Supplementary Materials

A Colorimetric LAMP Detection of *Xylella fastidiosa* in Crude Alkaline Sap of Olive Trees in Apulia as a Field-Based Tool for Disease Containment

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Supplementary Table S1: Optical density of the bacterial species used to assess the cLAMP assay specificity.

Supplementary Figure S1: Limit of detection of ddPCR by ten-fold dilution of plasmid DNA in the olive alkaline sap.

Supplementary Figure S2: Validation of cLAMP assay on field-grown olive samples by comparison with qPCR.

Supplementary Figure S3: Evaluation of ddPCR performance compared with qPCR.

Supplementary Figure S4: Comparison of the results obtained in cLAMP and ddPCR assays on infected olive alkaline crude extracts according the qPCR Cq values.

Supplementary Figure S5: Statistical analysis of data correlation (qPCR *vs* ddPCR).
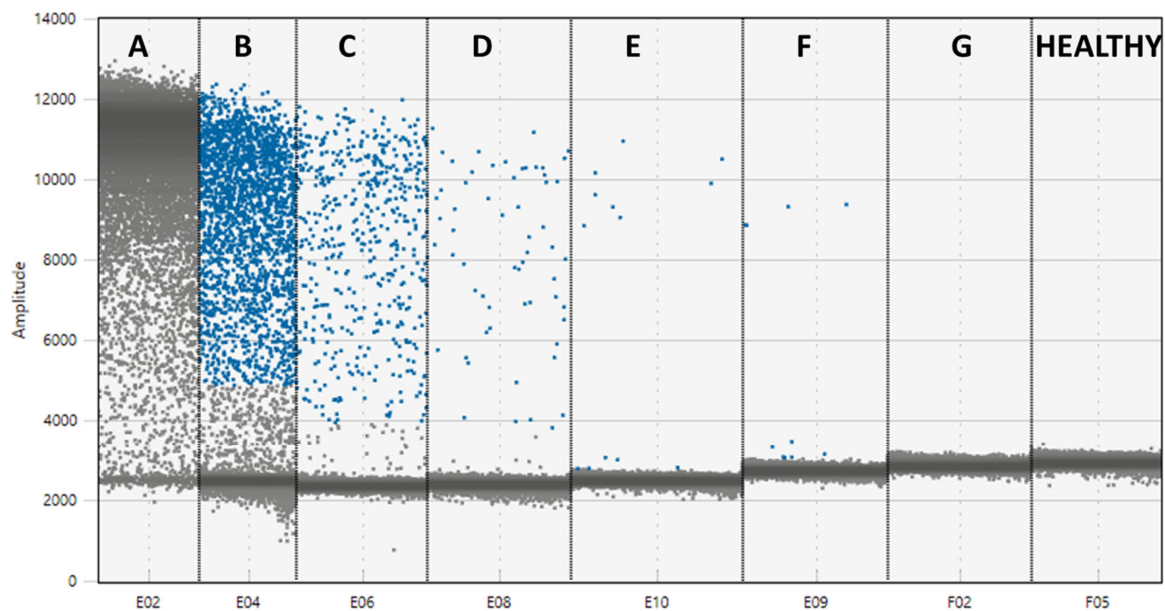
Supplementary Figure S6: Receiver Operating Characteristic (ROC) curves for qPCR, ddPCR and cLAMP.

Supplemetary Table S1: Optical density of the non-target bacterial species used to assess the cLAMP assay specificity.

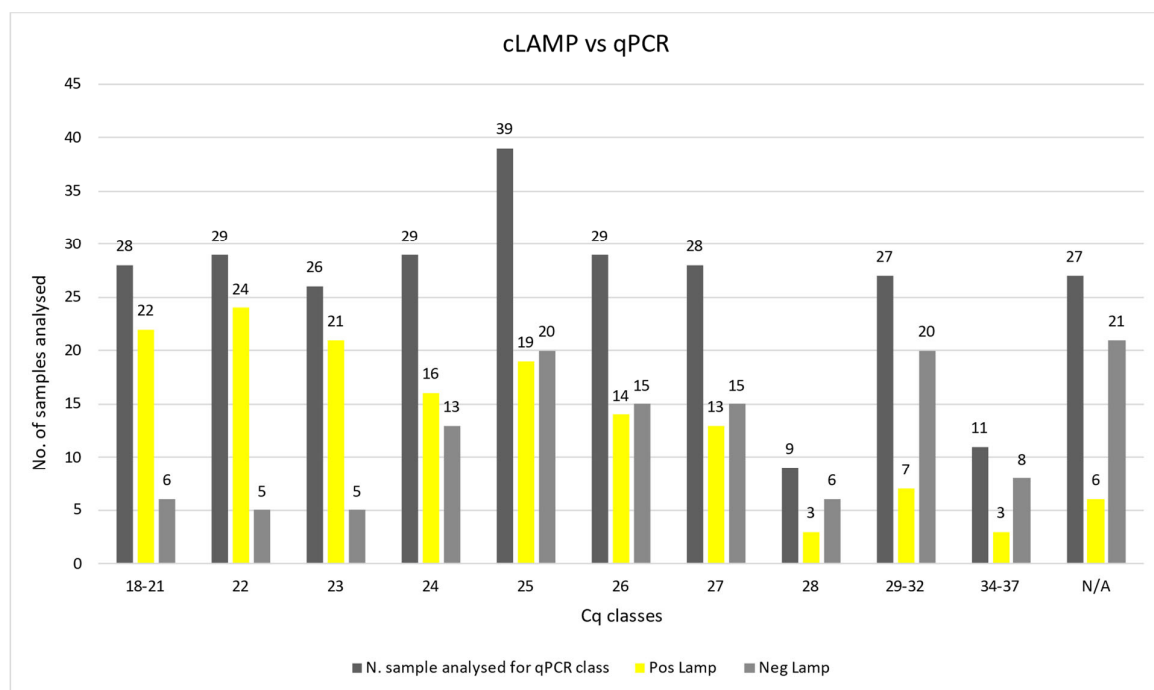| Bacterial specimens	OD$_{600}$ value
Xanthomonas campestris pv campestris	0.859
Pseudomonas marginalis	1.676
Paraburkholderia phytofirmans strain *PsJN*	1.062
Rahnella aquatilis	1.586
Escherichia coli strain *BL21(DE3)*	1.652

Supplementary Table S1. OD value at 600 nm (OD$_{600}$) of non-target bacterial suspensions used for testing the cLAMP primers specificity.

Supplementary Figure S1: Limit of detection of ddPCR by ten-fold dilution of plasmid DNA in the olive alkaline sap.
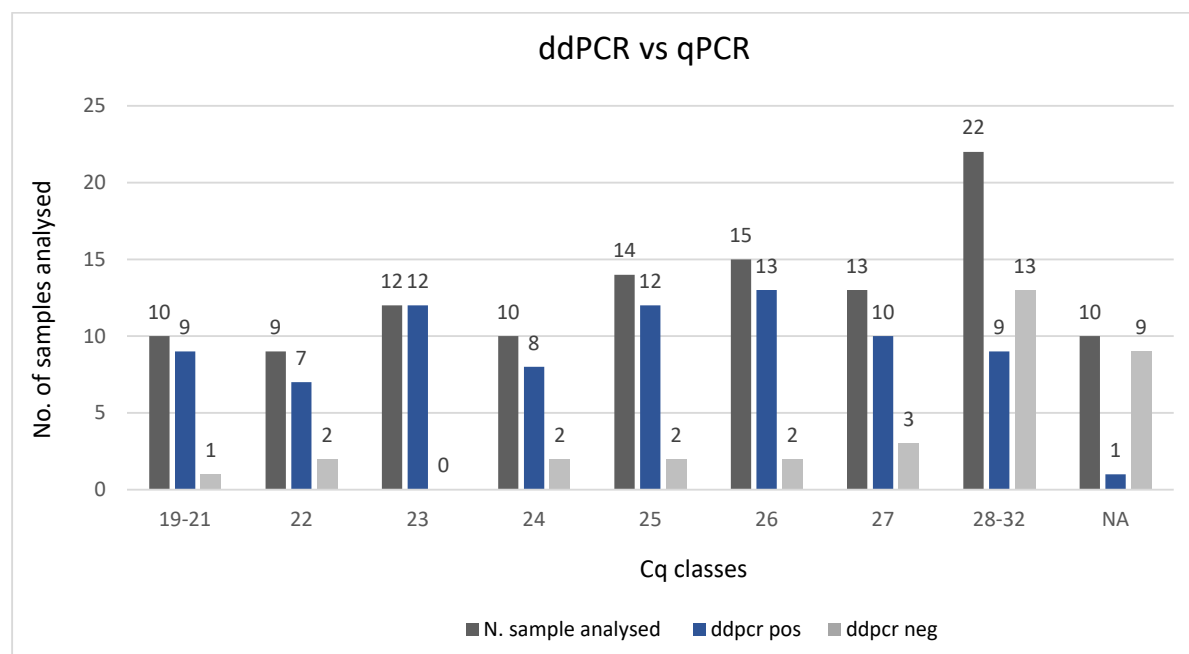

Supplementary Figure S1. Limit of detection (LOD) of *Xfp* obtained by ddPCR with plasmid DNA tenfold diluted in alkaline crude olive sap from from 9 ng/µL [lane A] to 9 fg/µL [lane G]. ddPCR optimized parameters are 600/300nM primer/probe concentration, 2 µL of crude extract and 45 end-point PCR cycles. Blue dots represent the positive amplification droplets above the horizontal threshold line. Grey dots represent the negative droplet background with no amplification. **Lane A**: The copious number of positive droplets saturated the fluorescence signal making Poisson algorithm invalid; **Lane G**: dilution with no positive events. **Healthy**: Negative Internal Control.

Supplementary Figure S2: Validation of cLAMP assay on field-grown olive samples by comparison with qPCR.
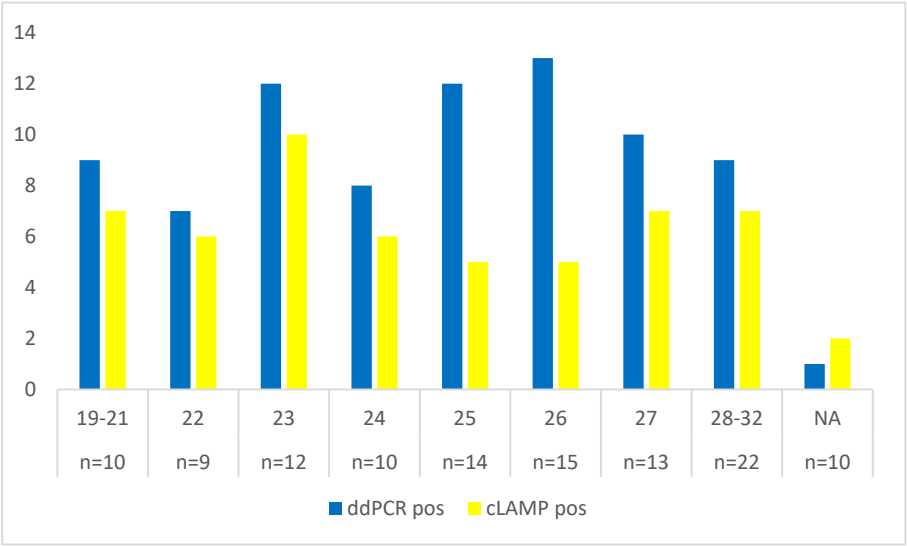

Supplementary Figure S2. Comparison of results of cLAMP and qPCR assays. The total number of olive samples tested in qPCR is in dark grey. The molecular template used for qPCR was purified DNA from each olive tree sample. Positive and negative olive samples in the cLAMP assay are reported in yellow and light grey columns, respectively. The template employed in cLAMP was alkaline crude sap.

Supplementary Figure S3: Evaluation of ddPCR performance compared with qPCR.
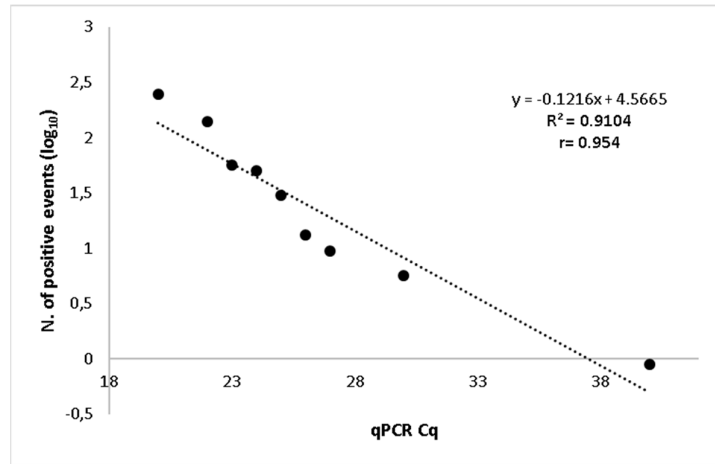

Supplementary Figure S3. Comparison of ddPCR and qPCR assays. The number of total olive samples tested in qPCR is indicated in dark grey. The template used in qPCR was made up of purified DNA. Positive and negative olive samples in ddPCR are indicated with blue and light grey columns, respectively. The templates employed in ddPCR reactions were the same alkaline extract tested in cLAMP.

Supplementary Figure S4: Comparison of the results obtained in cLAMP and ddPCR assays on infected olive alkaline crude extracts according to the qPCR Cq values.
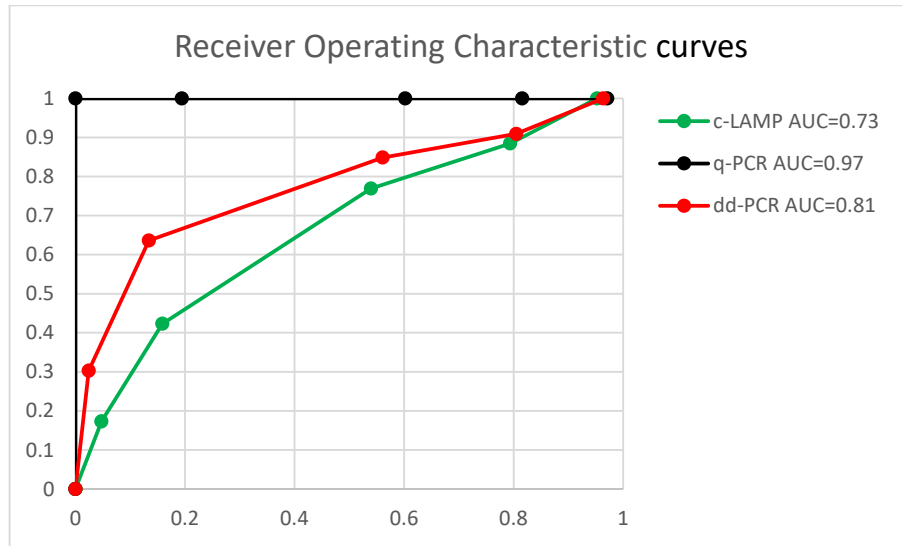

Supplementary Figure S4. Comparison of diagnostic parameters testing a subset of naturally infected olive trees. Total number of samples tested in qPCR, per each Cq range, are indicated below (n). Positive samples obtained in cLAMP (yellow column) and ddPCR (blue columns) assays are indicated.

Supplementary Figure S5: Statistical analysis of data correlation (qPCR *vs* ddPCR)

Supplementary Figure S5. Linear regression curve calculated plotting the $\log_{10}$-transformed average number of positive events (droplets) obtained in ddPCR (y-axis) on the Cq values resulting from qPCR (x-axis). The regression equation is reported together with its determination coefficient (R^2). A Pearson's correlation analysis was performed, whose coefficient (r), found to be significant at the 0.001 α-level (p-value < 0.001), is also reported. When Cq values were available as a bulk range, as in the case of 19-21 and 28-32, the mean values of the range, i.e. 20 and 30, respectively, were retained in the analyses.

Supplementary Figure S6: Receiver Operating Characteristic (ROC) curves for qPCR, ddPCR and cLAMP

Supplementary Figure S6. Receiver Operating Characteristic (ROC) curve analysis performed to evaluate the performance of the qPCR (black curve), ddPCR (red curve) and cLAMP (green curve) assays for the detection of *Xfp* in naturally infected olive trees. The resulting area under the curve (AUC) values were 0.73, 0.81 and 0.97 for cLAMP, ddPCR and qPCR, respectively, and are also reported. Sensitivity values (y axis) vs specificity ones (x axis) for each assay are plotted.