

Information pest: *Xylella fastidiosa*

Xylella fastidiosa is a regulated plant pathogen in many parts of the world and was included in European legislation as a quarantine pest.

Xylella fastidiosa is a Gram-negative bacterium which causes many important plant diseases such as Pierce's disease in grapevine, citrus veinal chlorosis, almond leaf scorch, phony peach and leaf scorch on a large range of ornamental plants and shade trees. Leafhoppers of the subfamily *Cicadellinae* and family *Cercopidae* (*Hemiptera*) are the most common known vectors.

Xylella fastidiosa was first described by Newton Pierce in 1892 on grapevine in USA. Until recently, it was mainly distributed throughout the Americas but there have now been reports of outbreaks in Asia and Europe.

Introduction

The qPCR *Xylella fastidiosa* set has been developed by Qualiplante based on Harper and al., 2010, Erratum 2013. A verification was performed by Qualiplante (data not published) and the performance characteristics of the set are the same as the original publication.

The *X. fastidiosa* target sequence is located in the gene coding for the 16S rRNA-processing *RimM* protein. The Internal Control (IC) has been designed on the COX gene.

The qPCR *Xylella fastidiosa* set enables the detection of all the 4 main subspecies of *Xylella fastidiosa*:

- *X. fastidiosa* subsp. *fastidiosa*,
- *X. fastidiosa* subsp. *multiplex*,
- *X. fastidiosa* subsp. *sandyi*,
- and *X. fastidiosa* subsp. *pauca*.

without any distinction, by Real-Time PCR.

No cross reactions were observed from other bacterial species as *Xanthomonas axonopodis* pv. *aurantifolii*, *X. campestris* pv. *citri*, *X. arboricola* pv. *fragariae*, *Pseudomonas syringae* pv. *persicae*, *Pantoea agglomerans*, *Agrobacterium tumefaciens* and *Spiroplasma citri*.

The *X. fastidiosa* specific probe is labelled with FAM® fluorophore. An internal control (IC) specific probe, labelled with Cy5® fluorophore, permits user to validate the DNA extraction step from the plant.

The real-time test of Harper et al., 2010, Erratum 2013 is recommended by the European and Mediterranean Plant Protection Organization (www.eppo.int) - PM7/24, Bulletin (2019) 49 (2), 175–227.

This product should be used only for research purposes.

Intended use

The qPCR set is validated for the simultaneous detection of *Xylella fastidiosa* (Xfast) and internal control (IC) in Real-Time PCR. Suitable tissues are plant petioles and central veins, non-woody branches

or stems, vascular tissues of woody-branches (xylem).

If the sample is symptomatic, sample organs with leaf burns and/or chlorosis and/or desiccants.

- for plant species with large petioles (e.g., coffee trees, mulberry trees, grapevines...), sampling is carried out on different leaves, branches or stems (minimum 5 leaves).
- for plant species with small petioles (ex: polygales, olive trees...) or without petiole and with small central vein (ex: rosemary...), sampling is carried out on different leaves, branches or stems (minimum 25 leaves).

During the period from March to July (in the European area), **in the absence of symptoms**, for *Prunus spp.* with deciduous leaves, it is recommended to collect the vascular tissues of woody branches (xylem), by removing the bark with a scalpel.

Set format and content

Two sets are available for 24 and 96 tests.

Article N°	Product name
7Xfastq2	qPCR <i>Xylella fastidiosa</i> set 24
7Xfastq9	qPCR <i>Xylella fastidiosa</i> set 96

Content	set 24	set 96
Direct Master Mix	24 tests 7Xfastq2-DM-	2x48 tests 7Xfastq9-DM-
Positive Control	3 tests 7Xfasq2-PC-	8 tests 7Xfasq9-PC-
Negative Control	3 tests 7Xfasq2-NC-	8 tests 7Xfasq9-NC-

Storage conditions

This set can be shipped at room temperature but upon receipt it should be stored immediately at the recommended storage temperature: **from -30 ° C to -10 ° C**.

Avoid prolonged exposure to light and repeated freeze and thaw cycles.

Shelf life

If the set is correctly stored, at constant-temperature freezer, its performance is guaranteed until the expiration date indicated on the tubes label.

Materials and equipment (not provided)

- DNA extraction tools and reagents
- Nuclease-free filter tips and micropipettes
- Optical grade nuclease-free tubes/plate
- Disposable latex or vinyl gloves
- Thermal-cycler for Real-Time PCR with filters calibrated for FAM® and Cy5®

Nucleic acids extraction

Extract DNA from samples according to your usual protocol. Upon request, Qualiplante can recommend you an extraction method.

Reaction set-up

- Slowly thaw **Direct Master Mix** by placing it on ice or at 4°C.
- Shake briefly **Direct Master Mix** and spin down the liquid.
- Add 18 µl of **Direct Master Mix** (without DNA template) to each PCR tubes or wells of an optical-grade PCR plate.
- Add 2 µl of DNA template to the **Direct Master Mix**. Do not forget to prepare a PCR tube or well of an optical-grade PCR plate for the **Positive Control** and the **Negative Control**.

Components	Volume/PCR tube or well
DNA template or Positive control or Negative control	2 µl
Direct Master Mix	18 µl
Total Volume / PCR tube or well	20 µl

In order to confirm the absence of any reagent's contamination, we strongly recommend including a no-template control (e.g. DEPC water) in the assay.

Run and thermal cycling

- Seal carefully the PCR tubes or PCR plate. Centrifuge briefly to collect components at the bottom of the PCR tubes or wells of the plate. Protect from light before thermocycling.
- Load the PCR tubes or plate into the thermal-cycler and follow the thermal cycling below:

Steps	Temp (°C)	Time	Cycle(s)
Enzyme activation	95°C	12 min	1
Denaturation	95°C	15 sec	40
Annealing and elongation	62°C	45 sec	

Results analysis

The reaction for *Xylella fastidiosa* will generate a specific FAM[®]-labeled amplification curve. The reaction for Internal Control will generate a specific Cy5[®]-labeled amplification curve in all the wells except those that contains the no-template control.

Fig.1: Example of an amplification curve relative to a *Xylella fastidiosa* positive sample.

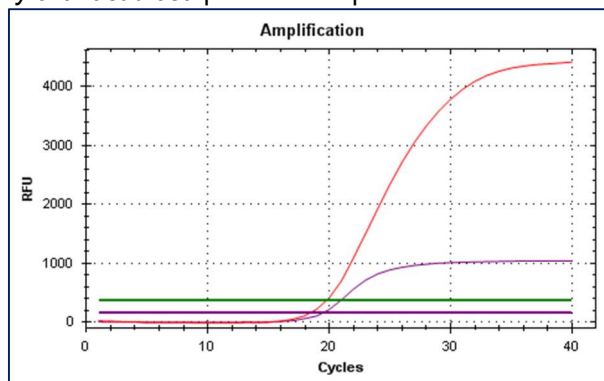


fig.1 shows the amplification curves associated to a *Xylella fastidiosa*-infected sample or **Positive Control** (red curve) and the relative **Internal Control** (violet curve).

Fig.2: Example of an amplification curve relative to a healthy olive tree sample.

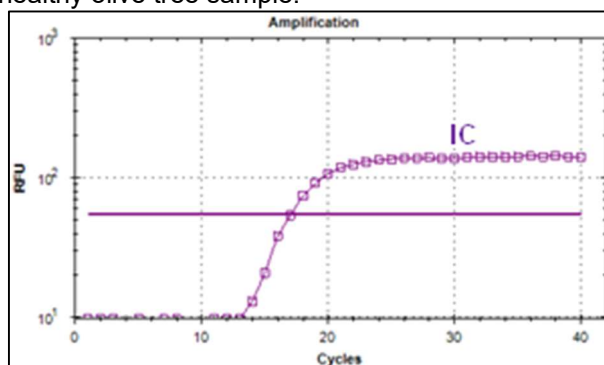


fig.2 shows the amplification curve associated to a healthy sample or the **Negative Control** (violet curve). Only the **Internal Control** curve must appear (violet curve).

ANALYSIS VALIDATION

The PCR plate is validated only when:

- ✓ the **Positive Control** generates an amplification curve for the two fluorophores FAM[®] and Cy5[®]. The Cycle threshold (Ct) value of the FAM[®]-labeled amplification curve should be below or equal to 35 (**fig.1**).
- ✓ the **Negative Control** does not generate any curve associated to the fluorophore FAM[®] but it generates a curve associated to the fluorophore Cy5[®] (**fig. 2**).
- ✓ the amplification plot associated to the IC is present in all the wells except in the no-template control^(*).
- ✓ the no-template control does not generate any curve for the two fluorophores FAM[®] and Cy5[®] ^(*).

^(*) An amplification curve may sometimes appear for the fluorophore Cy5[®] in the no-template control. In this case, the experiment is validated only if the Ct value is higher than 28.

RESULTS INTERPRETATION

When all the previous conditions are performed, the amplification results are interpreted as indicated in the **tab. 1**, by considering for each sample the Ct of the

curve generated by the FAM[®] fluorophore specific to *Xylella fastidiosa* and the Cy5[®] fluorophore specific to the IC.

The columns of the **tab. 1** refer to the Ct of the IC amplification and the lines refer to the Ct of *Xylella fastidiosa* amplification.

FAM fluorophore	Cy5 fluorophore	
	Ct IC < 25	Ct IC ≥ 25
Ct ≤ 35	Positive	Positive
35 < Ct ≤ 38	Indeterminate (*)	Unreliable (**)
Ct > 38	Negative	Unreliable (**)
No Ct	Negative	Unreliable (**)

tab.1 shows the results interpretation

(*) The concentration of bacteria could be very low in this sample, approaching the limit of detection. The results may not be reproducible.

(**) Some inhibitors of the *Taq* polymerase might be present in this sample. Repeat the analysis by diluting the DNA of this sample to 1:5 or to 1:10. If the Ct value of IC is still ≥ 25 after repeating the analysis on diluted sample, the DNA extraction must be repeated.

Special handling instructions

This set was designed to be used by laboratory staff trained to follow the usual molecular biology precautions. Always perform the tests in a nuclease-free work environment. Always wear gloves when handling samples containing DNA and the components of the set. Do not touch any set components with an ungloved hand. Use appropriate laboratory disposable parts. Use nuclease-free tubes and filter tips to avoid degradation and cross-contamination. Do not use components from sets with different batch numbers in the same test procedure. Do not interchange reagents with other sets. To avoid cross-contamination, use separate rooms for (a) nucleic acids extraction, (b) preparation of the Master Mix and (c) amplification. To avoid cross-contamination and obtain reliable results, it is essential to strictly follow the protocol in this manual. Avoid unnecessary freeze-thaw cycles of the set components. Do not use reagents after their expiration date.

Warranty and Responsibilities

Qualiplante SAS guarantees the buyer exclusively concerning the quality of reagents and of the components used to produce the Sets. Any product not fulfilling the specifications included in the product sheet will be replaced. This warranty limits Qualiplante SAS responsibility to the replacement of the product. No other warranties, of any kind, express or implied-are provided by Qualiplante SAS.

Qualiplante SAS is not responsible and cannot anyway be considered responsible or jointly responsible for possible direct and indirect damages resulting of the use and/or the misuses of the Sets. The user consciously and under her/his own responsibilities decides for the utilization purposes of the Sets and uses it the way she/he considers most suitable in order to reach her/his goals and/or objectives. Qualiplante SAS is not responsible for the data resulting from the use of the Sets, for the utilization that the user independently decides to make of them or for the direct or indirect damages possibly resulting from the disclosure or transmission of the data themselves to third parties under any form or circumstance. This clause is automatically accepted by the user when purchasing the Sets. Some of the applications which may be performed with this product may be covered by applicable patents in certain countries. The purchase of this product does not include or provide a license to perform patented applications. Users may be required to obtain a license depending on the country and/or application. Qualiplante SAS does not encourage the unlicensed use of patented applications. The Sets may require the use of *Taq* Polymerase enzyme, DNA binding components and fluorochromes/quencher, often registered as trademark by companies. The product, equipment and information included in the Sets consist of assembled reagents. The Sets are designed for the services supply, quality control or any other application that is not exclusively an internal company's research and requires a specific license for PCR and Real-Time PCR use. The license and authorization for PCR and Real-Time PCR use are not included in the Sets. The user is responsible for setting prefixed goals, choosing whether or not to perform the PCR or Real-Time PCR reaction and to apply for register her/his own license.

The Sets have been internally tested by our quality control. Any responsibility is waived if the warranty of quality control does not refer to the specific Sets. The user is personally responsible for data that she/he will obtain and/or she/he will supply to third parties using these Sets. Once the sealed package is opened the user accepts all the conditions without fail; if the package is still sealed the set can be returned and the user can be refunded.

Sets components are intended, developed, designed, and sold for Research Purpose Only. Product claims are subject to change.

Troubleshooting

Post-PCR data analysis shows no amplification, or amplification plots look grossly abnormal:

Possible causes	Corrective actions
Evaporation of the sample due to inadequate sealing of the plate	Repeat the test using the appropriate tools to seal correctly the plate
Consumables are not appropriate for the method	Repeat the test using consumables recommended by the thermal cycler supplier
The quality of nucleic acid extracted is low	Repeat the extraction step. Ensure that the method of extraction has been performed correctly. In any doubt, contact us
Abnormal amplification	Centrifuge the plate briefly to spin down the contents and eliminate any air bubbles

No amplification reaction is observed in the positive control well, while other samples are positive:

Possible causes	Corrective actions
The positive control provided with the set was not added into the reaction well	Repeat the test. If the problem persists, contact us

An amplification plot is observed in the negative control well:

Possible causes	Corrective actions
Contamination of the negative control or the Master Mix with target-positive nucleic acid	Repeat the test by applying appropriate quality procedures to prevent contamination. Seal the plate correctly