Real-time LAMP rapid diagnostic method for *X. fastidiosa* in plant material and insect vectors

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The Need

Ideal diagnostic test suitable for developing should be:

"Reliable"

- Affordable
- Sensitive
- Specific
- User-friendly
- Robust and rapid
- Equipment free
- Deliverable to the end user

LAMP (LOOP MEDIATED ISOTHERMAL AMPLIFICATION)

Nucleic Acids Research, 2000, Vol. 28, No. 12 e63

Loop-mediated isothermal amplification of DNA

Tsugunori Notomi[1,3,*], Hiroto Okayama[2], Harumi Masubuchi[1], Toshihiro Yonekawa[1], Keiko Watanabe[1], Nobuyuki Amino[3] and Tetsu Hase[1]

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- Originally reported by **Notomi *et al*** in 2000 of EIKEN Chemical Co. Ltd., Japan

(http://www.eiken.co.jp/en/)

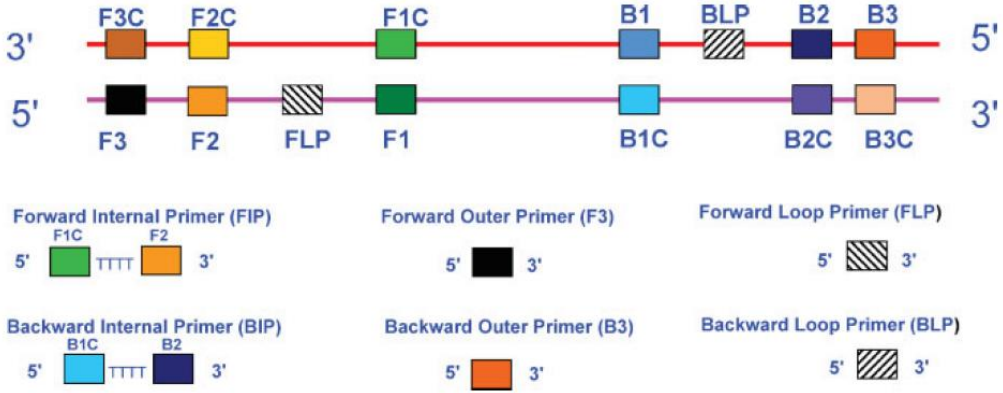
Eiken Chemical Co., Ltd.

Eiken GENOME SITE

- As of 17th May 2017, PubMed database has listed more than 1668 articles on this topic

Design of primers

4 primers based on the 6 distinct regions of the target gene: the F3c, F2c and F1c regions at the 3' side and the B1, B2 and B3 regions at the 5' side



(http://loopamp.eiken.co.jp/e/lamp/primer.html)

LAMP characteristics

- ***Bst DNA polymerase*** with strand displacement activity at 65℃
- No need for a step to denature double stranded into a single stranded form
- The amplification efficiency is extremely high
- Reduced total cost not require special reagents or sophisticated equipment

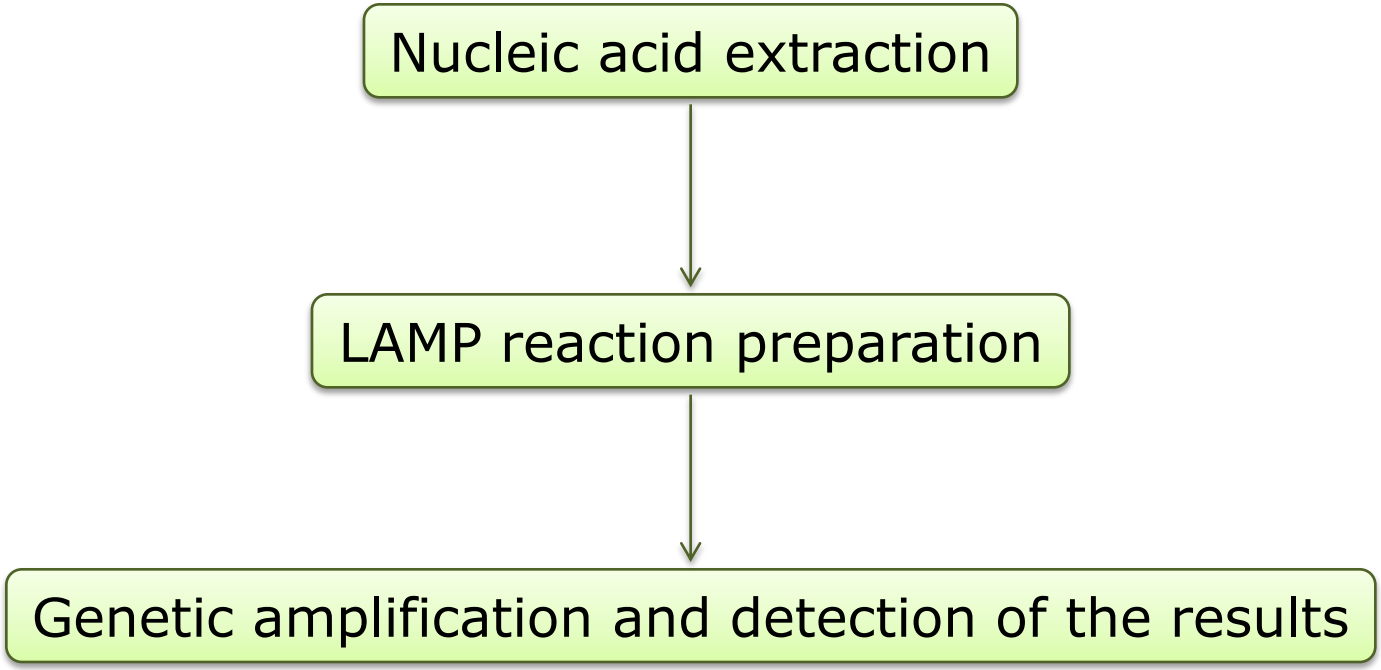
LAMP vs. PCR

- Amplification specificity is extremely high as LAMP requires 4/6 oligonucleotide primers that recognize 6/8 distinct regions on the target DNA
- Detection limit : LAMP $\geq$ PCR and RT-PCR
- Detection time : LAMP $<$ PCR and RT-PCR
- LAMP reaction: accelerated by two loop primers
- PCR reagent recommended storage temperature is -20^{o}C, LAMP reagents can be stored at 4° C and can shipped at ambient temperature.
- Crude DNA preparation can be used as LAMP template DNA.

Examples of LAMP application

Disease	Pathogen	References
Tomato and potato late blight	*Phytophthora infestans*	Hansen et al., 2016
Fusarium wilt of chickpea	*Fusarium oxysporum* f.sp. ciceris	Ghosh et al., 2015
Grape powdery Mildew	*Erysiphe necator*	Thiessen *et al.,* 2013
Fire blight	*Erwinia amylovora*	Temple and Johnson, 2011; Bühlmann *et al*., 2012; Moradi *et al.*, 2012
Citrus Bacterial Canker	*Xanthomonas spp.*	Rigano *et al*., 2010
Grey Mould	*Botrytis cinerea*	Tomlinson *et al*., 2010
Pierce's disease, citrus veinal chlorosis, almond leaf scorch, Olive Quick Decline	*Xylella fastidiosa*	Harper *et al.,* 2010 Yaseen *et al.*, 2015

Procedure


Nucleic acid extraction
LAMP reaction preparation
Genetic amplification and detection of the results

START THE APP

Sample cutting 4-5 olive leaf peduncle or transfer the insect from 95% alcohol tube to filter paper, leave them to dry for 5 minutes.

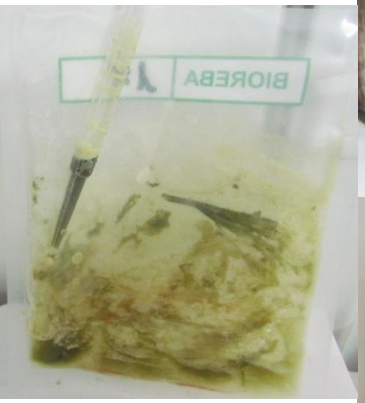
Insert the tube in icgene

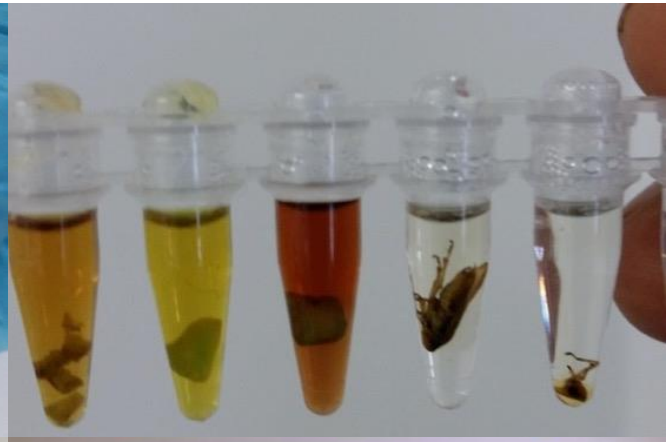
Run the extraction procedure for extraction 10’ at 65° C

Add 22,5ul of LAMP master mix to each primer mix tube + 30ul of mineral oil + 2,5ul of the extracted DNA of the first step

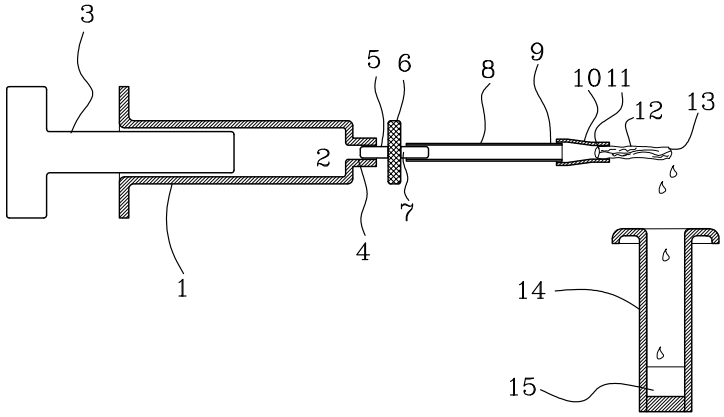
Run the amplification

SIMPLE AND RAPID DNA EXTRACTION FROM INSECT AND PLANT MATERIAL





CIHEAM/MAIB Patent number WO2017017555A1



(57) Abstract: Method and apparatus for the extraction of lymph from plant material, the method comprising: - preparing a sample (12) of plant material having a first (11) and a second (13) end, the ends being longitudinally spaced from each other and each having a predetermined cross section, - injecting the sample, through its first end (11), with a predetermined amount of extraction liquid and - collecting, at the second end (13) of the sample, the liquid exiting therefrom.

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SHORT NOTES

Identification of three potential insect vectors of *Xylella fastidiosa* in southern Italy

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DOI: 10.14601/Phytopathol_Mediterr-15250

RESEARCH PAPERS

On-site detection of *Xylella fastidiosa* in host plants and in "spy insects" using the real-time loop-mediated isothermal amplification method

Thaer YASEEN[1], Sandro DRAGO[2], Franco VALENTINI[1], Toufic ELBEAINO[1], Giuseppe STAMPONE[2], Michele DIGIARO[1] and Anna Maria D'ONGHIA[1]

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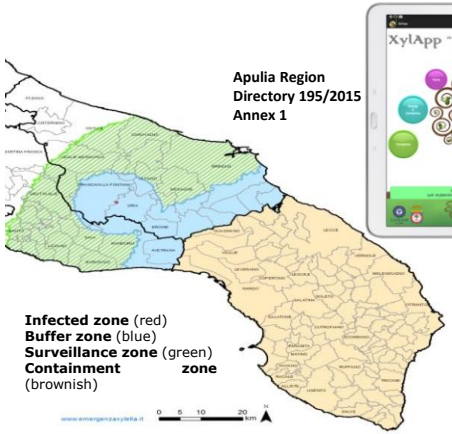
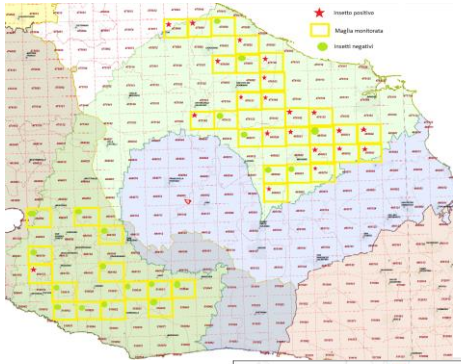
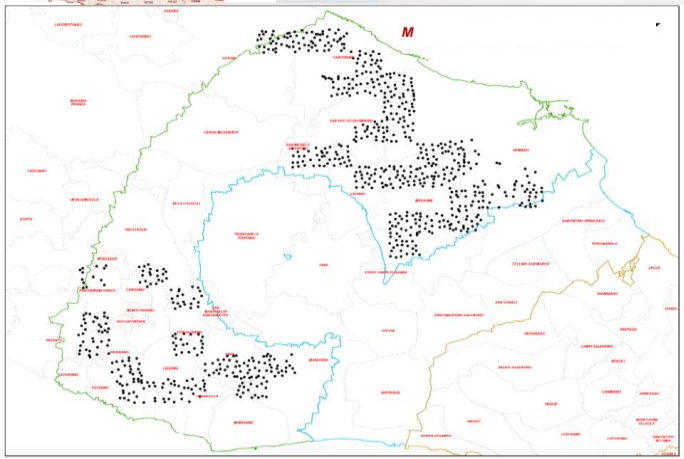
MONITORING OF XYLELLA FASTIDIOSA IN THE PATHOGEN-FREE AREA USING THE SPY INSECTS APPROACH

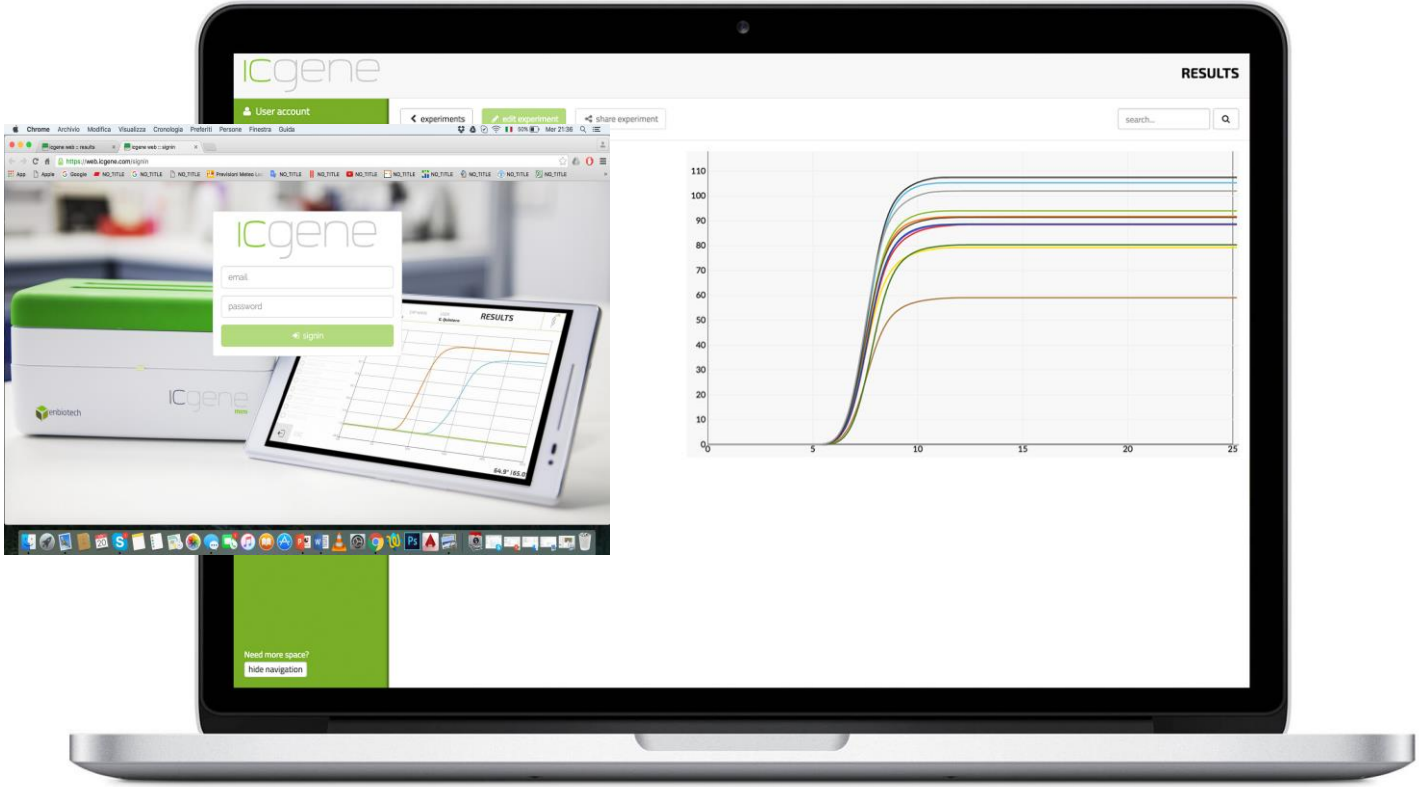




Poster N. 8.6

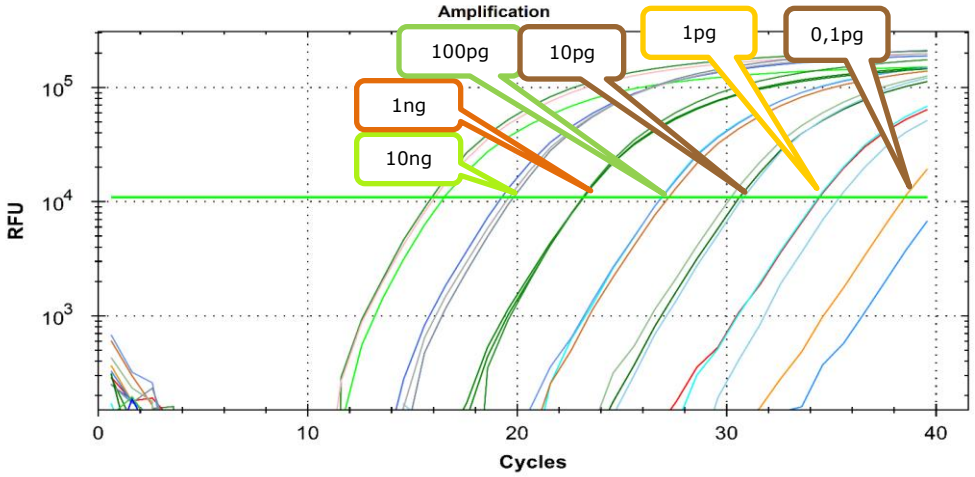


Possibility to send the results a real time to a server to collect the data and the all related information



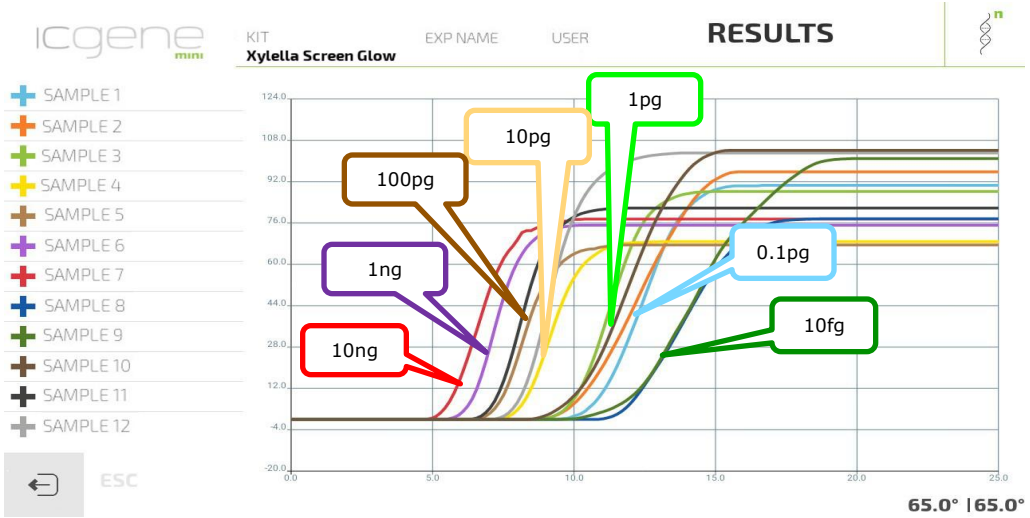
Sensetivity

RT-PCR



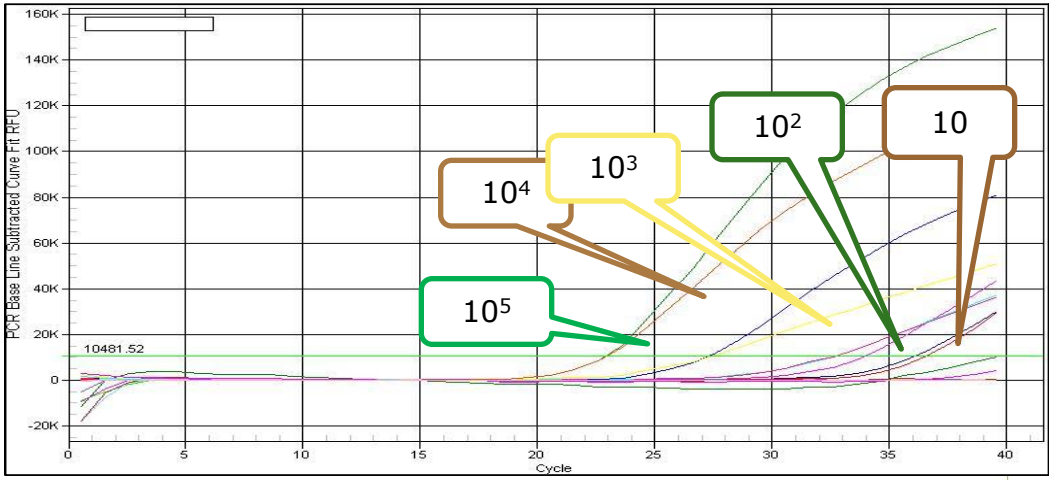
***X. fastidiosa* DNA diluted 1:10**

RT-LAMP

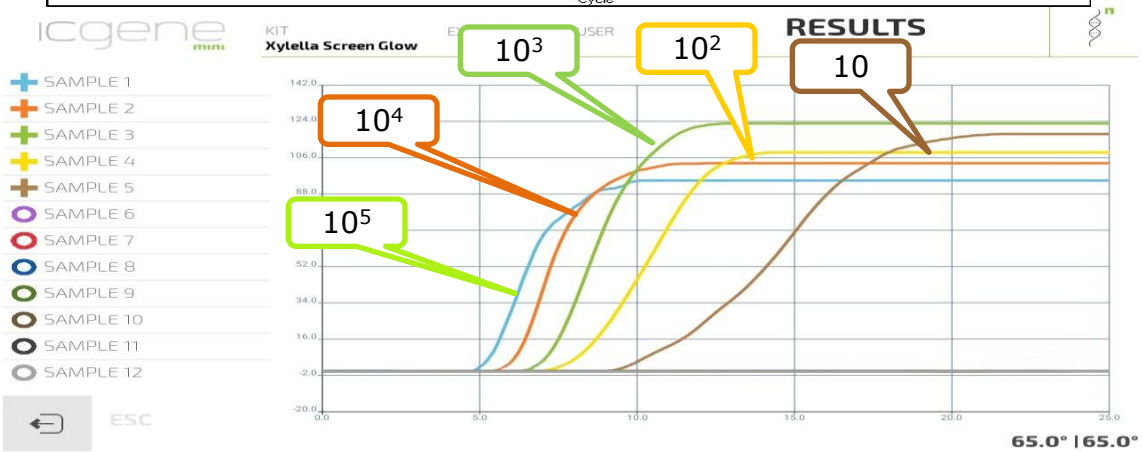


SERIAL DILUTION OF THE PATHOGEN FROM 10^5 UP TO 10 CELLS

RT-PCR



RT-LAMP



ANALYTICAL SENSITIVITY IN THE RING TEST

Technique	CONCENTRATION cfu/ml						
	10^7	10^6	10^5	10^4	10^3	10^2	10
ELISA (Agritest/Loewe)	√	√	√	√			
PCR RST31/33	√	√	√	√			
qPCR	√	√	√	√	√	√	
Lamp (enbiotech) on sap	√	√	√	√	√		

Serial dilutions prepared by spiking a bacterial suspension with an OD_{600} of 0.5, corresponding to ca. 10^8 CFU/ml, to get a panel of artificially contaminated samples ranging from 10^7 to 10 CFU/ml.

REAL TIME LAMP ADVANTAGE

- User friendly method, easy to handle, only a a simple portable equipment is required;
- Ready-to-use extraction system that allows for total DNA extraction in only a few minutes and without the use of sophisticated laboratory instruments
- The only method molecular can work with crud extract
- Very short time of execution (less than 40 min) including extraction
- Its cost is lower than qPCR or the conventional PCR
- More sensitive than qPCR
- Possibility to send the results a real time to a server to collect the data and the all related information
- The only efficient method for on-site detection Xf in the possible vectors and other spy insects which can harbor the bacterium.

REAL TIME LAMP ADVANTAGE

- Possibility of performing genetic tests directly on site
- Stable Kit at room temperature, transportation at room temperature and stored at +4° C
- Automatic interpretation of results

- We recommend the use of RT-LAMP method for insect vector monitoring in buffer and healthy area, as well as for quarantine cross borders control.

FAO REGIONAL TCP PROJECT
TCP/RAB/3601

Title: ***Strengthening preventive measures for the introduction and spread of Xylella fastidiosa– Olive Quick Decline Syndrome in NENA countries***

Launched in August 2016 in Tunis & still ongoing

BENEFICIARY COUNTRIES

Algeria

Egypt

Jordan

Libya

Lebanon

Morocco

Palestine

Syria

Tunisia

CIHEAM

Thank you

efsa European Food Safety Authority

UIB Universitat de les Illes Balears

European Commission

PONTE Pest Organisms Threatening Europe

XF ACTORS

Euphresco