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Xylella fastidiosa diagnostic methods used @ NIB

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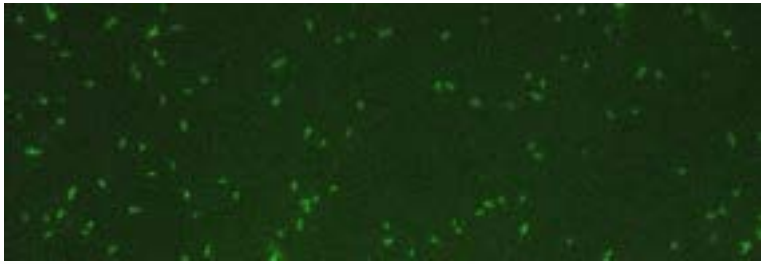
Past activities

- *Targeted reserach project on grapevine diseases*
  - *Xylophilus ampelinus*
  - *Agroacterium*
  - *Xylella fastidiosa monitoring*

1) **Selective media PW and PD2**
2) **Indirect immunofluorescence test**



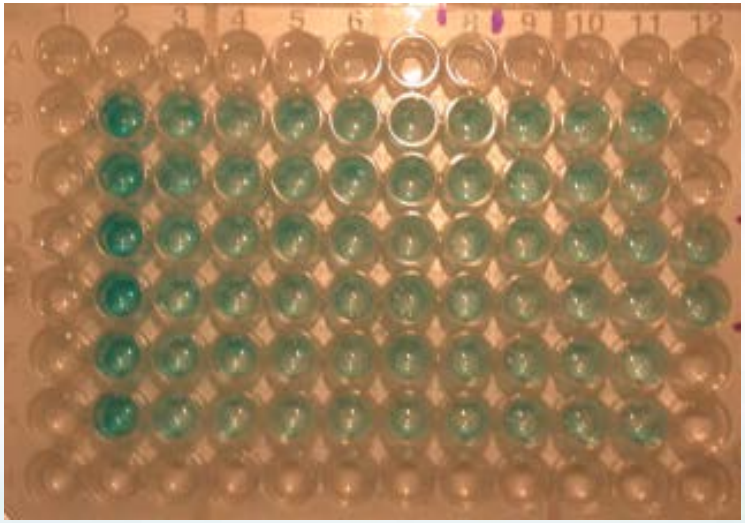
▪Campanharo et al. 2002. Growth optimization procedures for the phytopathogen *Xylella fastidiosa*. Current Microbiology, 46: 99-102

▪ Carbajal et al. 2004. Indirect immunofluorescence microscopy for direct detection of *Xylella fastidiosa* in xylem sap. Current Microbiology, 49: 372-375

▪ Normes OEPP/EPPO Standards. 2004. Diagnostic protocols for regulated pests, *Xylella fastidiosa*. Bulletin OEPP/EPPO Bulletin 34: 187-19

3) Enzyme-linked immunosorbent assay

- **DAS ELISA (Agdia Inc.)**
- **min 105 cells/mL extract detected in spiked samples**
  - **leaves**
  - **Leaf veins and petioles**
  - **Vacuum extract from shoots**
- **no cross-reactivity with *Agrobacterium* spp. and *X. ampelinus***



4) Real-time PCR

Schaad et al., 2002, Phytopath, 92(7): 721-728

Francis et al EJPP, 115(2): 203-213., 2006.

Real-Time Polymerase Chain Reaction for One-Hour On-Site Diagnosis of Pierce's Disease of Grape in Early Season Asymptomatic Vines

N. W. Schaad, D. Opgenorth, and P. Gaush

First and third authors: U.S. Department of Agriculture-Agricultural Research Service, Foreign Disease-Weed Science Research Unit, Ft. Detrick, MD 21702; and second author: California Department of Food and Agriculture, Sacramento 95832.

Genome-based PCR primers for specific and sensitive detection and quantification of *Xylella fastidiosa*

Marta Francis[1], Hong Lin[2], Juan Cabrera-La Rosa[3], Harshavardhan Doddapaneni[4], and Edwin L. Civerolo[2,*]

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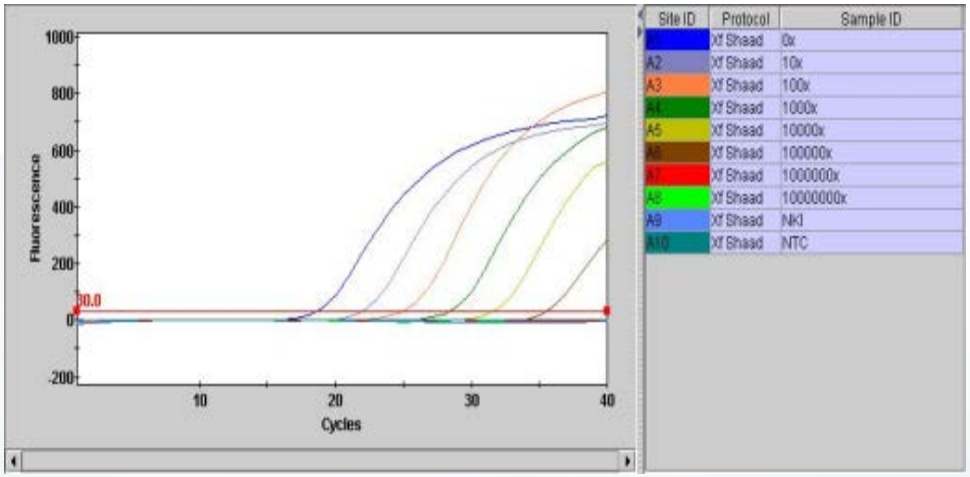

Xf conc [log cells/mL]	Av FAM Cq	qPCR	ELISA
8	18.46	+	NA
7	21.94	+	NA
6	24.87	+	+
5	28.23	+	+
4	31.41	+	-
3	35.18	+	-
2	0	-	-
1	0	-	-

Current testing scheme

- sample preparation modified from EPPO guidelines (2004), info from International Symposium on the European outbreak of Xylella fastidiosa in olive (Gallipoli, IT, 2014-10-21/24) and information obtained from other diagnostic laboratories
- Screening tests selective isolation on M115 media and, in parallel, DNA extraction and testing with real-time PCR using primers and probes developed by Schaad et al. (2002) and Francis et al. (2006)
- Spiking (olive, coffee, oleander, Liquidamber, rosemary, Polygala myrtifolia, Spartium junceum)
- Unconcentrated and 10-times concentrated samples

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Development of LAMP and Real-Time PCR Methods for the Rapid Detection of *Xylella fastidiosa* for Quarantine and Field Applications

S.J. Harper, L.I. Ward, and G.R.G. Clover

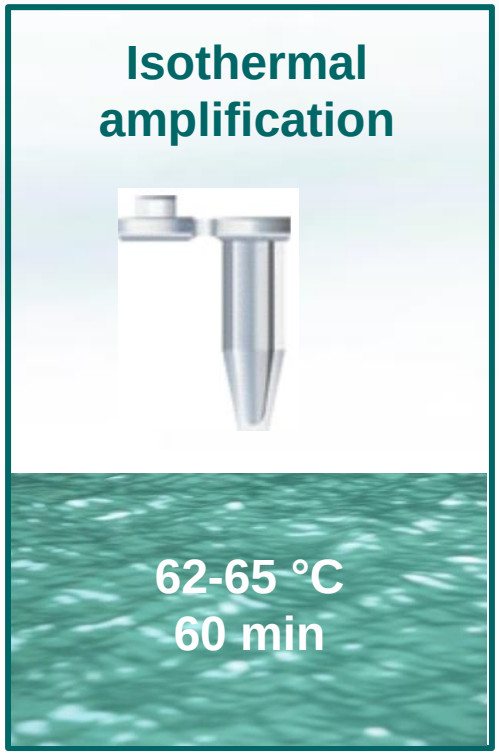
PHYTOPATHOLOGY 1282-1288 Vol. 100, No. 12, 2010

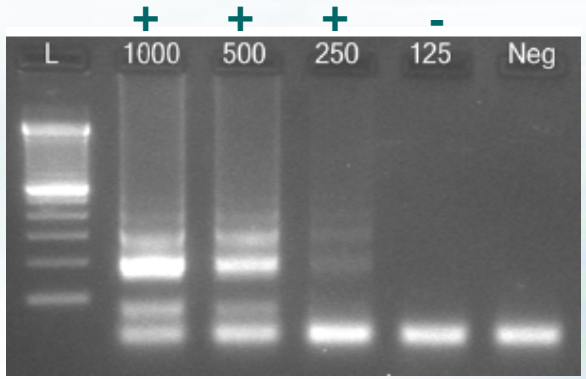
rimM

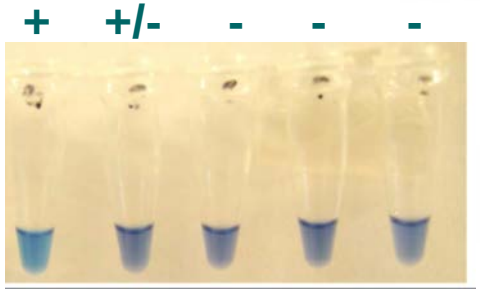
(i) kodira protein, dejavnik zorenja ribosomov

(ii) Conserved in genomes of *X. fastidiosa*

(iii) Different from sequences of rim in Xanthomonas





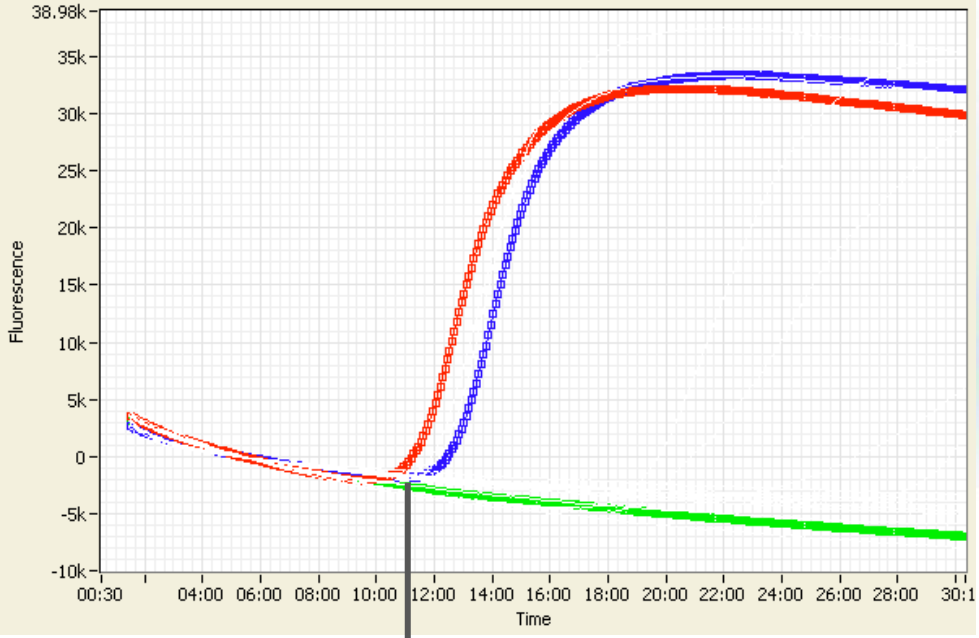


real-time LAMP

Slika: P. Kogovšek, NIB

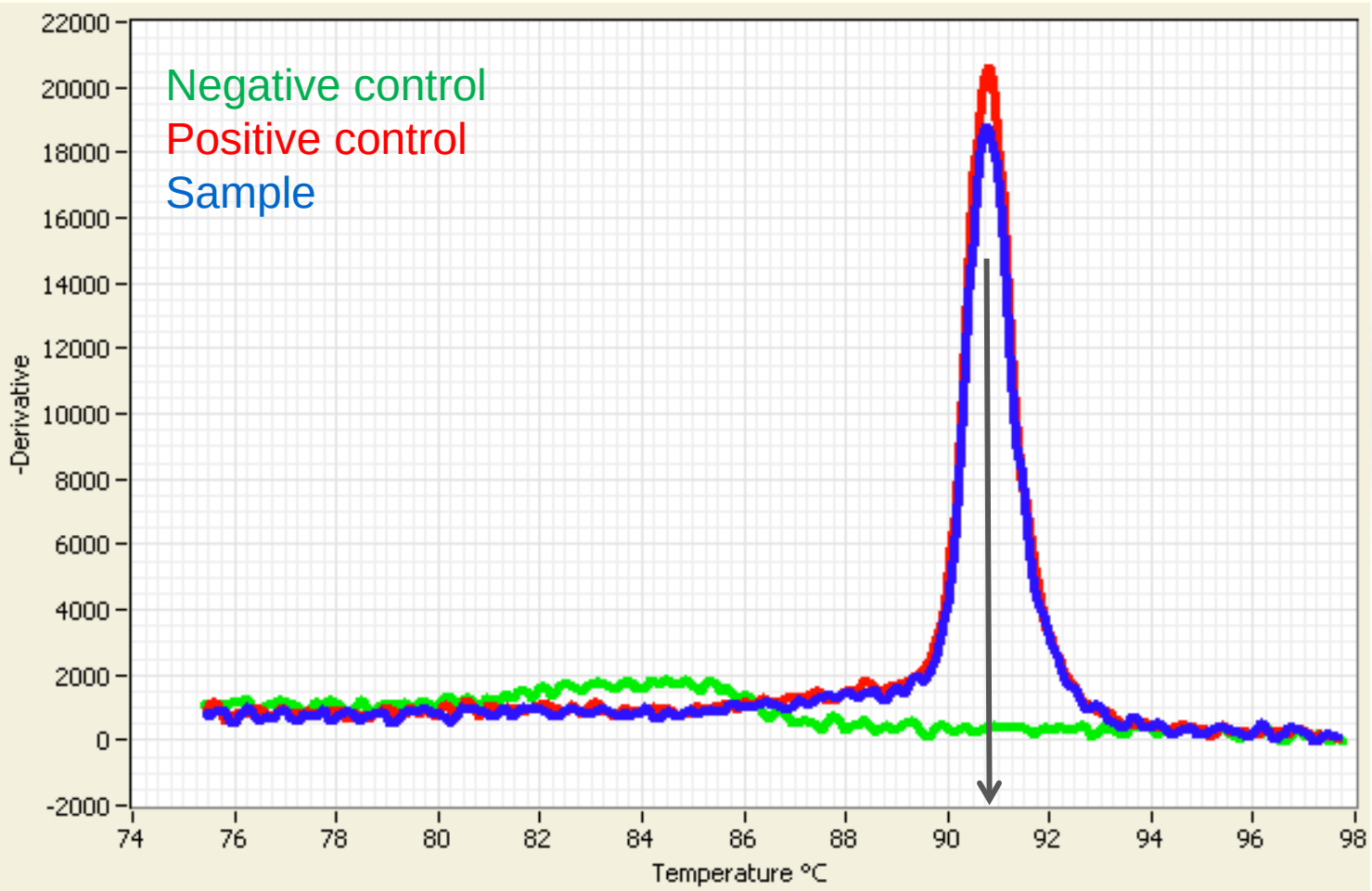
- Genie II (Optigene)
- **interkalirajoče barvilo** v reakciji
- Spremljamo **fluorescenco**

Tp = čas pozitivnosti



Tp = 12’

Tm


Negative control
Positive control
Sample
-Derivative
22000
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
-2000
74
76
78
80
82
84
86
88
90
92
94
96
98
Temperature °C

LAMP on serial dilutions

- *Xylella fastidiosa* subsp. *multiplex* LMG 9063 (ATCC 35871, *Prunus salicina*)

teoretična koncentracija log(kopij/mL)	qPCR			LAMP								
	kopij/ 2qPCR	Cq		kopij/ 1LAMP	*X. fastidiosa*		DNA from olive leaves					
		Schaad	Francis				undiluted		1:10		1:100	
					Tp	Tm	Tp	Tm	Tp	Tm	Tp	Tm
9.3	628824	19.8	22.4	786029	10:15	88.2	?	88.1	12:15	88.3	13:30	88.1
8.3	62882	23.5	26.5	78603	11:30	88.3	?	88.1	13:30	88.3	15:45	88.0
7.3	6288	27.1	30.4	7860	12:45	88.2	?	88.1	16:30	88.2	?	88.0
6.3	629	30.8	35.2	786	14:45	88.1	neg	neg	?	88.1	?	88.0
5.3	63	35.6	39.4	**79**	?	88.0	neg	neg	neg	neg	neg	neg
4.3	6	39.1	neg (45)	8	neg	neg	neg	neg	neg	neg	neg	neg
3.3	1	neg (45)	neg (45)	1	NA	NA	NA	NA	NA	NA	NA	NA

positive Tp & Tm | positive Tm

- As expected LAMP is **less sensitive than qPCR**
- Sensitivity of LAMP simialr to **serological methods**

sample	matrix	health status	dilution -1 Tp	Tm	result
1	olive	**pos**	**12.6**	**89.0**	pos
2	olive	neg	0	80.1	neg
3	olive	**pos**	**12.5**	**88.7**	**pos**
4	olive	neg	0	79.9	neg

sample	matrix	health status	dilution -1 Tp	Tm	result
5	oleander	pos	0	79.9	neg
6	oleander	**pos**	**18.6**	**88.3**	**pos**
7	oleander	neg	0	80.1	neg
8	oleander	neg	0	80.0	neg

sample	matrix	health status
9	cherry	neg
10	cherry	**pos**
11	cherry	neg
12	cherry	**pos**

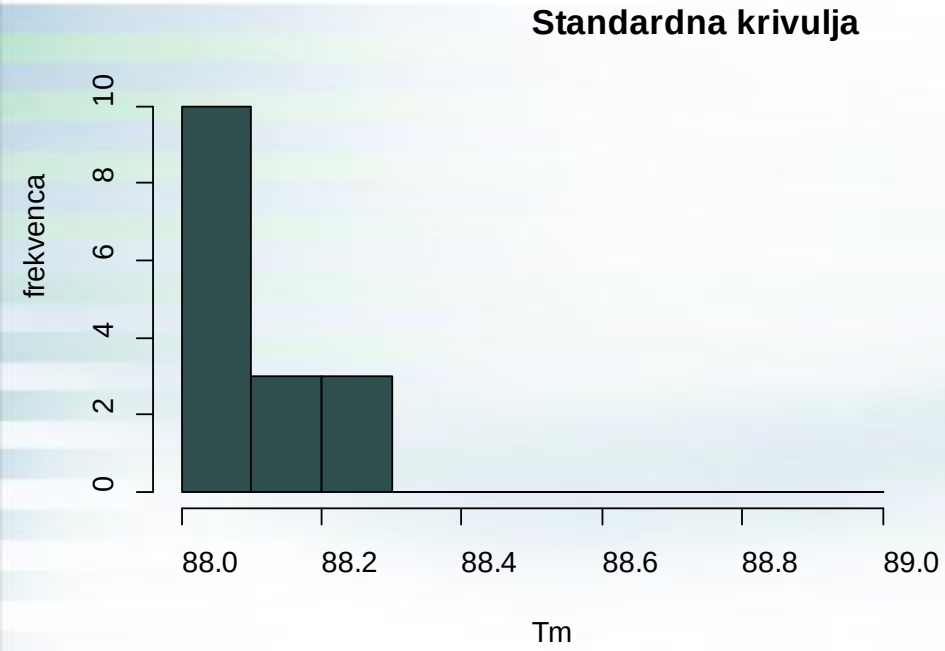
sample	matrix	health status
13	almond	neg
14	almond	neg
15	almond	**pos**
16	almond	**pos**

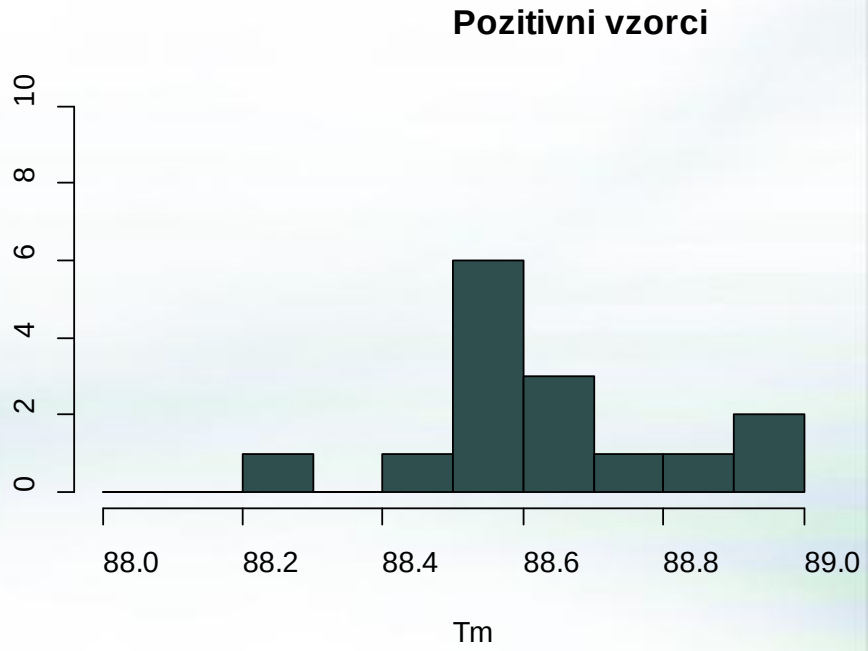
sample	matrix	health status	Tp	Tm	result
17	*Polygala myrtifolia*	**pos**	**17.6**	**88.6**	**pos**
19	*Polygala myrtifolia*	neg	0	79.9	neg
20	*Polygala myrtifolia*	neg	0	80.4	neg
21	*Acacia saligna*	**pos**	**19.6**	**88.9**	**pos**
22	*Acacia saligna*	neg	0	79.9	neg
23	*Acacia saligna*	pos	0	80.0	neg
24	*Acacia saligna*	neg	0	79.9	neg

- 12/12 negative and 9/11 positive samples correctly identified with LAMP
- 91 % diagnostic sensitivity
- 2 false negative samples

Vzorec	qPCR (Schaad) 0x	10x	100x
5, oleander	**38.9**	neg (45)	neg (45)
	neg (45)	neg (45)	neg (45)
	38.8	neg (45)	neg (45)
23, *Acacia saligna*	**30.3**	**34.1**	neg (45)
	30.4	**32.8**	**36.7**
	30.7	**33.5**	neg (45)

Stability of Tm





- **expected Tm: 88.0 ≤ Tm ≤ 89.0**
- None of the negative samples has suitable Tm (no false positives)

- Tp (positive) = 13-20 minutes (analysis of 10x dilutions)
- Tp (stand. curve) = 10-17 minut

Reaction time: 20’

Tm analysis: 10’

- qPCR: 54 min – 1h 12 min

Conclusions

- **LAMP less sensitive than qPCR**
- **Faster**
- *Further testing needed*
- *From other systems: LAMP relatively resistant to amplification inhibitors* – **could sample preparation be simplified?**

Future perspectives?

- **Coordinated evaluation of sample preparation and isolation protocols**
- **Risk identification**
- **Scouting and sampling strategy**

Digital PCR

- Increased resistance
- Increased reliability of low level detection

WGS

- Days to genome
- Identification of outbreaks and novel introductions

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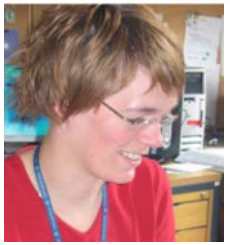

Špela Prijatelj-Novak, Lidija Matičič, Aleš Blatnik, Polona Kogovšek