% ethanol to avoid cross-contamination.

Applying extract to custom Go-Strips and running on the Franklin device

A total of 20 µl of the eluted DNA was transferred into each well of the custom Go-Strips, with one sample loaded per well. For each run, seven samples were analysed along with the two plasmid controls (10 µl of each plasmid control loaded into well 8) and the NEC (20 µl added into well 9). Void filling caps were applied and the Go-Strip tubes were flicked two to three times to ensure bubbles were removed from the bottom of each tube. The Go-Strips were then loaded into the Franklin device in the correct orientation (strip connections towards the back of the device). The device was turned on with Bluetooth enabled, and using the pre-installed Biomeme GoApp on the provided smartphone device, the OsHV-1 assay was selected and run (Figure 2D). If the smartphone was connected to the internet, the results would automatically sync to the desktop Biomeme Cloud Portal, or could be transferred later when internet access is available. Tests were run on the Franklin device without the power source connected (to mimic a field setting), and was only charged when the battery light indicated <20% battery life remained. Thermocycling conditions were initial denature at 95 °C for 60 s, followed by 45 cycles of amplification at 95 °C for 1 s and 60 °C for 20 S.

For a detailed guide to the Biomeme sample preparation, OsHV-1 run protocol and assay details, refer to Appendix 1 and Table 3.

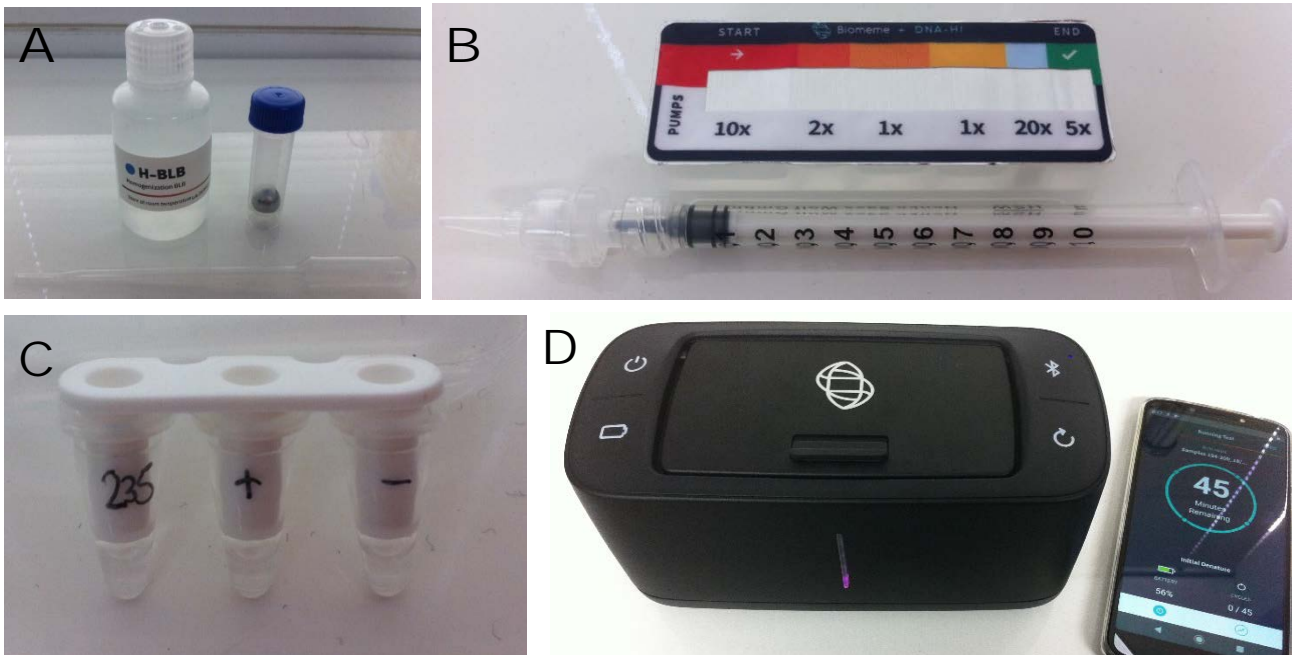


Figure 2. **A.** Homogenisation BLB buffer, 5 mL screw cap tube with a 1/2" stainless steel ball and 1 mL transfer pipette used for the sample homogenisation step; **B.** Biomeme M1 Sample Prep Cartridge for DNA and syringe with sample column attached for extracting purified nucleic acids from the tissue homogenates - requires no lab equipment, refrigeration, electricity, incubation or specialised extraction step; **C.** 20 ul of purified nucleic acid was added to custom Go-Strips and void filling caps applied; **D.** Go-Strips then placed in the Biomeme Franklin device (maximum of 3x 3 Go-Strips, 9 wells total) and a smartphone with the Biomeme GoApp pre-installed was used to run the custom OsHV-1 duplex assay protocol.

Biomeme Cloud Portal

Results for sample runs were viewed and downloaded from the Biomeme Cloud Portal (cloud.biomeme.com). A single threshold of 125 RFU was set for both targets, with the raw data plots inspected, including for the positive and negative controls, before the C_T results for samples were accepted and recorded.

Statistical methods

Latent class modelling (LCs) was used to estimate diagnostic sensitivity (DSe) and specificity (DSp) of each set of tests applied: the laboratory-based (AAHL) Jenkins and Martenot tests, and the field-based (Biomeme) Jenkins and Martenot tests. The model also provided estimates of true disease prevalence in the oyster tissue samples. For modelling, AAHL results for each test were considered positive where a detection occurred in at least one replicate sample of that test (i.e. results reported as either positive or indeterminate). The models were fit in a Bayesian framework allowing for covariance between each pair of tests, using code modified from Wang et al. (2019) (refer to Appendix 2). Prevalence was modelled by sampling set (see Table 2 for sampling dates) using a

logistic link. Differences in prevalence between sampling sets were assessed by examination of 95% credible intervals of the posterior predictions; covariate levels were considered to be different where the credible interval for the odds ratio between the covariates did not include 1. Priors for DSe and DSp of the AAHL tests were based on results of a LCM performed on a larger data set of results from testing of oysters from both SA and Tasmania (Deveney et al., 2019). Beta(215,8.97) priors were used for DSp of each AAHL test, reflecting 95% prior confidence that the true value is between 0.93 and 0.98, with mean 0.96. A Beta(16.6,2.92) prior was used for DSe of the AAHL Martenot test, reflecting 95% confidence of this being in the range 0.66 – 0.95 with mean 0.85, and a Beta(26.7,10.4) prior used for DSe of the AAHL Jenkins test, reflecting 95% confidence of this being in the range 0.56 – 0.84, with mean 0.72. Vague Beta(5,2) priors were used for DSe and DSp of each Biomeme test, reflecting 90% confidence that these parameters are > 0.5. Uniform priors were used for covariance, with bounds set based on minimum and maximum possible values, which depend on the test sensitivities and specificities (Gardner et al., 2000; Wang et al., 2019). Diffuse normal(0,1) priors were used for prevalence covariates. LCM fitting was performed using Markov chain Monte Carlo (MCMC) simulation in JAGS v. 4.3.0 (Plummer, 2017) with three chains for 10,000 iterations, thinned at a rate of 10, following 2,000 iterations for adaptation and 10,000 iterations for burn-in. JAGS was run using the 'R2jags' package (Su and Yajima, 2015) in R. Convergence was assessed by Gelman-Rubin convergence statistic, and confirmed by visual inspection of trace, density and autocorrelation plots generated using the 'MCMCplots' package (McKay Curtis 2015).

Posterior predictions of DSe and DSp of individual tests from the LCM were used to calculate the effective DSe and DSp of applying tests in combination, following Gardner et al. (2000). Where two different tests are applied to a sample, different case definitions can be applied to maximize either overall DSe or DSp. To maximize DSe, a sample is considered infected if either of two tests returns a positive, known as the 'or' case definition. Using this case definition, overall DSe (DSe_{1or2}) and DSp (DSp_{1or2}) are given by:

$$DSe_{1or2} = 1 - ((1 - DSe_1)(1 - DSe_2) + covp_{12}) \text{ and } DSp_{1or2} = DSp_1 \times DSp_2 + covn_{12}$$

Where DSe_1 and DSe_2 are the sensitivities of the two individual tests, $covp_{12}$ is the covariance between results of the two tests in infected samples, DSp_1 and DSp_2 are the specificities of the two individual tests, and $covn_{12}$ is the covariance between results of the two tests in uninfected samples. To maximize overall DSp, a sample is only considered infected where both tests return a positive, known as the 'and' case definition. In this case, overall DSe (DSe_{1and2}) and DSp (DSp_{1and2}) are given by:

$$DSe_{1and2} = DSe_1 \times DSe_2 + covp_{12} \text{ and } DSp_{1and2} = 1 - ((1 - DSp_1)(1 - DSp_2) + covn_{12})$$

Using these formulae, we calculated the effective DSe and DSp of the laboratory-based protocol with both Jenkins and Martenot tests, and of the Biomeme field-based protocol with both tests, using each case definition. From the combined test DSe values, we determined the number of samples (n) that would need to be tested to detect a disease prevalence of 2% and 5% with an overall detection likelihood of either 80% or 95% as:

$$n = \log(1 - Pdet) / \log(1 - Dse \times prev)$$

Where Pdet is the overall detection likelihood (0.8 or 0.95), DSe is the relevant combined test DSe, and prev is the target prevalence to detect (0.02 or 0.05).

Results

Biomeme trial run

From the Biomeme trial run, the ethanol preserved samples recorded a similar C_T value to the frozen tissue for both the Martenot B region and Jenkins C region tests (Table 4). Consistency in threshold results was also recorded between the Biomeme assessed ethanol preserved tissue and AAHL (laboratory-based qPCR) assessed ethanol preserved tissue for both tests (Table 4). The full ethanol preserved tissue collection ($n = 257$ samples) was therefore selected to run on the Biomeme Franklin, allowing for comparisons to then be made with the AAHL (laboratory-based qPCR) results.

Table 4. C_T values of ethanol preserved and frozen feral oyster samples used in the initial trial comparing the laboratory-based qPCR test for OsHV-1 (AAHL) with the field-based custom assay design (Biomeme chemistry and Franklin device) across two tests (Martenot B region and Jenkins C region).

| Sample No. | Preservation (E/F) | AAHL Mean C_T - Martenot | Biomeme C_T - Martenot | AAHL Mean C_T - Jenkins | Biomeme C_T - Jenkins |
|------------|--------------------|----------------------------|--------------------------|---------------------------|-------------------------|
| 55 | E | 31.88 | 33.64 | 30.58 | 30.47 |
| 77 | E | 30.68 | 30.86 | 29.75 | 27.60 |
| 187 | E | 24.49 | 30.98 | 23.81 | 26.95 |
| 55 | F | - | 30.73 | - | 27.37 |
| 77 | F | - | 32.62 | - | 29.34 |
| 187 | F | - | 32.02 | - | 27.71 |
| NQC-1 | - | 24.95 | 24.76 | - | - |
| NQC-3 | - | - | - | 23.69 | 22.76 |
| NEC | - | - | 0 | - | 0 |

Abbreviations: E, ethanol; F, frozen; NEC, negative extraction control; NQC-1, positive plasmid control for Martenot assay; NQC-3, positive plasmid control for Jenkins assay.

Diagnostic performance of Biomeme and laboratory-based qPCR

A summary of the test results for the 257 assessed oyster tissue samples are presented in Table 5, with 59 samples positive (C_T value recorded) for both the Biomeme platform and AAHL laboratory-based qPCR for one or both tests (Martenot B region and/or Jenkins C region), and 144 samples recorded as negative (no C_T value) for both the Biomeme platform and AAHL laboratory-based qPCR. The remaining 54 samples were recorded as negative for both tests by one platform (e.g. Biomeme platform/AAHL laboratory-based qPCR) but positive for one or both tests by the second platform. A higher proportion of positive results (across both target region tests) was returned by AAHL laboratory-based testing than by the Biomeme in samples from June 2018 through November 2018, while the Biomeme assay returned more positives than laboratory-based testing from the December 2018 and January 2019 sample sets. The majority of Biomeme detections (15/24) returned positives for both the Martenot B and Jenkins C region tests, with 7 samples returning a positive only for the Jenkins test, and two samples only for the Martenot test. In AAHL laboratory-based testing, 15/30 samples with a detection were positive by both tests, 12/30 returned a positive only for the

Martenot test, and 3/30 only for the Jenkins test. There were, therefore, more detections overall by the Martenot test from AAHL than Biomeme, but more detections by the Jenkins test by Biomeme than from AAHL.

Table 5. Summary of results from assessing 257 ethanol preserved feral oyster tissue samples by sample set across two OsHV-1 tests, Martenot B region and Jenkins C region, on the Biomeme platform and by AAHL using laboratory-based qPCR. Apparent prevalence calculated taking any sample with a positive result in any test as infected.

| Sample set | Jun-18 | Aug-18 | Sept-18 | Oct-18 | Nov-18 | Dec-18 | Jan-19 |
|---|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|
| Result (total) | | | | | | | |
| Laboratory + Biomeme (59) one or both tests | 9 | 6 | 5 | 3 | 8 | 24 | 4 |
| No detection (144) | 18 | 19 | 25 | 40 | 17 | 3 | 22 |
| Biomeme only (24) | 1 | 1 | 1 | 0 | 2 | 8 | 11 |
| Martenot only (2) | 0 | 1 | 0 | 0 | 1 | 0 | 0 |
| Jenkins only (7) | 0 | 0 | 0 | 0 | 1 | 2 | 4 |
| Both tests (15) | 1 | 0 | 1 | 0 | 0 | 6 | 7 |
| Laboratory only (30) | 3 | 6 | 6 | 3 | 5 | 4 | 3 |
| Martenot only (12) | 0 | 3 | 3 | 1 | 2 | 1 | 2 |
| Jenkins only (3) | 1 | 0 | 0 | 1 | 0 | 1 | 0 |
| Both tests (15) | 2 | 3 | 3 | 1 | 3 | 2 | 1 |
| Total detections (113) | 13 | 13 | 12 | 6 | 15 | 36 | 18 |
| Apparent prevalence | | | | | | | |
| Biomeme | 32.3% | 21.9% | 16.2% | 6.5% | 31.3% | 82.1% | 37.5% |
| Laboratory | 38.7% | 37.5% | 29.7% | 13.0% | 40.6% | 71.8% | 17.5% |
| Combined | 41.9% | 40.6% | 32.4% | 13.0% | 46.9% | 92.3% | 45.0% |
| Estimated true prevalence | | | | | | | |
| (95% credible intervals) | 0.38 (0.24 – 0.52) | 0.17 (0.05 – 0.41) | 0.27 (0.13 – 0.43) | 0.12 (0.04 – 0.24) | 0.42 (0.25 – 0.60) | 0.86 (0.72 – 0.95) | 0.36 (0.13 – 0.56) |

Diagnostic performance of each test as estimated by the LCM was similar across the two platforms (Table 6). DSe (mean and 95% credible intervals) of the Martenot B region test was 0.77 (0.65 – 0.90) for laboratory-based (AAHL) testing, and 0.64 (0.53 – 0.76) for Biomeme, and DSe of the Jenkins C region test was 0.69 (0.59 – 0.78) for laboratory-based (AAHL) testing, and 0.75 (0.62 – 0.87) for Biomeme. DSp was high (~0.95) in all cases (Table 6). For both laboratory-based and Biomeme testing, there was positive covariance between results of the two tests (Martenot and Jenkins) performed on the same platform for infected (+) samples, but not for uninfected (-) samples (Table 7). The 95% credible interval of all other covariance measures contained zero (Table 7), including covariances between the same test performed on different platforms, i.e. the two tests performed using the same platform were not conditionally independent, but results of tests (either the same or different) performed on different platforms did not show conditional dependence.

Table 6. Diagnostic performance of two OsHV-1 tests (Martenot and Jenkins) performed by AAHL (laboratory-based) and using the Biomeme platform estimated (mean and 95% credible intervals) from latent class model. *non-zero covariance.

| Parameter estimates | Laboratory | Biomeme |
|----------------------------------|---------------------|---------------------|
| Jenkins C region | | |
| DSe | 0.69 (0.59 – 0.78) | 0.75 (0.62 – 0.87) |
| DSp | 0.96 (0.94 – 0.98) | 0.94 (0.86 – 0.99) |
| Martenot B region | | |
| DSe | 0.77 (0.65 – 0.90) | 0.64 (0.53 – 0.76) |
| DSp | 0.94 (0.91 – 0.97) | 0.95 (0.88 – 0.99) |
| Covariance in + samples | 0.14 (0.06 – 0.21)* | 0.14 (0.07 – 0.21)* |
| Covariance in – samples | 0.01 (0.00 – 0.03) | 0.02 (0.00 – 0.08) |
| Combined ‘OR’ definition | | |
| DSe | 0.79 (0.67 – 0.92) | 0.77 (0.64 – 0.88) |
| DSp | 0.91 (0.88 – 0.95) | 0.92 (0.84 – 0.97) |
| Combined ‘AND’ definition | | |
| DSe | 0.67 (0.58 – 0.77) | 0.62 (0.51 – 0.74) |
| DSp | 0.98 (0.96 – 1.00) | 0.97 (0.90 – 1.00) |

Table 7. Covariances between each pair of tests. Top diagonal shows covariance in infected samples, lower diagonal shows covariance in uninfected samples. *non-zero covariance

| | Laboratory Jenkins | Laboratory Martenot | Biomeme Jenkins | Biomeme Martenot |
|------------------------|-----------------------|------------------------|-------------------------|-------------------------|
| Laboratory Jenkins | | 0.14 (0.06 – 0.21)* | -0.02 (-0.08 – 0.06) | 0.04 (0.00 – 0.11) |
| Laboratory Martenot | 0.01 (0.00 – 0.03) | | 0.03 (-0.03 – 0.14) | -0.01 (-0.08 – 0.05) |
| Biomeme Jenkins | 0.02 (0.00 – 0.04) | 0.00 (-0.01 – 0.01) | | 0.14 (0.07 – 0.21)* |
| Biomeme Martenot | 0.00 (0.00 – 0.01) | 0.01 (0.00 – 0.03) | 0.02 (0.00 – 0.08) | |

The effective DSe and DSp of applying both Martenot and Jenkins tests was very similar for both laboratory-based and Biomeme testing for either case definition (Table 6). Applying the ‘or’ case definition to maximise DSe results in effective DSe > 0.75 by either platform (Table 6). This interpretation results in a loss of DSp, but, due to the high specificity of the individual tests, effective DSp remains high (> 0.9). Applying the ‘and’ case definition maximizes DSp, and results in a DSp of

≥ 0.97, but with a reduction in DSe to < 0.7 (Table 6). The number of samples required to detect a given disease prevalence is, therefore, similar between the two testing platforms (Table 8). The number of samples required increases with decreasing target prevalence or where a greater overall likelihood of detection (higher survey sensitivity) is required, or where the ‘and’ case definition is applied, since this interpretation decreases effective DSe.

Disease prevalence in the Port River estimated by the LCM varied between sample sets (Table 5). Prevalence was higher in December than all other sample sets, with odds ratios ranging from 10.1 (95% credible interval 3.8 – 49.6) between December and January, to 66.4 (13.5 – 220.2) between December and October; prevalence was also higher in November than October (odds ratio 6.8, 95% credible interval 1.7 – 20.7). Credible intervals on prevalence estimates were narrower for sample sets for which there was greater congruence between results of laboratory-based and Biomeme testing, since agreement in results provides greater confidence in the prevalence estimate.

Table 8. Number of samples required to detect a disease prevalence of 2% or 5% with 80% or 95% likelihood (survey sensitivity) using AAHL laboratory-based or Biomeme platform with either case definition.

| Survey sensitivity | 0.8 | | 0.95 | |
|------------------------------|-----------------|--------------|-----------------|---------------|
| Target prevalence | 0.02 | 0.05 | 0.02 | 0.05 |
| ‘OR’ case definition | | | | |
| Laboratory | 103 (88 – 120) | 41 (35 – 48) | 190 (162 – 224) | 76 (64 – 89) |
| Biomeme | 105 (91 – 125) | 42 (36 – 50) | 195 (169 – 233) | 77 (67 – 92) |
| ‘AND’ case definition | | | | |
| Laboratory | 120 (105 – 139) | 48 (42 – 55) | 223 (195 – 258) | 89 (77 – 103) |
| Biomeme | 130 (108 – 159) | 52 (43 – 63) | 242 (201 – 296) | 96 (80 – 118) |

Discussion and conclusion

Diagnostic performance of the Biomeme platform was similar to that of laboratory-based qPCR assays. The Jenkins C region test returned more detections on the Biomeme platform than the Martenot B region test, while the opposite was true of laboratory testing, although resulting DSe estimates for each test from the LCM were similar, with 95% credible intervals overlapping. Different chemistry and methodology provide different relative test performance between the platforms, but a larger data set would be needed to elucidate any differences, and this study demonstrates that the differences in test performance are not large.

Tests performed on the same platform (either Biomeme or laboratory) were not conditionally independent for the infected sample case, but there was no dependence between tests, even for the same test, between platforms. This indicates that covariance between test results is due to the platform used, rather than to the test itself. Covariance could be due to the different extraction methods or to differences in the qPCR chemistry between platforms.

Where both tests are applied using the 'or' case definition, effective DSe is nearly identical between the two testing platforms, hence the number of samples needed to detect a given disease prevalence is also approximately equivalent. Using the 'and' case definition, effective DSe of the Biomeme platform is marginally lower than laboratory testing, although with considerable overlap of 95% credible intervals; this case definition is applied to maximise DSp, e.g. for confirmatory testing, so this interpretation is less likely to be applied for a field test.

The Biomeme platform returned more positive results across both tests than laboratory-based assay for the last two sample sets (December 2018 and January 2019), but not for earlier sets (June – November 2018). This suggests that the Biomeme platform may perform better with fresher material than material stored frozen for longer periods, but a much larger controlled study would be needed to confirm if this is the case. The LCM used assumes that DSe and DSp of each test are the same across sample sets, but that prevalence varies; it is not possible to estimate potential differences in DSe across sample sets unless prevalence in each sample set is known.

Implications

Rapid detection using the Biomeme system could facilitate more effective emergency responses to POMS outbreaks by providing rapid field diagnostic capability and improve disease management by expediting batch testing in farming areas to inform livestock translocations. In SA, where Pacific Oyster growing regions are isolated and feral populations of Pacific Oysters are small, growers have an unusual opportunity to prevent spread of disease from incursion sites. The oyster industry, however, relies on regular movements of livestock between regions, so an on farm diagnostic test could provide greater confidence of OsHV-1 freedom prior to stock movements for both regulators and industry. Yorke Peninsula farmers have oysters tested at Gribbles VetLab, Glenside, prior to moving them to other farming regions to gain the confidence of their buyers, which is not a regulatory requirement. If adopted, the rapid assessment tool developed by this study will assist management of this important disease. Uptake is likely to be driven by assessing cost-benefits. Costs of this on farm test would include \$US18K for purchase of the machine and approximately \$15/sample reagent and consumable costs. Training requirements would be minimal and can be achieved by SARDI providing a training day at the next SAOGA seminar or other relevant event.

Recommendations

The Australian case definition for a confirmed OsHV-1 microvariant infection is:

Suspect case

- Rapid onset of high mortality in Pacific oysters; or
- Positive result for OsHV-1 using a quantitative polymerase chain reaction (qPCR) test (Martenot et al. 2010) from one or more appropriate samples.

Confirmed case

- Positive result for OsHV-1 using the qPCR test; and
- Positive result with the C2/C6 conventional PCR (cPCR) test (Segarra et al. 2010); and
- Sequence analysis to confirm genotype as OsHV-1 microvariant (OsHV-1 microvariant exhibits a systematic deletion of 12–15 base pairs in ORF 4 of the genome (encompassed by the C2/C6 primers) in comparison with OsHV-1 (GenBank # AY509253)).

(Department of Agriculture, 2015) and as such samples must be submitted for the Segarra et al. (2010) C2/C6 cPCR for confirmation if a field diagnosis is made. Rapid response to detections of OsHV-1, however, should not be prevented by the time taken for a cPCR result to be received, and a detection using the Biomeme system should be treated as a Response Phase alert.

Extension and Adoption

The project was extended by proposing a change to the Australian case definition for a confirmed OsHV-1 microvariant infection to include detection using the Biomeme platform as triggering a Response Phase alert.

Further extension of the project may result in uptake of the Biomeme system among industry (e.g. South Australian Oyster Growers Association [SAOGA] members) and government (e.g. PIRSA Biosecurity SA staff) for use as a routine, everyday field-applicable surveillance tool, allowing for rapid detection of OsHV-1 microvariant infection in Pacific Oyster growing regions. This will then guide the activation of emergency response measures, providing an improved chance of containment and prevention of spread of the disease from incursion sites.

These results will be disseminated more broadly to the SAOGA members via a newsletter update and discussed through the SA POMS working group, with training to be provided to relevant staff if uptake of the system is recommended.

Project coverage

Presented to industry at the NSW Oyster meeting, 8 August 2019; Tasmanian industry meeting, 17 August 2019; SAOGA industry conference, 22 August 2019.

Project materials developed

Refer to Appendix 1 which provides a detailed protocol for preparing samples and using the Biomeme Franklin device to run the field-based qPCR test.

Appendix 1

Biomeme Franklin device – detailed protocol

Note: protocol detailed below is for preparing a single run on the Biomeme Franklin device using the custom OsHV-1 assay, equating to seven oyster tissue samples, one negative extraction control and one positive control.

1. Wipe down the bench area and forceps with 70% ethanol. Ensure lab coat and gloves are worn at all times while preparing samples. Approximately 25 mg of oyster tissue is required for this protocol. Subsample from an ethanol preserved sample or take a ~25 mg sample of mantle and gill from a sampled oyster and place in a new 1.5 mL DNA LoBind tube (Eppendorf, supplier – Southern Cross Science, cat. no. 0030108051) without any ethanol or other preservative. Store at -20 °C until required.
2. Label 8x 1.5 mL DNA LoBind tubes with the sample number on the lid of the tube, and sample number, 'elute' and the date on the side on the tube in preparation for step 11. Place in a tube rack and leave to the side.
3. Transfer 2 mL of homogenisation BLB buffer to the 5 mL blue screw cap tube containing a 1/2" stainless steel ball bearing using the 1 mL transfer pipette provided in the kit (see Figure 2A).
4. Add the oyster tissue subsample that is to be homogenised into the 5 mL blue cap tube using forceps, tightly screw on the blue tube cap and vigorously shake the tube with the ball bearing for 30 sec (or until the sample is sufficiently homogenised, this may take longer for some samples). Wipe forceps with 70% ethanol in between each sample transfer.
5. Remove the M1 Sample Prep Cartridge for DNA from the pouch, add the Sample Prep Column (filter) onto the end of the single-use 1 mL syringe and pierce two holes into the red section (labelled 'start') of the cartridge (one in foil section, one in red section above to minimise liquid splatter).
6. Using the 1 mL transfer pipette, transfer 1 mL of the homogenised solution from the 5 mL blue cap tube into the hole in the red foil section of the M1 Sample Prep Cartridge for DNA. Discard the transfer pipette and 5 mL blue cap tube containing the ball bearing and remaining homogenate.
7. Place the single-use 1 mL syringe with the Sample Prep Column (filter) into the hole in the red foil section of the M1 Sample Prep Cartridge for DNA and draw the fluid all the way up the syringe and pump slowly all the way back out. Repeat for a total of 10 pumps.
8. Push all the fluid out of the syringe before proceeding to the next compartment of the M1 Sample Prep Cartridge for DNA (do not transfer any liquid from one well of the cartridge to the next well).
9. Using the syringe, pierce a hole in the red-orange foil section of the M1 Sample Prep Cartridge for DNA and pump twice, then pierce a hole in the orange foil section and pump once, then

pierce a hole in the yellow foil section and pump once, then pierce a hole in the blue foil section and pump 20+ times very fast to dry the filter column.

10. Move to the last compartment of the M1 Sample Prep Cartridge for DNA and pierce a hole in the green foil section. Elute by slowly pumping for a total of 5 times. Note the number of pumps to perform in each coloured section of the cartridge are indicated on the bottom of the M1 Sample Prep Cartridge for DNA (see Figure 2B).
11. Draw all the liquid up the syringe and transfer the elute to the labelled 1.5 mL DNA LoBind tube from step 2.
12. Place the provided absorbent material over the holes in the now used M1 Sample Prep Cartridge for DNA, then dispose of the cartridge, single use 1 mL syringe and Sample Prep Column (filter) into the original pouch, seal and dispose of into a general waste bin.
13. Wipe down the bench area and forceps with 70% ethanol, then repeat steps 3-12 for the remaining six oyster tissue samples in the run. The final sample to prepare will be the negative extraction control (NEC), where no tissue is added to the 5 mL blue cap tube containing the homogenisation BLB buffer and stainless steel ball, vigorously shaken then the protocol is followed as previously from steps 5-12. Note if gloves have liquid droplets on them following sample preparation, discard and put on a new pair of gloves so there is no cross-contamination between samples.
14. Once all samples have been processed, open the pouch containing the custom OshV-1 strip tubes and place 3x three strips tubes (total of nine wells) into a rack. Orientate the tubes correctly in the rack by placing the strip connections away from you. Label tubes on the side with the sample number, NEC or pos.
15. Remove the foil seal from first 1x three Go-Strip tubes and using a pipette with a 100 µl filtered tip (Eppendorf, supplier – Southern Cross Science, cat. no. 0030077547), add 20 µl of the eluted sample from step 11 into the first well. Repeat for the remaining eluted samples (wells 1-7) and NEC (well 9), adding them into the corresponding labelled well of each strip tube. For well 8, add 20 µl of the prepared positive control (which will be supplied as aliquots of 2 µl of NQC-1 [Martenot B region positive plasmid control], 2 µl of NQC-3 [Jenkins C region positive plasmid control] and 16 µl of UltraPure DNase/RNase free water [Life Technologies, cat. no. 10977023]; to be stored at -20 °C until required). Remaining volume of sample elutes are then stored long-term at -20 °C.
16. Place void-filling caps over the Go-Strip tubes, with indent in the caps lining up with the strip connections, so the connections are visible. Place one finger on top of the caps with your thumb below and flick the tubes 2-3 times to mix the sample, ensuring no bubbles are present down the bottom of the tubes. Bubbles at the top of the tube are fine and won't interfere with the protocol (see Figure 2C).
17. Turn the Biomeme Franklin device on by holding down the power button (top left). Before loading strip tubes, check the device battery status by holding down the battery button (bottom left). Note if only one bar lights up and flashes, plug the device in to charge while performing the run.

18. Enable Bluetooth on the device by pressing the Bluetooth button (top right) and load the 3x three Go-Strip tubes into the device, making sure the orientation of the tubes is correct with the strip connections facing the back of the device.
19. Unlock the smartphone and click on the 'Biomeme GoApp'. Press 'Start Run', then 'select test'. Under user, select 'Oshv1' then press 'confirm'.
20. Next screen is to confirm Go-Strip quantity – keep this at three for testing nine samples - click 'confirm'.
21. Next screen is the run layout - select the last option '9 samples, 1 replicate each' and 'generate IDs'. 'Sample ID 1' will be the elute tube sample number. Add all sample ID's for oyster tissue samples 1-7, with sample 8 the positive control ('pos') and sample 9 the negative control ('NEC'). Press 'continue'.
22. Next screen is to select the folder to save the run into – select 'Samples' folder then 'save to current folder'. Enter run name as 'Samples X-X_date' and press 'confirm'.
23. As the Franklin device is already on, press 'continue' at the next screen. The app will then search for the device via the Bluetooth connection and 'Franklin-F9F32DE732BD' will appear below 'select thermocycler'. Click on this name to continue and click through remaining screens to start the run (ensuring strip tubes have already been loaded into the device in the correct orientation – connections facing the back of the device).
24. The custom OsHV-1 assay will start, taking ~45 mins (see Figure 2D). The device and smartphone can be left unattended during this time. Once the run is complete, ensure the results have been synced to the Biomeme Cloud Portal (press the 'sync' button, wifi must be on).
25. Remove the Go-Strip tubes from the device, discard and turn the device off using the power button (top left).

Appendix 2

JAGS code for latent class model

```
model{
  for(i in 1:n){
    logit(pi[i]) <- inprod(b_prev[],X[i,])
    for (k in 1:K){
      s1[i,k] <- se[k]^x[i,k]*((1-se[k])^(1-x[i,k]))
      s2[i,k] <- sp[k]^(1-x[i,k])*((1-sp[k])^x[i,k])
    }
    for (j in 1:K){
      for (h in 1:K){
        cop[i,j,h] <- c1[j,h]*(-1)^(x[i,j] + x[i,h])/(s1[i,j]*s1[i,h])
        con[i,j,h] <- c2[j,h]*(-1)^(x[i,j] + x[i,h])/(s2[i,j]*s2[i,h])
      }
    }
    eta[i] <- (prod(s1[i,1:K]) *(1+ sum(cop[i,,])))
    theta[i] <- (prod(s2[i, 1:K]) *(1+sum(con[i,,])))
    prob[i] <- pi[i]*eta[i] + (1-pi[i])*theta[i]
    z[i] ~ dpois( - log(prob[i]))
  }
  # Priors for Se and Sp of AAHL tests from Deveney et al. (2019)
  # EMAI (Jenkins) AAHL
  se[1] ~ dbeta(26.7,10.4)T(1-sp[1], )
  sp[1] ~ dbeta(215,8.97)
  # OIE (Martenot) AAHL
  se[2] ~ dbeta(16.6,2.92)T(1-sp[2], )
  sp[2] ~ dbeta(215,8.97)
  # Biomeme tests - vague priors
  #Jenkins
  se[3] ~ dbeta(5,2)T(1-sp[3], )
  sp[3] ~ dbeta(5,2)
  #Martenot
  se[4] ~ dbeta(5,2)T(1-sp[4], )
  sp[4] ~ dbeta(5,2)
  #Priors for covariance
  for (l in 1:(K-1)){
    for (h in (l+1):K){
      c1[l,h] ~ dunif((se[l]-1)*(1-se[h]), (min(se[l],se[h])-se[l]*se[h]))
      c2[l,h] ~ dunif((sp[l]-1)*(1-sp[h]), (min(sp[l],sp[h])-sp[l]*sp[h]))
      c1[h,l] <- 0
      c2[h,l] <- 0
    }
  }
}
```

```
}  
for (h in 1:K){  
  c1[h,h] <- 0  
  c2[h,h] <- 0  
}  
for (j in 1:Nc) {  
  b_prev[j] ~ dnorm(0,1) #Covariates for prevalence  
}  
}
```

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FRDC FINAL REPORT CHECKLIST

| | | | |
|---------------------------------|---|--------------|-------------------------------|
| Project Title: | Fast track validation of pond-side test kits for use on farms and in regions | | |
| Principal Investigators: | Sarah R. Catalano, Kathryn H. Wiltshire, Nick Moody, Peter Mohr, Ruth Muhonja, Nhu Y. Lieu, Marty Deveney | | |
| Project Number: | 2018/127 | | |
| Description: | This project investigated the sensitivity and specificity of the Biomeme Franklin platform for rapid detection of ostreid herpes virus 1 (OsHV-1) in Pacific Oyster (<i>Crassostrea gigas</i>) samples, with the aim of improving the time taken to detect and subsequent respond to POMS outbreaks in the environment. | | |
| Published Date: | XX/XX/XXXX (if applicable) | Year: | XXXX |
| ISBN: | XXXXX (if applicable) | ISSN: | XXXXXXXXXXXXX (if applicable) |
| Key Words: | Pacific Oyster (<i>Crassostrea gigas</i>), POMS, OsHV-1, qPCR, rapid pond-side test, Biomeme platform, Franklin device, diagnostic performance, disease surveillance | | |

Please use this checklist to self-assess your report before submitting to FRDC. Checklist should accompany the report.

| | Is it included (Y/N) | Comments |
|--|----------------------|--|
| Foreword (optional) | N | |
| Acknowledgments | Y | |
| Abbreviations | Y | |
| Executive Summary | Y | |
| – What the report is about | Y | |
| – Background – why project was undertaken | Y | |
| – Aims/objectives – what you wanted to achieve at the beginning | Y | |
| – Methodology – outline how you did the project | Y | |
| – Results/key findings – this should outline what you found or key results | Y | |
| – Implications for relevant stakeholders | Y | |
| – Recommendations | Y | |
| Introduction | Y | |
| Objectives | Y | |
| Methodology | Y | |
| Results | Y | |
| Discussion | Y | |
| Conclusion | Y | |
| Implications | Y | |
| Recommendations | Y | |
| Further development | Y | Refer to Recommendations section for next steps. Training and transfer to industry. |

| | | |
|---|---|---|
| Extension and Adoption | Y | |
| Project coverage | Y | Presented to industry at: NSW Oyster meetings, Foster, NSW 8 August 2019, Tasmanian industry meeting, 17 August 2019, SAOGA industry conference 22 August 2019. |
| Glossary | N | Refer to the Abbreviations table |
| Project materials developed | Y | See appendices. |
| Appendices | Y | |
| EXTENSION | | |
| Extension plan developed? | Y | |
| Extension undertaken? | Y | |
| If extension was undertaken, who was it undertaken with and was it successful? (Detail answer in comments section) | Y | |
| If No, then is further extension necessary? With who? How? (detail answer in comments section) | Y | Further implementation transfer and handover with SAOGA required. |