**Supplementary Information**

**Development and validation of X-*ComEC* qPCR, a novel assay for accurate universal detection of both *Xylella fastidiosa* and *Xylella taiwanensis***

Johanna Wong-Bajracharya1, John Webster1, Luciano A. Rigano2, Pragya Kant3, Anna Englezou1, Fridjof. Snijders1, Dor Agmon1, Rebecca Roach4, Cuiping Wang5, Monica Kehoe5, Rachel Mann3, Fiona E. Constable3, Nerida J. Donovan1, Toni A. Chapman1 \*

1 New South Wales Department of Primary Industries and Regional Development, Elizabeth Macarthur Agricultural Institute, New South Wales, Australia

2 Plant Health and Environment Laboratory, Ministry for Primary Industries, Auckland, New Zealand

3 Agriculture Victoria Research, Department of Energy, Environment and Climate Action, Victoria, Australia

4 Queensland Department of Agriculture and Fisheries, Queensland, Australia

5 Department of Primary Industries and Regional Development, Western Australia, Australia

**Correspondence:** Toni Chapman, [toni.chapman@dpi.nsw.gov.au](mailto:toni.chapman@dpi.nsw.gov.au)

**Address:** Elizabeth Macarthur Agricultural Institute, Woodbridge Rd, Menangle, NSW 2568

**Supplementary Table S1:** The Cq value of X-*ComEC* qPCR assay comparing to published qPCR assays in the analytical specificity testing using the exclusivity and inclusivity panel. nd = not detected; N/A = not available

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | **Assay** | **X-*ComEC* generic qPCR**  **(This study)** | **Harper Xf qPCR** | **Ouyang Xf qPCR** | **Dupas Xf qPCR** | **Agiletti Xf qPCR** | **Francis Xf qPCR** | **Ito & Suzaki generic qPCR** | **Ito & Chiaki generic qPCR** |
| **Isolate** | **Species** | **Subspecies** | **Cq value** | | | | | | | |
| **Exclusivity panel** | | | | | | | | | | |
| DAR65801 | *Stenotrophomonas maltophilia* | Not applicable | nd | nd | nd | nd | nd | nd | nd | nd |
| DAR72045 | *Stenotrophomonas maltophilia* | Not applicable | nd | nd | nd | nd | nd | nd | 29.44 | nd |
| DAR75512 | *Stenotrophomonas maltophilia* | Not applicable | nd | nd | nd | nd | nd | nd | nd | nd |
| DAR76132 | *Stenotrophomonas maltophilia* | Not applicable | nd | nd | nd | nd | nd | nd | 18.01 | 29.41 |
| DAR77232 | *Stenotrophomonas sp.* | Not applicable | nd | nd | nd | nd | nd | nd | 17.36 | 31.28 |
| DAR77233 | *Stenotrophomonas sp.* | Not applicable | nd | nd | nd | nd | nd | nd | 18.47 | 28.01 |
| DAR77234 | *Stenotrophomonas sp.* | Not applicable | nd | nd | nd | nd | nd | nd | 17.31 | 29.63 |
| DAR77236 | *Stenotrophomonas sp.* | Not applicable | nd | nd | nd | nd | nd | nd | 18.82 | 28.87 |
| DAR77237 | *Stenotrophomonas sp.* | Not applicable | nd | nd | nd | nd | nd | nd | nd | nd |
| 1622 B Strain | *Xanthomonas fuscans* | *aurantifolii* | nd | nd | nd | nd | nd | nd | nd | nd |
| P03-83 | *Xanthomonas alfalfae* | *citrumelo* | nd | nd | nd | nd | nd | nd | nd | nd |
| DAR41379 | *Xanthomonas vasicola* | *holcicola* | nd | nd | nd | nd | nd | nd | nd | nd |
| DAR73877 | *Xanthomonas vesicatoria* | unknown | nd | nd | nd | nd | nd | nd | nd | nd |
| DAR35705 | *Xanthomonas translucens* | *translucens* | nd | nd | nd | nd | nd | nd | nd | nd |
| DAR49849 | *Xanthomonas hortorum* | *carotae* | nd | nd | nd | nd | nd | nd | nd | nd |
| DAR33337 | *Xanthomonas arboricola* | unknown | nd | nd | nd | nd | nd | nd | nd | nd |
| DAR82645 | *Xanthomonas campestris* | *phaseoli* | nd | nd | nd | nd | nd | nd | nd | nd |
| DAR82580 | *Xanthomonas sp.* | unknown | nd | nd | nd | nd | nd | nd | nd | nd |
| DAR82611 | *Xanthomonas oryzae* | *oryzae* | nd | nd | nd | nd | nd | nd | nd | nd |
| DAR30526 | *Xanthomonas campestris* | *vitians* | nd | nd | nd | nd | nd | nd | nd | nd |
| VPRI41552 | *Xanthomonas campestris* | unknown | nd | nd | nd | nd | nd | nd | nd | nd |
| DAR82627 | *Xanthomonas campestris* | *pruni* | nd | nd | nd | nd | nd | nd | nd | nd |
| DAR82711 | *Xanthomonas campestris* | *cucurbitae* | nd | nd | nd | nd | nd | nd | nd | nd |
| DAR72015 | *Xanthomonas translucens* | *translucens* | nd | nd | nd | nd | nd | nd | nd | nd |
| NCPPB4612 | *Xylella taiwanensis* | Not applicable | 18.13 | nd | nd | nd | nd | nd | 18.72 | 32.4 |
| ICMP8731 | *Xylella fastidiosa* | *fastidiosa* | 22.14 | 23.9 | 26.6 | 17.56 | 27.45 | 32.55 | 19.99 | 30.13 |
| **Inclusivity panel** | | | | | | | | | | |
| CFBP7969 | *Xylella fastidiosa* | *fastidiosa* | 18.14 | 19.33 | 19.4 | 19.56 | 23.16 | 20.24 | N/A | N/A |
| CFBP7970 | *Xylella fastidiosa* | *fastidiosa* | 16.28 | 17.26 | 19.43 | 16.56 | 19.38 | 18.31 | N/A | N/A |
| CFBP8071 | *Xylella fastidiosa* | *fastidiosa* | 15.87 | 18.54 | 18.03 | 16.44 | 19.57 | 17.78 | N/A | N/A |
| CFBP8082 | *Xylella fastidiosa* | *fastidiosa* | 16.26 | 17.71 | 17.58 | 15.71 | 20.52 | 18.8 | N/A | N/A |
| ICMP15197 | *Xylella fastidiosa* | *fastidiosa* | 25.05 | 26.64 | 29.14 | 20.72 | 29.16 | 34 | N/A | N/A |
| ICMP8742 | *Xylella fastidiosa* | *fastidiosa* | 20.5 | 23.28 | 25.29 | 24.21 | 26.98 | 31.58 | N/A | N/A |
| ICMP8745 | *Xylella fastidiosa* | *fastidiosa* | 24.88 | 27.28 | 28.9 | 26.74 | 29.97 | 29.81 | N/A | N/A |
| NCPPB4432 | *Xylella fastidiosa* | *fastidiosa* | 17.11 | 17.65 | 18.32 | 17.38 | 22 | 20.26 | N/A | N/A |
| NCPPB4605 | *Xylella fastidiosa* | *fastidiosa* | 16.55 | 18.02 | 17.7 | 16.76 | 24.91 | 16.89 | N/A | N/A |
| CFBP8173 | *Xylella fastidiosa* | *multiplex* | 15.67 | 16.29 | 17.38 | 15.67 | 22.23 | 18.72 | N/A | N/A |
| ICMP8739 | *Xylella fastidiosa* | *multiplex* | 24 | 20.4 | 29 | 28.82 | 30.09 | 27.41 | N/A | N/A |
| ICMP8740 | *Xylella fastidiosa* | *multiplex* | 30.99 | 23.47 | 26.97 | 19.79 | 28.86 | 31.38 | N/A | N/A |
| NCPPB4604 | *Xylella fastidiosa* | *multiplex* | 17.24 | 18.46 | 18.83 | 15.72 | 28.39 | 20.47 | N/A | N/A |
| CFBP8072 | *Xylella fastidiosa* | *pauca* | 17.71 | 19.29 | 18.33 | 20.12 | 21.27 | 20.13 | N/A | N/A |
| CFBP8073 | *Xylella fastidiosa* | *pauca* | 16.54 | 17.06 | 18.14 | 14.92 | 21.72 | 20.73 | N/A | N/A |
| CFBP8477 | *Xylella fastidiosa* | *pauca* | 16.83 | 18.21 | 17.9 | 15.49 | 22.01 | 20.29 | N/A | N/A |
| CFBP8495 | *Xylella fastidiosa* | *pauca* | 17.18 | 18.13 | 19.03 | 16.85 | 24.21 | 20.76 | N/A | N/A |
| CFBP8077 | *Xylella fastidiosa* | *sandyi* | 16.91 | 17.24 | 17.45 | 16.8 | 21.96 | 20.38 | N/A | N/A |
| CFBP8524 | *Xylella fastidiosa* | *sandyi* | 15.42 | 15.64 | 15.73 | 14.57 | 17.93 | 16.92 | N/A | N/A |

**Supplementary Table S2:** The qPCR machine and reagents used by the test performance study participants

|  |  |  |
| --- | --- | --- |
| **Participant** | **qPCR machine model** | **qPCR reagent** |
| **A** | QuantStudio 5  (Applied Biosystem; Catalog number: A34322) | Immolase  (Meridian Bioscience; Catalog number: BIO-21047) |
| **B** | QuantStudio 3  (Applied Biosystem; Catalog number: A28567) | GoTaq qPCR systems  (Promega; Catalog number: A6001) |
| **C** | CFX96  (Biorad; Catalog number: 1854095) | Platinum Quantitative PCR SuperMix-UDG  (ThermoFisher; Catalog number: 11730017) |
| **D** | QuantStudio 5  (Applied Biosystem; Catalog number: A34322) | Immolase  (Meridian Bioscience; Catalog number: BIO-21047) |
| **E** | Rotor-Gene Q  (Qiagen; Catalog number: 9001862) | QuantiNova  (Qiagen; Catalog number: 208456) |

**Supplementary Table S3:** The amplification efficiency (%) of X-*ComEC* generic qPCR as compared with the published assay. N/A = not available because there were fewer than three valid detections across the dilution series.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Samples** | **Participant** | **X-ComEC generic qPCR (This study)** | **Harper Xf qPCR** | **Ouyang Xf qPCR** | **Dupas Xf qPCR** |
| ICMP 8731  *Xylella fastidiosa* subsp *fastidiosa* | A | 134.62 | 89.57 | 80.47 | 70.83 |
| B | 93.07 | 70.83 | 89.57 | 93.07 |
| C | 121.22 | 105.35 | N/A | 121.22 |
| D | 93.07 | 105.35 | 89.57 | 100.92 |
| E | 86.32 | 83.3 | 105.35 | 86.32 |
| ICMP 8739  *Xylella fastidiosa* subsp *multiplex* | A | 80.47 | 89.57 | 83.3 | 75.35 |
| B | 93.07 | 93.07 | 93.07 | 93.07 |
| C | 83.3 | 93.07 | N/A | 80.47 |
| D | 105.35 | 96.84 | 86.32 | 127.58 |
| E | 93.07 | 93.07 | 86.32 | 89.57 |
| CFBP8072 *Xylella fastidiosa* subsp *pauca* | A | 100.92 | 93.07 | 93.07 | 54.41 |
| B | 75.35 | 93.07 | 80.47 | 83.3 |
| C | 96.84 | 105.35 | N/A | 80.47 |
| D | 86.32 | 100.92 | N/A | 100.92 |
| E | 93.07 | 100.92 | 96.84 | 100.92 |
| NCPPB 4612 *Xylella taiwanensis* | A | 134.62 | N/A | N/A | N/A |
| B | 93.07 | N/A | N/A | N/A |
| C | 80.47 | N/A | N/A | N/A |
| D | 86.32 | N/A | N/A | N/A |
| E | 89.57 | N/A | N/A | N/A |

**Supplementary Table S4:** Results of inter-laboratory diagnostic sensitivity and limit of detection testing for the Xf (Harper) / Xt (X-ComEC) duplex qPCR assay. Mean Cq value ± Standard error <number of positive detection> is shown. nd = not detected

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Isolate** | **Concentration (ng/µl)** | **Participant A** | | **Participant B\*** | | **Participant C** | | **Participant D** | | **Participant E** | |
| **Harper XF-P** | **qPCR-Xt-*ComEC*-P** | **Harper XF-P** | **qPCR-Xt-*ComEC*-P** | **Harper XF-P** | **qPCR-Xt-*ComEC*-P** | **Harper XF-P** | **qPCR-*Xt-ComEC*-P** | **Harper XF-P** | **qPCR-*Xt-ComEC*-P** |
| ICMP 8731  *Xylella fastidiosa* subsp *fastidiosa* | 2.40E-01 | 23.83 ± 1.15 <3> | nd | 17.03 ± 1.08 <3> | nd | 27.38 ± 0.33 <3> | nd | 24.63 ± 0.15 <3> | nd | 22.00± 0.04 <3> | nd |
| 2.40E-02 | 28.57 ± 0.18 <3> | nd | 20.57 ± 0.13 <3> | nd | 31.88 ± 1.95 <3> | nd | 27.97 ± 0.15 <3> | nd | 26.41± 0.07 <3> | nd |
| 2.40E-03 | 32.76 ± 0.89 <3> | nd | 23.66 ± 0.51 <3> | nd | 34.4 ± 0.33 <3> | nd | 31.97 ± 0.64 <3> | nd | 30.21± 0.10 <3> | nd |
| 2.40E-04 | 35.84 ± 1.29 <2> | nd | 27.86 ± 1.04 <3> | nd | 38.02 ± 2.11 <3> | nd | nd | nd | 33.92± 0.26 <3> | nd |
| 2.40E-05 | nd | nd | 31.11 ± 0.89 <3> | nd | nd | nd | nd | nd | nd | nd |
| 2.40E-06 | 35.49 ± 0.91 <3> | nd | 34.48 ± 0.94 <2> | nd | nd | nd | nd | nd | nd | nd |
| 2.40E-07 | nd | nd | 36.41 ± 0.72 <2> | nd | nd | nd | nd | nd | nd | nd |
| 2.40E-08 | nd | nd | 36.13 ± 0.23 <2> | nd | nd | nd | nd | nd | nd | nd |
| 2.40E-09 | 35.52 ± 0.17 <2> | nd | 36.01 ± 1.74 <2> | nd | nd | nd | nd | nd | nd | nd |
| 2.40E-10 | nd | nd | 38.52 ± 0.41 <2> | nd | nd | nd | nd | nd | nd | nd |
| 2.40E-11 | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| 2.40E-12 | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| 2.40E-13 | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| 2.40E-14 | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| ICMP 8739  *Xylella fastidiosa* subsp *multiplex* | 2.40E-01 | 23.2 ± 1.01 <3> | nd | 21.65 ± 0.09 <3> | nd | 22.06 ± 1.29 <3> | nd | 21.4 ± 0.1 <3> | nd | 20.42± 0.05 <3> | nd |
| 2.40E-02 | 26.71 ± 0.12 <3> | nd | 25.89 ± 0.15 <3> | nd | 25.88 ± 1.45 <3> | nd | 25 ± 0.26 <3> | nd | 25.31± 0.17 <3> | nd |
| 2.40E-03 | 30.38 ± 1.88 <3> | nd | 29.5 ± 0.28 <3> | nd | 29.17 ± 1.56 <3> | nd | 28.6 ± 0.26 <3> | nd | 30.18± 0.16 <3> | nd |
| 2.40E-04 | 35.28 ± 1.03 <3> | nd | 33.41 ± 0.37 <3> | nd | 32.53 ± 2.1 <3> | nd | 32.9 ± 0.52 <3> | nd | 32.74± 0.38 <3> | nd |
| 2.40E-05 | nd | nd | 36.45 ± 1.94 <3> | nd | nd | nd | nd | nd | nd | nd |
| 2.40E-06 | nd | nd | 38.07 ± 0.07 <2> | nd | nd | nd | nd | nd | nd | nd |
| 2.40E-07 | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| 2.40E-08 | nd | nd | 38.12 ± 0.41 <2> | nd | nd | nd | nd | nd | nd | nd |
| 2.40E-09 | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| 2.40E-10 | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| 2.40E-11 | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| 2.40E-12 | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| 2.40E-13 | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| 2.40E-14 | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| CFBP8072 *Xylella fastidiosa* subsp  *pauca* | 2.40E-01 | 19.64 ± 0.01 <3> | nd | 21.63 ± 0.46 <3> | nd | 22.63 ± 0.57 <3> | nd | 21.27 ± 0.15 <3> | nd | 18.93± 0.19 <3> | nd |
| 2.40E-02 | 26.11 ± 2.1 <3> | nd | 26.32 ± 0.54 <3> | nd | 25.22 ± 0.56 <3> | nd | 25.03 ± 0.12 <3> | nd | 23.29± 0.04 <3> | nd |
| 2.40E-03 | 30.07 ± 0.33 <3> | nd | 31.14 ± 0.51 <3> | nd | 28.87 ± 0.25 <3> | nd | 29.6 ± 0.17 <3> | nd | 25.93± 0.04 <3> | nd |
| 2.40E-04 | 34.08 ± 0.14 <3> | nd | 32.77 ± 1.98 <3> | nd | 32.14 ± 1.21 <3> | nd | 34.4 ± 0.3 <3> | nd | 30.36± 0.14 <3> | nd |
| 2.40E-05 | nd | nd | nd | nd | nd | nd | 39.25 ± 0.35 <2> | nd | 34.10± 0.06 <3> | nd |
| 2.40E-06 | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| 2.40E-07 | nd | nd | 39.42 ± 0.05 <2> | nd | nd | nd | nd | nd | nd | nd |
| 2.40E-08 | nd | nd | 39.05 ± 0.07 <2> | nd | nd | nd | nd | nd | nd | nd |
| 2.40E-09 | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| 2.40E-10 | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| 2.40E-11 | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| 2.40E-12 | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| 2.40E-13 | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| 2.40E-14 | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| NCPPB 4612 *Xylella taiwanensis* | 2.40E-01 | nd | 18.56 ± 1.28 <3> | nd | 22.24 ± 0.07 <3> | nd | 17.98 ± 2.11 <3> | nd | 21 ± 0.1 <3> | nd | 20.35± 0.06 <3> |
| 2.40E-02 | nd | 25.74 ± 1.32 <2> | nd | 26.77 ± 0.07 <3> | nd | 25.01 ± 1.45 <3> | nd | 25.3 ± 0.1 <3> | nd | 23.72± 0.52 <3> |
| 2.40E-03 | nd | 30.75 ± 0.08 <3> | 32.92 ± 0.69 <3> | 33.19 ± 0.07 <3> | nd | 30.37 ± 2.17 <3> | nd | 28.83 ± 0.45 <3> | nd | 27.97± 0.08 <3> |
| 2.40E-04 | nd | nd | nd | 37.05 ± 0.4 <3> | nd | nd | nd | 33.67 ± 0.55 <3> | nd | 31.70± 0.12 <3> |
| 2.40E-05 | nd | nd | nd | nd | nd | nd | nd | nd | nd | 35.74± 0.43 <3> |
| 2.40E-06 | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| 2.40E-07 | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| 2.40E-08 | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| 2.40E-09 | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| 2.40E-10 | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| 2.40E-11 | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| 2.40E-12 | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| 2.40E-13 | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| 2.40E-14 | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |

\* Participant B had performed their testing on the qPCR-Xt-*ComEC*-P probe using a different fluorophore (VIC) than other participants (Cy5) due to incompatibility with their qPCR machine.

**Supplementary Table S5:** Results of independent follow-up validation on *Xylella taiwanensis* detection ability of X-*ComEC* generic qPCR and the published assays using freshly prepared *Xylella taiwanensis* DNA extract. SE = standard error; N/A = not available/applicable; nd = not detected.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | **X-ComEC generic qPCR** | | **Dupas Xf qPCR** | | **Harper Xf qPCR** | | **Ouyang Xf qPCR** | |
| **Isolate** | **Concentration (ng/µl)** | **mean Cq** | **Cq SE** | **mean Cq** | **Cq SE** | **mean Cq** | **Cq SE** | **mean Cq** | **Cq SE** |
| NCPPB 4612  *Xylella taiwanensis* | 2.40E-01 | 23.44 | 0.06 | nd | N/A | nd | N/A | nd | N/A |
| 2.40E-02 | 26.84 | 0.15 | nd | N/A | nd | N/A | nd | N/A |
| 2.40E-03 | 30.44 | 0.11 | nd | N/A | nd | N/A | nd | N/A |
| 2.40E-04 | 33.90 | 0.23 | nd | N/A | nd | N/A | nd | N/A |
| 2.40E-05 | nd | N/A | nd | N/A | nd | N/A | nd | N/A |
| 2.40E-06 | nd | N/A | nd | N/A | nd | N/A | nd | N/A |
| 2.40E-07 | nd | N/A | nd | N/A | nd | N/A | nd | N/A |
| 2.40E-08 | nd | N/A | nd | N/A | nd | N/A | nd | N/A |
| 2.40E-09 | nd | N/A | nd | N/A | nd | N/A | nd | N/A |
| 2.40E-10 | nd | N/A | nd | N/A | nd | N/A | nd | N/A |
| 2.40E-11 | nd | N/A | nd | N/A | nd | N/A | nd | N/A |
| 2.40E-12 | nd | N/A | nd | N/A | nd | N/A | nd | N/A |
| 2.40E-13 | nd | N/A | nd | N/A | nd | N/A | nd | N/A |
| 2.40E-14 | nd | N/A | nd | N/A | nd | N/A | nd | N/A |
| ICMP 8731  *Xylella fastidiosa*  (positive control) | N/A | 11.72 | 0.35 | 19.63 | 0.21 | 15.48 | 0.05 | 14.80 | 0.35 |