

**First International External Quality Assessment studies for
laboratory performance of molecular and serological diagnosis of *Xylella fastidiosa***

PRELIMINARY INFORMATION

Objective: The aim of this quality assessment study is to assess the efficiency and accurateness of molecular and serological methods used by expert laboratories performing *X. fastidiosa* diagnosis.

Experimental materials: Xf-free cuttings of olives, oleander and *Polygala myrtifolia* will be shipped to each laboratory. In addition, pre-spotted whatman Chr-3MM discs will be provided for preparing the artificial Xf-contaminated samples. The spotted paper discs (3mm in diameter) will contain heat-inactivated bacterial suspension of *X. fastidiosa* subsp. *pauca* strain CoDiRO. Instructions will be given to prepare for each plant matrice a bacterial serial dilution. A panel of 24 samples will be tested for each selected diagnostic method, with serial dilutions containing from 10^6 to 10^4 CFU/ml.

Procedure for sample processing: following the EPPO procedures at least 0.5-1g of leaf petioles should be homogenized in the proper buffer according to the different diagnostic protocols to be followed. Homex 6 (Bioreba) is one of the homogenizer commonly used for this purpose. Once the plant sap is obtained, the pre-spotted disc will be added for preparing the artificial infected sample.

Molecular tests: extraction of plant DNA, qPCR and PCR will be performed following the recommended EPPO procedures.

Serological test: ELISA will be performed using the kits commercially available.

REAGENTS THAT EACH LABORATORY NEEDS TO HAVE AVAILABLE

DNA EXTRACTION

I. PROTOCOL A

CTAB BUFFER

2% CTAB (Hexadecyl trimethyl-ammonium bromide) (any vendor)

Autoclaved 0.1M TrisHCl PH 8 (any vendor)

Autoclaved 20mM EDTA (any vendor)

Autoclaved 1.4M NaCl (any vendor)

Adjust PH to 8.0.

Additional Materials and Reagents

1. Microcentrifuge tubes 1,5-2ml (pre-sterilized, certified DNase & RNase free, any vendor)
2. Sterile filter (barrier) pipette tips (P10,P200, P1000, any vendor)
3. Molecular grade water (any vendor)
4. Ethanol (96-100%, any vendor)
5. Isopropanol (any vendor)
6. Chloroform (any vendor)

II. PROTOCOL B

DNeasy® mericon™ Food Standard Protocol (69514 - Qiagen)

Additional Materials and Reagents

1. Chloroform (any vendor)
2. Microcentrifuge tubes 1,5-2ml (pre-sterilized, certified DNase & RNase free, any vendor)
3. Sterile filter (barrier) pipette tips (P10,P200, P1000, any vendor)

III. PROTOCOL C

QuickPick™ SML Plant DNA Kit-based extraction (Bio-Nobile)

53022 QuickPick SML Plant DNA Kit, 96 Preps

24196 QuicPick Tips, 96 boxed, untreated

Additional Materials and Reagents

1. Microcentrifuge tubes 1,5ml (pre-sterilized, certified DNase & RNase free, any vendor)
2. Sterile filter (barrier) pipette tips (P10, P200, P1000, any vendor)
3. Magnet Pipet or automatized platform (BioSprint 15 Qiagen or KingFisher mL or Flex Thermo Scientific)

REAL TIME PCR:

Harper *et al.*, 2010; erratum 2013

Primers:

XF-F (forward) 5'-CAC GGC TGG TAA CGG AAG A-3'

XF-R (reverse) 5'-GGG TTG CGT GGT GAA ATC AAG-3'

XF-P (probe) 5' 6FAM -TCG CAT CCC GTG GCT CAG TCC-BHQ-1- 3'

2x qPCR master mix: TaqMan™ Universal PCR Master Mix (Applied Biosystem) (Cod. 4364338) or from other vendors

CONVENTIONAL PCR Minsavage et al. (1994):

PCR master mix for conventional PCR Invitrogen or other vendor

Forward primer RST31 5'-GCG TTA ATT TTC GAA GTG ATT CGA TTG C-3'

Reverse primer RST33 5'-CAC CAT TCG TAT CCC GGT G-3'

REAGENTS FOR SEROLOGICAL TESTS

- ELISA

Commercial kits with validation data available:

- Agritest srl, *Xylella fastidiosa* Elisa – Cat. No. K-27B
- Loewe Biochemica GmbH-Germany, *Xylella fastidiosa* Cat. No. 07119S

APPENDIX:

EXAMPLE OF CUTTINGS THAT WILL BE SHIPPED (XF-FREE MATERIALS)



PAPER DISCS SPOTTED WITH HEAT-INACTIVATED BACTERIAL SUSPENSION

