**Supplementary material**

**S1. Repeatability, preliminary reproducibility of the method and influence of matrix DNA concentration on amplification**

Repeatability (within series variations) and preliminary reproducibility (between series variations) were determined using plasmid dilutions (105 à 10-1 copies /µl, 8 replicates per condition), during three consecutive days. Intra and inter-series standard deviations were calculated, as well as variation coefficient of intermediate fidelity (i.e. fiability) as described in ISO 5725-2.

Impact of oyster DNA concentration on amplification efficiency was determined by adding different oyster DNA concentrations (5, 50, 150 et 250 ng/µl) to a serie of plasmid dilutions (101 to 103 copies/µl). For each condition, the difference (ΔCt) between the obtained and the control Cts was calculated as well as the ΔCt mean and the average deviation.

**S2 – Bayesian model used for DSe and DSp estimation**

Legend:

4 possible result patterns with two methods: --, -+,+-,++;

Method 1: new real-time PCR, Method 2: conventional PCR

r1=count in population 1in order --, -+,+-,++; r2=count in population 2 in order --, -+,+-,++ , r3=count in population 3 in the order: --, -+,+-,++;

n1, n2, n3= total count in population 1, 2 and 3;

Pr1, Pr2, Pr3=prevalence in population 1, 2 and 3;

se1 se2= sensibility method 1 and 2, sp1 sp2= specificity method 1 and 2,

p1[1]=p(--) in population 1, p1[4]=p(++) in population 1 ...etc ,

Cp12= covariance between method 1and 2 within the “diseased” class,

Cn12= covariance between method 1and 2 within the “non-diseased” class.

Bayesian model

model{

r1[1:4] ~ dmulti(p1[1:4], n1)

r2[1:4] ~ dmulti(p2[1:4], n2)

r3[1:4] ~ dmulti(p3[1:4], n3)

a[1] = (1-se1)\*(1-se2)+cp12

a[2] = (1-se1)\*se2-cp12

a[3] = se1\*(1-se2) -cp12

a[4] =se1\*se2+cp12

b[1] = sp1\*sp2+cn12

b[2] = sp1\*(1-sp2) -cn12

b[3] = (1-sp1)\*sp2 -cn12

b[4] = (1-sp1)\*(1-sp2)+cn12

p1[1:4] = (pr1\*a[1:4] + (1-pr1)\*b[1:4])

p2[1:4] = (pr2\*a[1:4] + (1-pr2)\*b[1:4])

p3[1:4] = (pr3\*a[1:4] + (1-pr3)\*b[1:4])

pr1 ~ dbeta(1,1)

pr2 ~ dbeta(1,1)

pr3 ~ dbeta(1,1)

sp1 ~ dbeta(1,1)

sp2 ~ dbeta(1,1)

se1 ~ dbeta(1,1)

se2 ~ dbeta(1,1)

cp12 ~ dnorm(0,5)

cn12 = 0

**Table S1.** *Crassostrea gigas* populations (origin and status with regard to the *Haplosporidium costale* presence) used for the determination of the diagnostic performances of the new Real Time PCR assay.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Prevalence of *H. costale* in populations** | **Number of individuals per population** | **Sampling site** | **Batch number** | **Sampling date** | **Number of individuals per batch** | **Age** | **Population origin** | **Context of sampling** |
| Free population | 170 | High biosecurity level area of Ifremer experimental facilities in Bouin  (Vendée) | 19/111 | 18/06/2019 | 40 | 1-2 years | Farm | Outside mortality |
| 19/190 | 20/08/2019 | 130 | 1-2 years | Farm | Outside mortality |
| Low prevalence  (<10%) | 181 | Bourgneuf bay  (Vendée) | 19/080 | 11/06/2019 | 119 | > 2 years | Wild | Outside mortality |
| 19/103 | 18/06/2019 | 62 | > 2 years | Wild | Outside mortality |
| Moderate prevalence  (10-30%) | 137 | Nursery of Ifremer experimental facilities in Bouin  (Vendée) | 19/030 | 28/03/2019 | 18 | 1-2 years | Farm | Mortality event |
| 19/073 | 11/06/2019 | 30 | < 1 year | Farm | Outside mortality |
| 19/075 | 11/06/2019 | 18 | 1-2 years | Farm | Outside mortality |
| 19/083 | 11/06/2019 | 7 | > 2 years | Farm | Mortality event |
| 19/084 | 11/06/2019 | 6 | > 2 years | Farm | Mortality event |
| 19/087 | 11/06/2019 | 12 | 1-2 years | Farm | Outside mortality |
| 19/089 | 11/06/2019 | 10 | 1-2 years | Farm | Outside mortality |
| 19/090 | 11/06/2019 | 13 | > 2 years | Farm | Outside mortality |
| 19/091 | 11/06/2019 | 13 | 1-2 years | Farm | Outside mortality |
| 19/092 | 11/06/2019 | 10 | 1-2 years | Farm | Outside mortality |

**Table S2.** Estimation of the detection limits (in blue in the table) of the new Real Time PCR assay using DNA plasmidic suspensions.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Dilution** | **Number of positive samples**  **(8 samples /dilution)** | | | **Total** |
| **Test 1** | **Test 2** | **Test 3** |
| 10 copies/µl | 8 | 8 | 8 | 24 |
| **5 copies/µl** | **8** | **8** | **8** | **24** |
| **2.5 copies/µl** | **7** | **8** | **7** | **22** |
| 1.25 copies/µl | 6 | 6 | 8 | 20 |
| 0.75 copies/µl | 2 | 3 | 2 | 7 |
| 0.375 copies/µl | 4 | 2 | 1 | 7 |

**Table S3.** Repeatability and preliminary reproducibility of the new Real-Time assay. 10-fold serial dilutions of plasmidic DNA were tested in 8 replicates x 3 PCR runs (N =24 PCR tests for each dilution). Repeatability and preliminary reproducibility were based on the Ct values of the 24 replicates tested for each dilution.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| DNA plasmidic concentration (copies/µl) | **105** | **104** | **103** | **102** | **10** | **1** | **0.1** |
| % of positive results (N positives/ N tested) | 100%  (24/24) | 100%  (24/24) | 100%  (24/24) | 100%  (24/24) | 100%  (24/24) | 88%  (21/24) | 29%  (7/24) |
| Ct mean value (n=24) | 20.97 | 24.09 | 27.28 | 30.57 | 34.15 | 37.50 | - |
| Intra assay standard deviation | 0.30 | 0.44 | 0.34 | 0.73 | 0.58 | 0.70 | - |
| Inter assay standard deviation | 0.16 | 0.00 | 0.00 | 0.29 | 0.16 | 0.46 | - |
| Intermediate Precision (IP) | 0.34 | 0.44 | 0.34 | 0.79 | 0.61 | 0.84 | - |
| Coefficient of IP variation (%) | 1.64 | 1.82 | 1.25 | 2.58 | 1.77 | 2.24 | - |

**Table S4.** Influence of *Crassostrea gigas* DNA matrix concentration **[C]** on the Real Time PCR results. The Ct control corresponds to [C] DNA =0 and ΔCt correspond to Ctobtained-Ctcontrol.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **[C] DNA (ng/µL)** | **[C] plasmid (cps/µL)** | **Ct mean** | **ΔCt** | **ΔCt mean** | **ΔCt average deviation** |
| **0** | **1000** | 27,24 | 0 | 0 | 0 |
| **100** | 30,49 | 0 |
| **10** | 33,53 | 0 |
| **5** | **1000** | 27,42 | 0,18 | 0,62 | 0,46 |
| **100** | 30,86 | 0,37 |
| **10** | 34,85 | 1,32 |
| **50** | **1000** | 27,34 | 0,11 | 0,31 | 0,14 |
| **100** | 30,84 | 0,35 |
| **10** | 34,00 | 0,47 |
| **150** | **1000** | 27,04 | -0,20 | 0,24 | 0,29 |
| **100** | 30,82 | 0,33 |
| **10** | 34,12 | 0,59 |
| **250** | **1000** | 27,42 | 0,19 | 0,38 | 0,13 |
| **100** | 31,02 | 0,53 |
| **10** | 33,96 | 0,42 |