Final Report

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Q-DETECT +
Euphresco: Q-DETECT II – test performance study of LAMP assays

Project Duration:

Start date:	2015
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2. Executive Summary

Project Summary

Q-DETECT II – test performance study of LAMP assays.

A test performance study (TPS) involving 26 European laboratories was conducted to evaluate the robustness and reliability of loop mediated isothermal amplification (LAMP) assays. These assays were for the detection of the quarantine listed species *Liriomyza huidobrensis* (a leaf mining insect) and *Clavibacter michiganensis* subsp. s*epedonicus* (CMS, a bacterial potato pathogen).

The *L. huidobrensis* assay performed well with the main challenge being the interpretation of the results from non-target genera and DNA extraction from small insect parts. Many participants had problems with contamination of the CMS LAMP assay, and a decrease in the limit of detection for the assay was found. Some users found the correct interpretation of LAMP results difficult, and variation was seen upon the use of different amplification platforms.

The TPS has highlighted that thorough training prior to implementation of a new diagnostic method would be beneficial, even in the hands of experienced diagnostic laboratories. Useful information has been gathered around the requirements for user instructions/protocols and the formulation of TPS material. Overall the feedback from the participants was positive and LAMP was viewed as a useful new tool, with the speed of testing and simple DNA extraction protocols particularly attractive to laboratories.

3. Report

Introduction

Implementation of new methods into diagnostic laboratories performing regulatory testing requires the methods to be fully validated and assessed through a test performance study. New methods should confer an advantage or provide an improvement compared to existing protocols, be reliable, cost-effective and easy to apply. The purpose of this test performance study (TPS) was to assess the robustness of rapid, simple, crude DNA extraction protocols combined with loop mediated isothermal amplification (LAMP) for the detection of a range of quarantine organisms.

The TPS was for the detection of *Liriomyza huidobrensis* and *Clavibacter michiganensis* subsp. *sepedonicus* (CMS) using LAMP. LAMP assays have also been developed for use as internal controls to allow the correct interpretation of results. TPS partners were provided with 4 LAMP assays, testing was conducted on different laboratory equipment with different personnel on sets of blind samples provided by the TPS organiser. Twentysix laboratories working on the molecular detection of plant pests and pathogens participated in the study.

Methods

Sample sets were prepared consisting of blind samples; 4 for the *L. huidobrensis* and 5 for CMS. For *L. huidobrensis* an adult, a single wing of an adult and a pupae were selected for use along with an aphid leg of a non-target species. The samples were shipped in ethanol for preservation. For CMS healthy potatoes were inoculated with high, medium and low levels of CMS alongside a healthy potato sample. The samples were freeze dried in vials to ensure stability during shipping.

Control assays provided included an assay for generic species detection of *Liriomyza* (*Liriomyza* control) and for plant host DNA (cytochrome oxidase I, COX) for use with the CMS assay. These assays are used in conjunction with specific pathogen detection assays to allow confirmation of negative results. All material used within the study was evaluated for homogeneity and was non-infectious. LAMP assays were manufactured by OptiGene Limited in a kit format as lyophilised reagents. Samples, positive control DNA and a detailed protocol were distributed to the participating laboratories.

Results

Results for the participating laboratories have been randomised to ensure confidentiality. Nine of the participating laboratories used a Genie® II instrument to run the assays and the remaining 14 laboratories used real-time PCR instruments (various models). Of the 26 participating partners 23 returned the results promptly and in the format requested. Original data submitted by participants is presented in Appendix 1 (*Liriomyza huidobrensis* and *Liriomyza* control assays) and Appendix 2 *(Clavibacter michiganensis* subsp. *sepedonicus* and COX assays).

Liriomyza huidobrensis

Of the 26 participating laboratories 21 returned results for *L. huidobrensis* identification. Results are summarised in **Table 1**, as the participants reported them.

Laboratory 1 is the organising laboratory; two other laboratories reported the results with all samples and controls as expected. Participants experienced two main problems with the testing; DNA extraction of insect parts and the interpretation of sample L-4 which was a non-target aphid species. Thirteen of the laboratories reported results which indicate problems with the DNA extraction of the insect parts; ideally a whole insect would be used in LAMP testing, however in some situations testing part of an insect maybe required. Five laboratories successfully extracted DNA from the *Liriomyza* wing (sample L-2). Three laboratories obtained a false positive result for the aphid leg sample with the *L. huidobrensis* assay and two for the *Liriomyza* control assay. Eight laboratories interpreted the aphid sample as negative and all other laboratories reported it as a test failure. The instruction manual was ambiguous in this regard, and did indicate that a negative control assay result should be interpreted as a test failure. Two laboratories obtained the results that were expected but interpreted them as assay failures due to errors interpreting the results. Six laboratories experienced false positive results or contamination of the assays.

Clavibacter michiganensis subsp. *sepedonicus*

Of the 26 participating laboratories 23 returned results for CMS, the results are summarised in **Table 2**, as the participants reported them. Laboratory 1 is the organising laboratory; 18 of the laboratories reported false positive results for either the negative (no-template) control sample and/or the healthy potato sample (sample C-1). This is probably due to processing contamination from the vials with infected samples; contamination of samples is a risk in LAMP and precautions should be taken to avoid this. Sample C-3 was heavily infected with CMS and contamination may have been introduced during rehydrating and processing of this sample. Four laboratories did not have contamination. Of these, three failed to detect sample C-5, which contained CMS at levels just above the limit of detection for the assay, and one also failed to detect sample C-2 which contained CMS at readily detectable levels.

Table 1: Test performance study results for *Liriomyza huidobrensis* as interpreted and reported by participants. Please note that participant numbers have been randomised and do not relate to those in the research consortium partners secition of this report.

Sample / Participant		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**
Negative control		-	F			-		F	-		-	-	F	F	-	-	-	F	-	-	F	-	-	-		-	-
Sample L-1	*L. huidobrensis* pupae	+	+			-		+	+		+	+	+	F	+	+	+	F	+	+	+	+	-	+		+	+
Sample L-2	*L. huidobrensis* wing	+	-			F		F	+		-	-	F	F	-	+	-	F	F	F	F	+	-	-		-	+
Sample L-3	*L. huidobrensis* adult	+	+			+		+	+		+	+	+	F	+	+	+	F	+	+	+	+	+	+		+	+
Sample L-4	Aphid leg	-	F			F		F	-		-	-	F	F	-	F	-	F	F	F	F	-	F	-		F	F
Positive control		+	+			+		+	+		+	+	+	F	+	+	+	F	+	+	+	+	+	+		+	+

Table 2: Test performance study results for *Clavibacter michiganensis* subsp. s*epedonicus* as interpreted and reported by participants

Sample / Participant		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**
Negative control		-	F			+	F	F	+	-	-	+	F	F	F	F	F	F	-	-	-	-	F	-		-	+
Sample C-1	Healthy potato	-	F			+	+	F	+	-	+	+	+	F	F	F	+	+	+	-	+	+	F	-		-	+
Sample C-2	Potato + 10^{4} CMS	+	F			+	+	+	+	-	+	+	+	F	F	F	+	+	+	+	+	+	F	+		+	+
Sample C-3	Potato + 10^{8} CMS	+	F			+	+	+	+	+	+	+	+	F	F	F	+	+	+	+	+	+	F	+		+	+
Sample C-4	Potato + 10^{6} CMS	+	F			+	F	+	+	+	+	+	+	F	F	F	+	+	+	+	+	+	F	+		+	+
Sample C-5	Potato + 10^{2} CMS	+	F			+	F	+	+	-	+	+		F	F	F	+	+	+	-	+	+	F	-		-	+
Positive control		+	F			+	+	+	+	+	+	+	+	F	F	F	+	+	+	+	+	+	F	+		+	+

Key:

Indicates the correct result reported

Indicates correct results but interpreted differently than expected

Indicates an incorrect result reported

Indicates sensitivity issues for target pathogen or extraction failure of insect wing

'+' indicates positive results, '-' indicates negative result and 'F' indicates failure

Discussion

The purpose of the TPS was to evaluate the robustness and reliability of LAMP assays as a precursor to the more routine deployment of LAMP for pest and pathogen identification. Interest in the TPS far exceeded expectations, with 26 laboratories participating.

The *L. huidobrensis* assay performed well and identified two areas challenging to end users. Firstly successful DNA extraction and manipulation of small samples (i.e. less than whole adults/pupae) was found to be difficult. However in deployment, the majority of samples would be whole insects. Secondly interpretation of the results from non-target genera where both the control assay (specific to the *Liriomyza* genus primarily) and the target assays are negative. Here, a negative result may be due to extraction failure or the presence of a non-target genus that is not detected by the control assay. With non-specialists selecting samples for testing this may result in inconclusive test results requiring confirmation.

Many participants had problems with contamination of the CMS LAMP assay. For those that didn't, detect of the lowest concentration of CMS failed although this was just above the limit of detection for the assay. The CMS test samples were distributed in glass vials with rubber stoppers (required for preservation of the samples during shipping); however processing of these vials was found to be challenging without causing contamination of the samples. These vials are not representative of the sample process workflow within the routinely testing laboratory. However this does demonstrate the sensitivity of LAMP testing and the high likelihood of contamination of samples/testing unless stringent good laboratory practice and contamination control measures are in place. The observed reduction in the assay limit of detection was likely an effect due to the lyophilisation of the LAMP reagents. This process is undergoing further optimisation to minimise the impact on final assay performance.

Useful lessons were learnt around requirements for sample storage/processing and the influence this may have upon final results. For example, the vials required to allow freeze-drying of the CMS samples and the need for insect samples to be shipped in ethanol as a preservative both caused challenges for participants during testing, and this was reflected in the TPS results. However these issues are in fact an artefact of the requirement for homogeneous samples stable for shipping at ambient temperatures, and are not variables that would be present in the routing deployment of LAMP testing in the laboratory.

Across all assays tested, some users found the interpretation of LAMP results challenging, particularly the correct assessment of the two parts of LAMP results (the time to positive and the anneal temperature) and the combined analysis of the pathogen specific and control assays. Variation in the anneal temperature was seen depending upon the detection platform used demonstrating this would need determining on a case by case basis. Furthermore interpretation of results from real-time PCR platforms compared to Genie® devices was found to be more difficult.

Feedback from participants indicated that the simple and rapid DNA extraction processes were attractive in terms of time reduction, especially when combined with the short run time of LAMP assays, and that LAMP testing was easy to conduct. The

TPS has provided very useful information and identified critical points of LAMP testing that can be built upon to further enable the deployment of LAMP.

Appendix 1 - *Liriomyza huidobrensis and Liriomyza* control participant data

Participant 1

Well	Sample	*Liriomyza huidobrensis* (Block A)		*Liriomyza* control (Block B)		Interpretation of test results (Positive/ Negative/Test failure)
		Run number:		Run number:		
		Amplification	Anneal	Amplification	Anneal	
		Tp (mm:ss)	°C	Tp (mm:ss)	°C	
1	Negative control					Neg
2	Sample L-1	21:00	80.69	8:30	84.95	Pos
3	Sample L-2	21:55	80.83	8:45	84.01	Pos
4	Sample L-3	18:45	80.97	7:00	84.95	Pos
5	Sample L-4				76.36	Neg
6						
7						
8	Positive control	17:3	80.88	6:45	85.00	Pos

Participant 2

Well	Sample	*Liriomyza huidobrensis* (Block A)		*Liriomyza* control (Block B)		Interpretation of test results (Positive/ Negative/Test failure)
		Run number:		Run number:		
		Amplification	Anneal	Amplification	Anneal	
		Tp (mm:ss)	°C	Tp (mm:ss)	°C	
1	Negative control		83.92		no amplification	test failure
2	Sample L-1		81.53		85.71	positive
3	Sample L-2		82.92		85.41	negative
4	Sample L-3		81.53		85.81	positive
5	Sample L-4		82.82		no amplification	test failure
6						
7						
8	Positive control		81.53		85.71	positive

Participant 3
No response

Participant 4
No response

Participant 5

Well	Sample	*Liriomyza huidobrensis* (Block A)		*Liriomyza* control (Block B)		Interpretation of test results (Positive/ Negative/Test failure)
		Run number:	*633*	Run number:	*633*	
		Amplification	Anneal	Amplification	Anneal	
		Tp (mm:ss)	°C	Tp (mm:ss)	°C	
1	Negative control	-	-	-	-	negative
2	Sample L-1	-	-	10:15	85.08	negative
3	Sample L-2	-	81.88	-	-	test failure
4	Sample L-3	24:07	80:38	08:45	85.13	positive
5	Sample L-4	-	-	-	-	test failure
6						
7						
8	Positive control	21:15	80.83	07:19	85.22	positive

Participant 6

Well	Sample	*Liriomyza huidobrensis* (Block A)		*Liriomyza* control (Block B)		Interpretation of test results (Positive/ Negative/Test failure)
		Run number:	*1*	Run number:	*1*	
		Amplification	Anneal	Amplification	Anneal	
		Tp (mm:ss)	°C	Tp (mm:ss)	°C	
1	Negative control	no data	no data	no data	no data	Test failure
2	Sample L-1	no data	no data	no data	no data	Test failure
3	Sample L-2	no data	no data	no data	no data	Test failure
4	Sample L-3	no data	no data	no data	no data	Test failure
5	Sample L-4	no data	no data	no data	no data	Test failure
6						
7						
8	Positive control	no data	no data	no data	no data	Test failure

Participant 7

Well	Sample	*Liriomyza huidobrensis* (Block A)		*Liriomyza* control (Block B)		Interpretation of test results (Positive/ Negative/Test failure)
		Run number:	*1*	Run number:	*1*	
		Amplification	Anneal	Amplification	Anneal	
		Tp (mm:ss)	°C	Tp (mm:ss)	°C	
1	Negative control		pos		neg	
2	Sample L-1		pos		pos	
3	Sample L-2		pos		neg	
4	Sample L-3		pos		pos	
5	Sample L-4		pos		neg	
6						
7						
8	Positive control		pos		pos	

Participant 8

Well	Sample	*Liriomyza huidobrensis* (Block A)		*Liriomyza* control (Block B)		Interpretation of test results (Positive/ Negative/Test failure)
		Run number:		Run number:		
		Amplification	Anneal	Amplification	Anneal	
		Tp (mm:ss)	°C	Tp (mm:ss)	°C	
1	Negative control	13:07	82.65	Undet	76.86	Negative
2	Sample L-1	15:31	81.05	6:61	85.33	Positive
3	Sample L-2	25:34	80.47	8:39	85.33	Positive
4	Sample L-3	12:97	80.96	5:74	85.33	Positive
5	Sample L-4	20:05	83.34	25:83	85.03	Negative
6						
7						
8	Positive control	12:31	81.05	4:47	85.33	Positive

Participant 9

Well	Sample	*Liriomyza huidobrensis* (Block A)		*Liriomyza* control (Block B)		Interpretation of test results (Positive/ Negative/Test failure)
		Run number:		Run number:		
		Amplification	Anneal	Amplification	Anneal	
		Tp (mm:ss)	°C	Tp (mm:ss)	°C	
1	Negative control					Test failure
2	Sample L-1					Test failure
3	Sample L-2					Test failure
4	Sample L-3					Test failure
5	Sample L-4					Test failure
6						
7						
8	Positive control					Test failure

Participant 10

Well	Sample	*Liriomyza huidobrensis* (Block A)		*Liriomyza* control (Block B)		Interpretation of test results (Positive/ Negative/Test failure)
		Run number:	*Lh*	Run number:	*L*	
		Amplification	Anneal	Amplification	Anneal	
		Tp (mm:ss)	°C	Tp (mm:ss)	°C	
1	Negative control	27:60	n	n	n	**negative**
2	Sample L-1	18:90	81.7	7:80	85.7	**positive** for L.h.
3	Sample L-2	38:51	n	19:35	85.6	**negative** for L.h.
4	Sample L-3	19:02	81.7	7:01	85.8	**positive** for L.h.
5	Sample L-4	20:06	82.8	n	n	**negative** for Liriomyca spec.
6						
7						
8	Positive control	15:56	81.7	5:68	85.7	**positive** for L.h.

Participant 11

Well	Sample	*Liriomyza huidobrensis* (Block A)		*Liriomyza* control (Block B)		Interpretation of test results (Positive/ Negative/Test failure)
		Run number:	*LOG gen2-1047_0511.gen*	Run number:	*LOG gen2-1047_0511.gen*	
		Amplification	Anneal	Amplification	Anneal	
		Tp (mm:ss)	°C	Tp (mm:ss)	°C	
1	Negative control	neg	neg	neg	75.81	negative
2	Sample L-1	20:30	81.15	07:45	85.31	positive[1]
3	Sample L-2	neg	neg	neg	77.75	negative
4	Sample L-3	20:00	81.1	08:00	85.37	positive[1]
5	Sample L-4	neg	neg	neg	75.56	negative
6	empty well	not tested	not tested	not tested	not tested	not tested
7	empty well	not tested	not tested	not tested	not tested	not tested
8	Positive control	18:00	81.25	07:15	85.46	positive[1]

Participant 12

Well	Sample	*Liriomyza huidobrensis* (Block A)		*Liriomyza* control (Block B)		Interpretation of test results (Positive/ Negative/Test failure)
		Run number:		Run number:		
		Amplification	Anneal	Amplification	Anneal	
		Tp (mm:ss)	°C	Tp (mm:ss)	°C	
1	Negative control	21:81	82	0	-	
2	Sample L-1	22:31	81	13:07	85	
3	Sample L-2	28:26	82.5	36:34	-	
4	Sample L-3	17:29	81	7:17	85	
5	Sample L-4	21:3	82.5	-	-	
6						
7						
8	Positive control	13:88	81	6:4	85	

Participant 13

Well	Sample	*Liriomyza huidobrensis* (Block A)		*Liriomyza* control (Block B)		Interpretation of test results (Positive/ Negative/Test failure)
		Run number:	*GEN2-1190_0893.gen*	Run number:	*GEN2-1190_0893.gen*	
		Amplification	Anneal	Amplification	Anneal	
		Tp (mm:ss)	°C	Tp (mm:ss)	°C	
1	Negative control	29:30	78.00	-	-	Test failure
2	Sample L-1	24:15	80.48	12:45	85.09	Test failure
3	Sample L-2	29:30	81.75	11:15	85.09	Test failure
4	Sample L-3	20:30	80.85	8:30	85.09	Test failure
5	Sample L-4	-	-	-	-	Test failure
6						
7						
8	Positive control	17:45	80.78	6:30	85.04	Test failure

Participant 14

Well	Sample	*Liriomyza huidobrensis* (Block A)		*Liriomyza* control (Block B)		Interpretation of test results (Positive/ Negative/Test failure)
		Run number:	*1*	Run number:	*1*	
		Amplification	Anneal	Amplification	Anneal	
		Tp (mm:ss)	°C	Tp (mm:ss)	°C	
1	Negative control	33:8	83.5	-	-	Negative
2	Sample L-1	22:79	82.1	9:52	86	Positive
3	Sample L-2	35:75	80.7	-	-	Negative
4	Sample L-3	19:97	81.7	9:5	86	Positive
5	Sample L-4	31:27	80.7	-	-	Negative
6						
7						
8	Positive control	17:48	81.7	7:87	86	Positive

Participant 15

Well	Sample	*Liriomyza huidobrensis* (Block A)		*Liriomyza* control (Block B)		Interpretation of test results (Positive/ Negative/Test failure)
		Run number:		Run number:		
		Amplification	Anneal	Amplification	Anneal	
		Ct	ºC	Ct	ºC	
1	Negative control	33:71	80,2 / 83	N/A	None	Negative
2	Sample L-1	18:37	81.2	7:45	85.2	Positive
3	Sample L-2	22:75	81 / 82,8	9:6	85.2	Positive
4	Sample L-3	17:49	81.2	6:1	85.2	Positive
5	Sample L-4	25:19	82.4	N/A	None	Test failure
6	Positive control	13:89	81	5:59	85.2	Positive
7						
8						

Participant 16

Well	Sample	*Liriomyza huidobrensis* (Block A)		*Liriomyza* control (Block B)		Interpretation of test results (Positive/ Negative/Test failure)
		Run number:		Run number:		
		Amplification	Anneal	Amplification	Anneal	
		Tp (mm:ss)	ºC	Tp (mm:ss)	ºC	
1	Negative control	29:62	83	N/A	-	negative
2	Sample L-1	18:66	81	10:52	85	positive
3	Sample L-2	31:08	82.5	N/A	-	negative
4	Sample L-3	17:03	81	7:15	85	positive
5	Sample L-4	25:84	82.5	N/A	-	negative
6						
7						
8	Positive control	14:44	81	5:29	85.5	positive

Participant 17

Well	Sample	*Liriomyza huidobrensis* (Block A)		*Liriomyza* control (Block B)		Interpretation of test results (Positive/ Negative/Test failure)
		Run number:		Run number:		
		Amplification	Melt Temp	Amplification	Melt Temp	
		Cq	°C	Cq	°C	
1	Negative control	none	none	none	none	test failure
2	Sample L-1	none	none	none	76.00	test failure
3	Sample L-2	none	none	none	76.60	test failure
4	Sample L-3	none	none	none	77.00	test failure
5	Sample L-4	none	none	none	76.00	test failure
6						
7						
8	Positive control	none	none	none	76.00	test failure

Participant 18

Well	Sample	*Liriomyza huidobrensis* (Block A)		*Liriomyza* control (Block B)		Interpretation of test results (Positive/ Negative/Test failure)
		Run number:	*0033*	Run number:	*0033*	
		Amplification	Anneal	Amplification	Anneal	
		Tp (mm:ss)	°C	Tp (mm:ss)	°C	
1	Negative control					negative
2	Sample L-1	23:00	80.82	09:30	85.08	positive
3	Sample L-2					test failure
4	Sample L-3	21:45	80.33	07:30	85.09	positive
5	Sample L-4					test failure
6						
7						
8	Positive control	17:15	80.17	06:15	84.89	positive

Participant 19

Well	Sample	*Liriomyza huidobrensis* (Block A)		*Liriomyza* control (Block B)		Interpretation of test results (Positive/ Negative/Test failure)
		Run number:		Run number:		
		Amplification	Anneal	Amplification	Anneal	
		Tp (mm:ss)	°C	Tp (mm:ss)	°C	
1	Negative control					
2	Sample L-1	20:45	80.72	7:45	85.23	Positive
3	Sample L-2					Test failure
4	Sample L-3	19:45	80.84	7:45	85.14	Positive
5	Sample L-4					Test failure
6						
7						
8	Positive control	18:15	80.91	7:00	85.04	

Participant 20

Well	Sample	*Liriomyza huidobrensis* (Block A)		*Liriomyza* control (Block B)		Interpretation of test results (Positive/ Negative/Test failure)
		Run number:		Run number:		
		Amplification	Anneal	Amplification	Anneal	
		Tp (mm:ss)	°C	Tp (mm:ss)	°C	
1	Negative control	16:65*	83.80	26:13	84.80	test failure: possible contamination. Test repeated for samples: negative control, L-2 and L-4 but results were the same
2	Sample L-1	20:97	81.00	8:75	85.00	
3	Sample L-2	17:73*	83.40	none	none	test failure, no control signal. When preparing the sample there were doubts if the arthropod leg was still in the tube after ethanol removal.
4	Sample L-3	14:58	81.00	4:95	85.00	
5	Sample L-4	18:05*	83.40	none	none	test failure, no control signal. When preparing the sample there were doubts if the arthropod leg was still in the tube after ethanol removal.
6						
7						
8	Positive control	14:07	81.00	5:23	85.20	

Participant 21

Well	Sample	*Liriomyza huidobrensis* (Block A)		*Liriomyza* control (Block B)		Interpretation of test results (Positive/ Negative/Test failure)
		Run number:		Run number:		
		Amplification	Anneal	Amplification	Anneal	
		Tp (mm:ss)	°C	Tp (mm:ss)	°C	
1	Negative control	negative	-	**16:13**	**84.8**	negative*[1]
2	Sample L-1	25:13	80.1	9:28	84.6	positive
3	Sample L-2	28:58	79.8	28:18	84.2	positive*[2]
4	Sample L-3	21:43	80.2	8:43	84.5	positive
5	Sample L-4	negative	-	23:13	84.4	positive for the genus *Liriomyza**[3]
6	Positive control	17:58	80.2	7:28	84.7	positive
7						
8						

Participant 22

Well	Sample	*Liriomyza huidobrensis* (Block A)		*Liriomyza* control (Block B)		Interpretation of test results (Positive/ Negative/Test failure)
		Run number:	*1*	Run number:	*1*	
		Amplification	Anneal	Amplification	Anneal	
		Tp (mm:ss)	°C	Tp (mm:ss)	°C	
1	Negative control	26	83	_	_	Negative
2	Sample L-1	32	83.2	9	85.86	Negative
3	Sample L-2	36	83.46	30	85.96	Negative
4	Sample L-3	17	81.74	7	85.96	Positive
5	Sample L-4	40	83.56	_	_	Test failure (*L. huidobrensis* negative… but *Liriomyza* control is also negative)
6						
7						
8	Positive control	15	81.76	7	86.06	Positive

Participant 23

Well	Sample	*Liriomyza huidobrensis* (Block A)		*Liriomyza* control (Block B)		Interpretation of test results (Positive/ Negative/Test failure)
		Run number:	*1*	Run number:	*1*	
		Amplification	Anneal	Amplification	Anneal	
		Tp (mm:ss)	°C	Tp (mm:ss)	°C	
1	Negative control				75.86	Test has to be repeated
2	Sample L-1	29:15	80.43	08:15	85.20	Test has to be repeated
3	Sample L-2				76.15	Test has to be repeated
4	Sample L-3	20:30	81.03	08:15	85.20	Test has to be repeated
5	Sample L-4				76.17	Test has to be repeated
6					76.07	Test has to be repeated
7					76.06	Test has to be repeated
8	Positive control	18:15	81.13	07:00	85.30	Test has to be repeated

Participant 24
No results

Participant 25

Well	Sample	*Liriomyza huidobrensis* (Block A)		*Liriomyza* control (Block B)		Interpretation of test results (Positive/ Negative/Test failure)
		Run number:		Run number:		
		Amplification	Anneal	Amplification	Anneal	
		Tp (mm:ss)	°C	Tp (mm:ss)	°C	
1	Negative control	negative		negative		Negative
2	Sample L-1	22:28	ca. 80.3	09:58	ca. 84.4	Positive
3	Sample L-2	negative		negative		Negative
4	Sample L-3	21:28	ca. 80.0	07:13	ca. 84.4	Positive
5	Sample L-4	Negative		Negative		Negative/ Test failure?
6	Positive control	18:13	ca. 80.5	07:13	ca. 84.4	Positive
7						
8						

Participant 26

Well	Sample	*Liriomyza huidobrensis* (Block A)		*Liriomyza* control (Block B)		Interpretation of test results (Positive/ Negative/Test failure)
		Run number:		Run number:		
		Amplification	Anneal	Amplification	Anneal	
		Tp (mm:ss)	°C	Tp (mm:ss)	°C	
1	Negative control	-	95.07	-	95.4	negative
2	Sample L-1	21:09	80.59	8:58	85.5	positive for pathogen
3	Sample L-2	18:68	82.45	13:57	85.1	positive for pathogen
4	Sample L-3	20:88	81.09	7:49	85.49	positive for pathogen
5	Sample L-4	-	94.78	-	95.3	test failure
6						
7						
8	Positive control	14:91	81.47	5:53	85.52	positive

Appendix 2 - *Clavibacter michiganensis subsp. sepedonicus* and plant control participant data

Participant 1

Well	Sample	*Clavibacter michiganensis subsp. sepedonicus* (Block A)		Plant control (COX) (Block B)		Interpretation of test results (Positive/ Negative/Test failure)
		Run number:		Run number:		
		Amplification	Anneal	Amplification	Anneal	
		Tp (mm:ss)	°C	Tp (mm:ss)	°C	
1	Negative control				76.45	Neg
2	Sample C-1			19:15	85.45	Neg
3	Sample C-2	11:15	89.61	16:15	85.40	Pos
4	Sample C-3	5:30	89.61	15:45	85.45	Pos
5	Sample C-4	7:45	89.52	19:00	85.20	Pos
6	Sample C-5	18:15	89.42	17:30	85.40	Pos
7					76.30	
8	Positive control	6:15	89.86	14:15	85.85	Pos

Participant 2

Well	Sample	*Clavibacter michiganensis subsp. sepedonicus* (Block A)		Plant control (COX) (Block B)		Interpretation of test results (Positive/ Negative/Test failure)
		Run number:		Run number:		
		Amplification	Anneal	Amplification	Anneal	
		Tp (mm:ss)	°C	Tp (mm:ss)	°C	
1	Negative control		89.89		no amplification	test failure-contamination
2	Sample C-1		89.79		85.83	test failure
3	Sample C-2		89.99		85.23	test failure
4	Sample C-3		89.69		85.83	test failure
5	Sample C-4		89.69		85.73	test failure
6	Sample C-5		89.89		85.83	test failure
7						
8	Positive control		89.79		85.93	test failure

Participant 3
No response

Participant 4
No response

Participant 5

Well	Sample	*Clavibacter michiganensis subsp. sepedonicus* (Block A)		Plant control (COX) (Block B)		Interpretation of test results (Positive/ Negative/Test failure)
		Run number:	*634*	Run number:	*634*	
		Amplification	Anneal	Amplification	Anneal	
		Tp (mm:ss)	°C	Tp (mm:ss)	°C	
1	Negative control	21:57	88.91	-	-	contamination CMS/negative COX
2	Sample C-1	24:18	89.07	19:51	85.1	positive
3	Sample C-2	20:04	88.86	19:17	84.74	positive
4	Sample C-3	05:24	88.84	19:18	84.69	positive
5	Sample C-4	07:55	89.1	21:38	84.84	positive
6	Sample C-5	09:56	88.68	20:25	84.99	positive
7						
8	Positive control	05:41	89.12	14:45	85.24	positive

Participant 6

Well	Sample	*Clavibacter michiganensis subsp. sepedonicus* (Block A)		Plant control (COX) (Block B)		Interpretation of test results (Positive/ Negative/Test failure)
		Run number:	*1*	Run number:	*1*	
		Amplification	Anneal	Amplification	Anneal	
		Ct	°C	Ct	°C	
1	Negative control	12:31	89.20	no amplification	none	Test failure
2	Sample C-1	12:11	89.00	10:43	85.20	Positive
3	Sample C-2	13:32	89.20	19:39	85.20	Positive
4	Sample C-3	5:07	89.00	19:84	85.20	Positive
5	Sample C-4	11:67	89.20	no amplification	none	Test failure
6	Sample C-5	13:08	89.20	no amplification	none	Test failure
7						
8	Positive control	4:73	89.00	14:75	85.20	Positive

Participant 7

Well	Sample	*Clavibacter michiganensis subsp. sepedonicus* (Block A)		Plant control (COX) (Block B)		Interpretation of test results (Positive/ Negative/Test failure)
		Run number:	*1*	Run number:	*1*	
		Amplification	Anneal	Amplification	Anneal	
		Tp (mm:ss)	°C	Tp (mm:ss)	°C	
1	Negative control		pos		neg	
2	Sample C-1		pos		neg	
3	Sample C-2		pos		pos	
4	Sample C-3		pos		pos	
5	Sample C-4		pos		pos	
6	Sample C-5		pos		pos	
7						
8	Positive control		pos		pos	

Participant 8

Well	Sample	*Clavibacter michiganensis subsp. sepedonicus* (Block A)		Plant control (COX) (Block B)		Interpretation of test results (Positive/ Negative/Test failure)
		Run number:		Run number:		
		Amplification	Anneal	Amplification	Anneal	
		Tp (mm:ss)	°C	Tp (mm:ss)	°C	
1	Negative control	11:21	89.82	7:84	79.06	Positive CMS / Negative COX
2	Sample C-1	7:82	89.62	32:14	84.54	Positive
3	Sample C-2	7:40	89.52	20:10	84.94	Positive
4	Sample C-3	3:93	89.42	4:30	84.74	Positive
5	Sample C-4	6:11	89.49	9:19	85.04	Positive
6	Sample C-5	8:72	89.52	8:11	84.94	Positive
7						
8	Positive control	4:60	89.62	6:60	85.24	Positive

Participant 9

Well	Sample	*Clavibacter michiganensis subsp. sepedonicus* (Block A)		Plant control (COX) (Block B)		Interpretation of test results (Positive/ Negative/Test failure)
		Run number:		Run number:		
		Amplification	Anneal	Amplification	Anneal	
		Tp (mm:ss)	ºC	Tp (mm:ss)	ºC	
1	Negative control	0	0	0	0	Negative
2	Sample C-1	33:73	92	0	0	Negative
3	Sample C-2	0	0	0	0	Negative
4	Sample C-3	22:73	90	27:03	85	Positive
5	Sample C-4	11:57	90	0	0	Positive
6	Sample C-5	36:44	92	0	0	Negative
7	COX control	36:40	90	15:88	85	Positive
8	Positive control	29:92	90	0	0	Positive

Participant 10

Well	Sample	*Clavibacter michiganensis subsp. sepedonicus* (Block A)		Plant control (COX) (Block B)		Interpretation of test results (Positive/ Negative/Test failure)
		Run number:	*Cms*	Run number:	*COX*	
		Amplification	Anneal	Amplification	Anneal	
		Tp (mm:ss)	ºC	Tp (mm:ss)	ºC	
1	Negative control	n	n	n	n	negative
2	Sample C-1	17:10	89.8	19:78	85.7	positive
3	Sample C-2	28:99	89.7	20:90	85.7	positive
4	Sample C-3	4:95	89.8	20:62	85.7	positive
5	Sample C-4	9:45	89.8	22:99	85.6	positive
6	Sample C-5	17:48	89.7	21:25	85.6	positive
7						
8	Positive control	5:80	89.8	15:06	85.7	positive

Participant 11

Well	Sample	*Clavibacter michiganensis subsp. sepedonicus* (Block A)		Plant control (COX) (Block B)		Interpretation of test results (Positive/ Negative/Test failure)
		Run number:	*LOG gen2-1047_0512.gen*	Run number:	*LOG gen2-1047_0512.gen*	
		Amplification	Anneal	Amplification	Anneal	
		Tp (mm:ss)	°C	Tp (mm:ss)	°C	
1	Negative control	23:45	89.03	neg	75.96	Test failure[2]
2	Sample C-1	25:45	88.88	29:15	84.12	Test failure[2]
3	Sample C-2	16:00	89.18	neg	84.24	Test failure[2]
4	Sample C-3	6:00	89.08	neg	84.29	Test failure[2]
5	Sample C-4	13:30	89.22	23:15	84.67	Test failure[2]
6	Sample C-5	29:15	89.23	25:30	84.82	Test failure[2]
7	reaction mix added only	23:15	89.08	neg	75.51	Test failure[2]
8	Positive control	6:15	89.47	15:15	85.41	Test failure[2]

Participant 12

Well	Sample	*Clavibacter michiganensis subsp. sepedonicus* (Block A)		Plant control (COX) (Block B)		Interpretation of test results (Positive/ Negative/Test failure)
		Run number:		Run number:		
		Amplification	Anneal	Amplification	Anneal	
		Tp (mm:ss)	°C	Tp (mm:ss)	°C	
1	Negative control	17:06	89	0	0	
2	Sample C-1	15:35	89	18:74	85	
3	Sample C-2	9:2	89	23:1	85	
4	Sample C-3	4:15	89	16:7	85	
5	Sample C-4	8:09	89	20:87	85	
6	Sample C-5	broken				
7						
8	Positive control	6:4		13:88	85	

Participant 13

Well	Sample	*Clavibacter michiganensis subsp. sepedonicus* (Block A)		Plant control (COX) (Block B)		Interpretation of test results (Positive/ Negative/Test failure)
		Run number:	*GEN2-1190_0894.gen*	Run number:	*GEN2-1190_0894.gen*	
		Amplification	Anneal	Amplification	Anneal	
		Tp (mm:ss)	°C	Tp (mm:ss)	°C	
1	Negative control	23:00	88.87	-	-	Test failure
2	Sample C-1	11:15	88.88	20:30	84.94	Test failure
3	Sample C-2	10:15	88.98	19:45	84.65	Test failure
4	Sample C-3	5:45	88.98	18:15	84.85	Test failure
5	Sample C-4	9:45	89.02	22:00	84.52	Test failure
6	Sample C-5	-	-	19:30	84.62	Test failure
7						
8	Positive control	6:00	89.02	16:00	85.09	Test failure

Participant 14

Well	Sample	*Clavibacter michiganensis subsp. sepedonicus* (Block A)		Plant control (COX) (Block B)		Interpretation of test results (Positive/ Negative/Test failure)
		Run number:	*1*	Run number:	*1*	
		Amplification	Anneal	Amplification	Anneal	
		Tp (mm:ss)	°C	Tp (mm:ss)	°C	
1	Negative control	18:76	90.7	-	-	Test Failure
2	Sample C-1	17:46	90.8	26:35	86	Test Failure
3	Sample C-2	12:91	90.3	32:49	86	Test Failure
4	Sample C-3	5:93	90.3	28:22	85.5	Test Failure
5	Sample C-4	9:56	90.3	24:87	85.5	Test Failure
6	Sample C-5	23:09	90.3	25:66	85.5	Test Failure
7						
8	Positive control	7:5	90.4	22:59	85.8	Test Failure

Participant 15

Well	Sample	*Clavibacter michiganensis subsp. sepedonicus* (Block A)		Plant control (COX) (Block B)		Interpretation of test results (Positive/ Negative/Test failure)
		Run number:		Run number:		
		Amplification	Anneal	Amplification	Anneal	
		Ct	ºC	Ct	ºC	
1	Negative control	19:86	89.4	N/A	None	Test failure, NC positive for CMS
2	Sample C-1	19:19	89.4	22:37	85.2	
3	Sample C-2	9:35	89.2	18:45	85.2	
4	Sample C-3	4:47	89.2	19:69	85.2	
5	Sample C-4	9:69	89.2	19:37	85.2	
6	Sample C-5	20:34	89.2	20:29	85.2	
7	Positive control	5:13	89	14:57	85.4	
8						

Participant 16

Well	Sample	*Clavibacter michiganensis subsp. sepedonicus* (Block A)		Plant control (COX) (Block B)		Interpretation of test results (Positive/ Negative/Test failure)
		Run number:		Run number:		
		Amplification	Anneal	Amplification	Anneal	
		Tp (mm:ss)	ºC	Tp (mm:ss)	ºC	
1	Negative control	14:53	89	N/A	-	Test failure
2	Sample C-1	8:37	89	18:23	85	positive
3	Sample C-2	25:68	88.5	17:89	85	positive
4	Sample C-3	4:02	89	19:24	85	positive
5	Sample C-4	7:27	89	19:89	85	positive
6	Sample C-5	7:21	89	19:03	85	positive
7						
8	Positive control	5:01	89	13:84	85	positive

Participant 17

Well	Sample	*Clavibacter michiganensis subsp. sepedonicus* (Block A)		Plant control (COX) (Block B)		Interpretation of test results (Positive/ Negative/Test failure)
		Run number:		Run number:		
		Amplification	Melt Temp	Amplification	Melt Temp	
		Cq	°C	Cq	°C	
1	Negative control	22:44	89.40	0	none	handling failure
2	Sample C-1	29:72	89.60	19:46	85.40	positive
3	Sample C-2	8:78	89.40	22:6	85.40	positive
4	Sample C-3	4:4	89.40	17:25	85.40	positive
5	Sample C-4	8:12	89.40	22:13	85.20	positive
6	Sample C-5	19:03	89.40	21:3	85.40	positive
7						
8	Positive control	5:28	89.4	13:38	85.40	positive

Participant 18

Well	Sample	*Clavibacter michiganensis subsp. sepedonicus* (Block A)		Plant control (COX) (Block B)		Interpretation of test results (Positive/ Negative/Test failure)
		Run number:	*0034*	Run number:	*0034*	
		Amplification	Anneal	Amplification	Anneal	
		Tp (mm:ss)	°C	Tp (mm:ss)	°C	
1	Negative control					negative
2	Sample C-1	16:45	89.6	18:30	84.88	positive
3	Sample C-2	11:15	88.67	21:15	84.74	positive
4	Sample C-3	05:45	88.82	18:30	84.79	positive
5	Sample C-4	08:45	88.85	25:45	84.43	positive
6	Sample C-5	20	88.8	21	84.73	positive
7						
8	Positive control	7:15	88.96	16:30	84.98	positive

Participant 19

Well	Sample	*Clavibacter michiganensis subsp. sepedonicus* (Block A)		Plant control (COX) (Block B)		Interpretation of test results (Positive/ Negative/Test failure)
		Run number:		Run number:		
		Amplification	Anneal	Amplification	Anneal	
		Tp (mm:ss)	°C	Tp (mm:ss)	°C	
1	Negative control					
2	Sample C-1			18:00	84.79	negative
3	Sample C-2	14:15	88.86	17:15	84.92	positive
4	Sample C-3	5:30	89.09	20:00	84.86	positive
5	Sample C-4	8:15	88.92	16:00	85.00	positive
6	Sample C-5	29:30		26:30	84.41	negative
7						
8	Positive control	6:00	89.17	13:45	85.09	

Participant 20

Well	Sample	*Clavibacter michiganensis subsp. sepedonicus* (Block A)		Plant control (COX) (Block B)		Interpretation of test results (Positive/ Negative/Test failure)
		Run number:		Run number:		
		Amplification	Anneal	Amplification	Anneal	
		Tp (mm:ss)	°C	Tp (mm:ss)	°C	
1	Negative control	none	none	none	none	negative
2	Sample C-1	19:56	89.20	28:19	84.80	positive
3	Sample C-2	13:57	89.00	22:64	85.00	positive
4	Sample C-3	3:36	89.20	17:05	85.00	positive
5	Sample C-4	7:56	89.20	21:18	85.00	positive
6	Sample C-5	22:24	89.00	25:66	84.80	positive
7						
8	Positive control	5:54	89.20	17:90	85.20	positive

Participant 21

Well	Sample	*Clavibacter michiganensis subsp. sepedonicus* (Block A)		Plant control (COX) (Block B)		Interpretation of test results (Positive/ Negative/Test failure)
		Run number:		Run number:		
		Amplification	Anneal	Amplification	Anneal	
		Tp (mm:ss)	°C	Tp (mm:ss)	°C	
1	Negative control	negative	-	negative	-	negative
2	Sample C-1	24:15	88.8	20:30	84.5	positive
3	Sample C-2	22:00	88.5	22:00	84.4	positive
4	Sample C-3	6:45	88.5	21:45	84.5	positive
5	Sample C-4	11:15	88.8	19:30	84.6	positive
6	Sample C-5	22:15	88.5	23:15	84.6	positive
7	Positive control	7:00	88.8	13:15	84.8	positive
8						

Participant 22

Well	Sample	*Clavibacter michiganensis subsp. sepedonicus* (Block A)		Plant control (COX) (Block B)		Interpretation of test results (Positive/ Negative/Test failure)
		Run number:	*1*	Run number:	*1*	
		Amplification	Anneal	Amplification	Anneal	
		Tp (mm:ss)	°C	Tp (mm:ss)	°C	
1	Negative control	29	90.46	_	_	Positive for Cm (problem!)
2	Sample C-1	20	90.10	30	86.66	Test failure
3	Sample C-2	16	90.20	33	86.60	Test failure
4	Sample C-3	5	90.30	23	86.80	Test failure
5	Sample C-4	8	90.30	30	86.80	Test failure
6	Sample C-5	17	90.36	40	86.90	Test failure
7						
8	Positive control	6	90.50	18	87.10	Test failure

Participant 23

Well	Sample	*Clavibacter michiganensis subsp. sepedonicus* (Block A)		Plant control (COX) (Block B)		Interpretation of test results (Positive/ Negative/Test failure)
		Run number:		Run number:		
		Amplification	Anneal	Amplification	Anneal	
		Tp (mm:ss)	°C	Tp (mm:ss)	°C	
1	Negative control	no Tp		no Tp		Negative
2	Sample C-1	no Tp		23:07	84.65	Negative
3	Sample C-2	13:45	88.89	18:15	84.66	Positive
4	Sample C-3	05:45	89.1	21:50	84.71	Positive
5	Sample C-4	09:41	89.15	23:18	84.58	Positive
6	Sample C-5	no Tp		18:13	84.82	Negative
7		no Tp		no Tp		Negative
8	Positive control	06:18	89.07	13:49	84.98	Positive

Participant 24
No results

Participant 25

Well	Sample	*Clavibacter michiganensis subsp. sepedonicus* (Block A)		Plant control (COX) (Block B)		Interpretation of test results (Positive/ Negative/Test failure)
		Run number:		Run number:		
		Amplification	Anneal	Amplification	Anneal	
		Tp (mm:ss)	°C	Tp (mm:ss)	°C	
1	Negative control	negative		negative		Negative
2	Sample C-1	negative		21:04	ca. 84.2	Negative
3	Sample C-2	14:49	ca. 88.0	28:49	ca. 84.2	Positive
4	Sample C-3	06:04	ca. 88.0	18:19	ca. 84.2	Positive
5	Sample C-4	09:34	ca. 88.3	20:19	ca. 84.2	Positive
6	Sample C-5	negative		18:49	ca. 84.2	Negative
7	Positive control	06:49	ca. 88.3	14:49	ca. 84.2	Positive
8						

Participant 26

Well	Sample	*Clavibacter michiganensis subsp. sepedonicus* (Block A)		Plant control (COX) (Block B)		Interpretation of test results (Positive/ Negative/Test failure)
		Run number:		Run number:		
		Amplification	Anneal	Amplification	Anneal	
		Tp (mm:ss)	ºC	Tp (mm:ss)	ºC	
1	Negative control	15:24	89.71	-	94	negative control Block A is positive
2	Sample C-1	17:36	89.15	26:58	85.06	positive for pathogen
3	Sample C-2	13:45	89.51	27:92	85.06	positive for pathogen
4	Sample C-3	5:76	89.54	16:74	85.08	positive for pathogen
5	Sample C-4	11:88	89.46	31:18	84.89	positive for pathogen
6	Sample C-5	16:4	89.28	26:68	84.99	positive for pathogen
7						
8	Positive control	6:16	89.69	19:1	85.46	positive