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Federal Office for Agriculture FOAG
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Swiss Federal Plant Protection Service SPPS

EPPO Workshop for inspectors

LAMP based identification of quarantine insects at the airports of Zürich and Geneva

13. – 14.12.2017

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Head of Plant Protection Inspectors
Swiss Federal Plant Protection Service SPPS
Federal Office for Agriculture

Overview

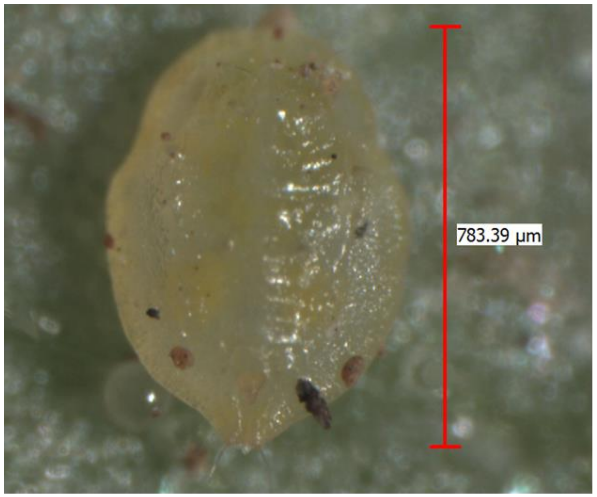
1. Introduction
2. Implementation at the airports
3. Conclusion
4. Outlook

1. Introduction

Why do we need a tool for on-site diagnosis:

- The morphological on-site identification can be difficult

- Especially for insects species at developmental stages (e. g. eggs and larvae)



Bemisia tabaci

1. Introduction

Why do we need a tool for on-site diagnosis:

- The morphological on-site identification can be difficult

- Especially for insects species at developmental stages (e. g. eggs and larvae)

- If plant health inspectors detect insects suspected to be QOs, samples are sent to Agroscope for DNA barcoding analysis

  - Shipment of samples to the laboratory and DNA barcoding analysis require 2-3 working days

1. Introduction

Why do we need a tool for on-site diagnosis:

- Plant imports are often perishable goods (e. g. fresh fruits or vegetables)

Mentha arvensis from Vietnam with *Bemisia tabaci*

1. Introduction

Why do we need a tool for on-site diagnosis:

- Plant imports are often perishable goods (e. g. fresh fruits or vegetables)
- Import delay can result in economic damage

➢ Perishable goods can not wait!

Solution:

Rapid molecular tests for on-site identification

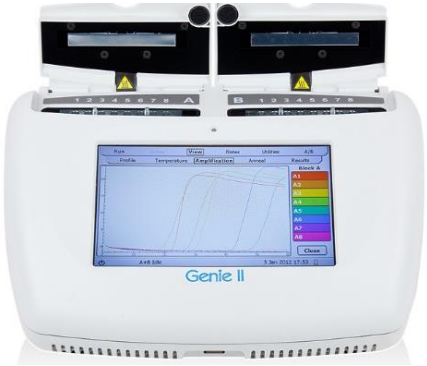
1. Introduction

Method to be used:

LAMP = **L**oop Mediated Isothermal **Amp**lification

- Isothermal DNA amplification
- Robust against amplification inhibitors
- Highly specific (six primers)
- Can be performed on a portable device (Genie II, Optigene®)

→ Suitable for on-site application



Source: optigene.co.uk

1. Introduction

Source: optigene.co.uk

Method to be used:

LAMP = **L**oop Mediated Isothermal **Amp**lification

▪In the framework of the EU-Project Q-Detect (WP7), LAMP-assays were developed for several Quarantine pests:

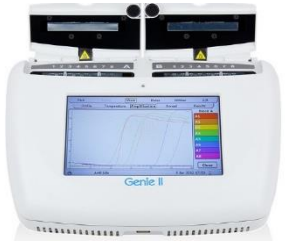
- *Different Bactrocera species*
- Bemisia tabaci
- *Different Liriomyza*
- *Thrips palmi*

They represent more than 70 % of the intercepted quarantine insects per year.

Validation of the assays by Agroscope showed:

- 383 samples were tested
- 97.2% of the results were positive
- **No wrong-positive results**
- Low amount of false-negative results (2.8%) → redesign of primers

1. Introduction



Source:
optigene.co.uk

Requirement for a two-stage identification system

Limitations of DNA amp. based test

- DNA amplification based methods only detect pre-defined targets
- Rare false-negative results due to previously undescribed pest biotypes are to be expected (biotypes not included in the primer design process)

Solution:

To ensure 100% sensitivity of the identification system, LAMP - negatively tested specimen are analyzed by DNA barcoding

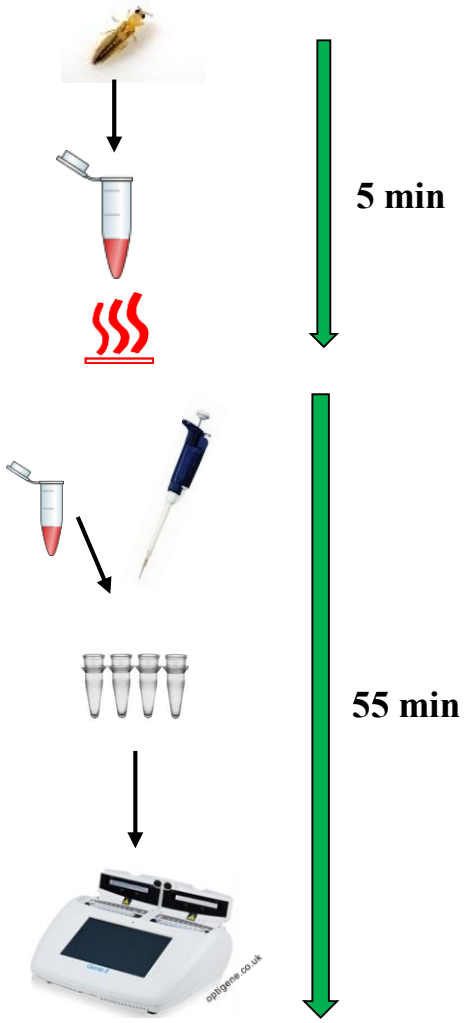
Important: In case of a positive LAMP results → Inspector can directly reject infested commodities

1. Introduction

Modification for on-site application

Goal: Performable for Plant health inspectors with minimal laboratory training

- Simplification of handling (**1-pipetting-step**)
- Production of pre-mixed LAMP kits
- Staining of chemicals (Cresol Red)

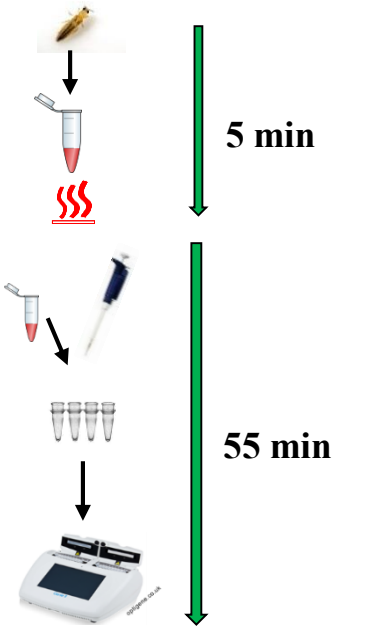


Source:
Eppendorf-Tube, https://openclipart.org
PCR-Tubes, www.vwr .com
Pipette, http://bmskgroup.com
T. palmi: http://mrec.ifas.ufl.edu

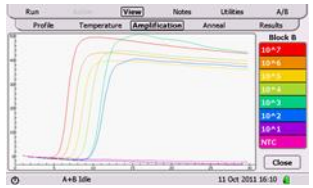
1. Introduction

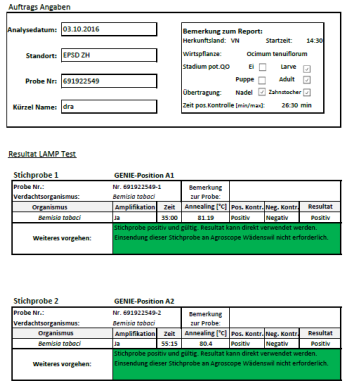
Modification for on-site application

<u>Goal:</u> Performable for Plant health inspectors with minimal laboratory training



- Simplification of handling (**1-pipetting-step**)
- Production of pre-mixed LAMP kits
- Staining of chemicals (Cresol Red)
- Simple interpretation of results





1. Introduction

Modification for on-site application

Bericht - LAMP Importuntersuchung

Auftrags Angaben

Analysedatum: 24.11.2017

Standort: EPSD ZH

Probe Nr: 932984420

Kürzel Name: sdc

Bemerkung zum Report:

Herkunftsland: XA Startzeit: 14:35

Wirtspflanze: Ocimum basilicum green

Stadium pot.QO Ei ☐ Larve ☑

Puppe ☐ Adult ☐

Übertragung: Nadel ☐ Zahnstocher ☑

Zeit pos.Kontrolle [min/max]: 27:30 min

1. Introduction

Resultat LAMP Test

Stichprobe 1 **GENIE-Position A1**

Probe Nr.:	Nr. 932984420-1		Bemerkung zur Probe:			
Verdachtsorganismus:	*Bemisia tabaci*					
Organismus	**Amplifikation**	**Zeit**	**Annealing [°C]**	**Pos. Kontr.**	**Neg. Kontr.**	**Resultat**
Bemisia tabaci	Ja	56:15	81.35	Positiv	Negativ	Positiv
Weiteres vorgehen:	Stichprobe positiv und gültig. Resultat kann direkt verwendet werden. Einsendung dieser Stichprobe an Agroscope Wädenswil nicht erforderlich.					

Stichprobe 2 **GENIE-Position A2**

Probe Nr.:	Nr. 932984420-2		Bemerkung zur Probe:			
Verdachtsorganismus:	*Bemisia tabaci*					
Organismus	**Amplifikation**	**Zeit**	**Annealing [°C]**	**Pos. Kontr.**	**Neg. Kontr.**	**Resultat**
Bemisia tabaci	Ja	50:00	81.8	Positiv	Negativ	Positiv
Weiteres vorgehen:	Stichprobe positiv und gültig. Resultat kann direkt verwendet werden. Einsendung dieser Stichprobe an Agroscope Wädenswil nicht erforderlich.					

2. Implementation at the airports

Installation of workstation:

2. Implementation at the airports

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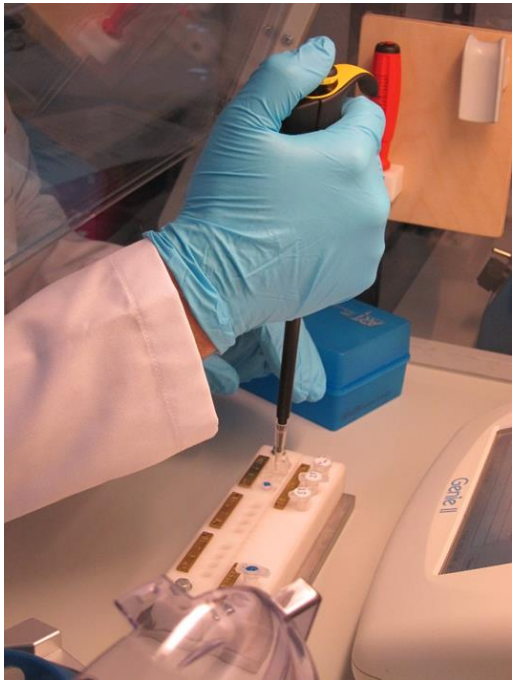
2. Implementation at the airports

Knowledge transfer, training and certification:

- Theoretical basics of LAMP Method
- Introduction into basic laboratory work
- Guided performance of LAMP assay
- Safety instructions
- Test for certification



Zertifikat

Hiermit bestätigen wir, dass Frau

Annette Rudin Moor

Die GENIE II® LAMP-Analyse Qualitätskontrolle am Flughafen Zürich erfolgreich durchgeführt und bestanden hat.

Bern, 19.10.2015

Dr. Andreas von Felten
Bundesamt für Landwirtschaft

Dr. Jürg E. Frey
Agroscope Wädenswil

Simon Blaser
Bundesamt für Landwirtschaft

2. Implementation at the airports

Validation:

Phase I:

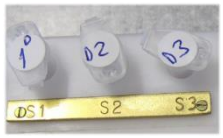
- First 10 samples per organisms and point of entry (ZH/GE) were checked at Agroscope by Sanger sequencing
- If the all 10 results per organism are correct → test will be considered as validated

Phase II:

- Positive result → inspectors can directly apply measures based on the LAMP result
- Negative result → sample must be sent to Agroscope for further analyses

Quality control:

- Blind-test



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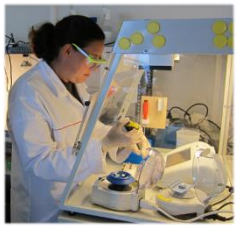


2. Implementation at the airports

Validation data airport of ZH (09/2016):

LAMP Kit	No. of analyses	No. of correct-positive results	No. of false-positive results	No. of correct-negative results	No. of false-negative results
Bactrocera Triplex [1]	15	12	0	3	0
B. tabaci	15	15	0	0	0
Liriomyza sativae	6	1	0	5	0
Liriomyza duplex [2]	7	6	0	1	0
T. palmi	3	3	0	0	0
Total	46	37	0	9	0

[1] includes *B. dorsalis* group, *B. latifrons / cucurbitae*, *B. correcta / zonata*

[2] includes *L. trifolii / huidobrensis*

2. Implementation at the airports

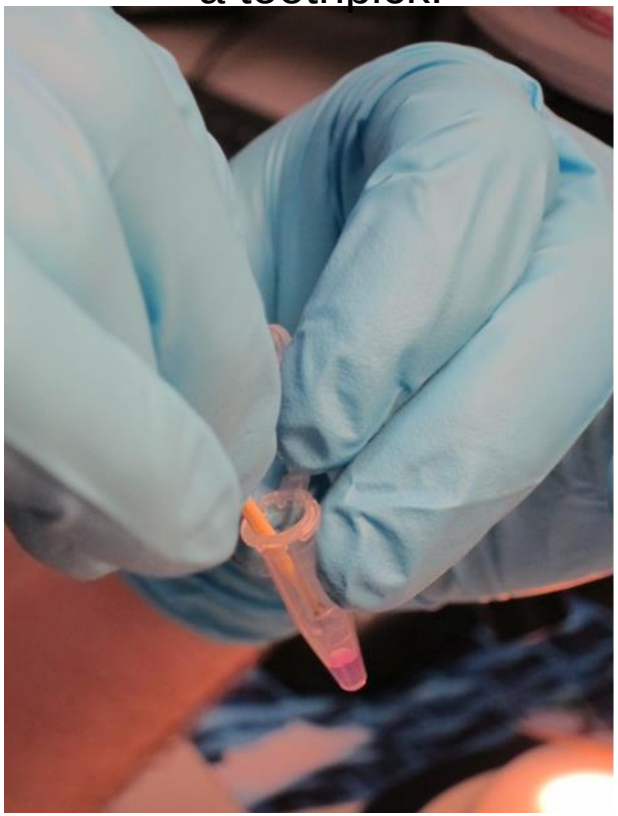
Information about analysis performed 2017:

POE	Bactrocera Triplex	Bremisia tabaci	Liriomyza sativae	Liriomyza Duplex	Thrips palmi	Result
Geneva	6	2	0	0	5	True positive
	0	0	0	0	0	True negative
Zürich	6	8	0	1	6 (1)	True positive
	1	0	2	1	0	True negative

3. Conclusions

Preparation of samples is challenging:

- Caching a *Thrips* and transferring it into the reaction solution with a toothpick.



➢ **Adapted procedure:**

Use of acupuncture needle

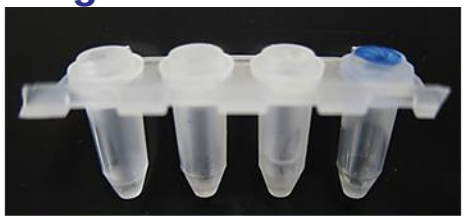
3. Conclusions

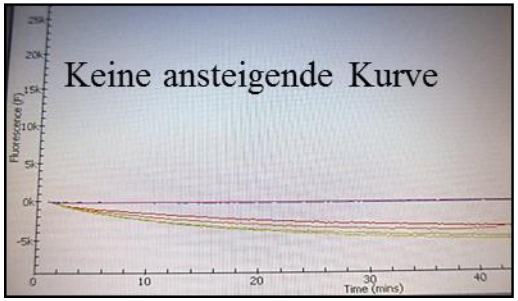
Kit had to be adapted (early stage):

1. Kit

3x Samples

1x negativ control

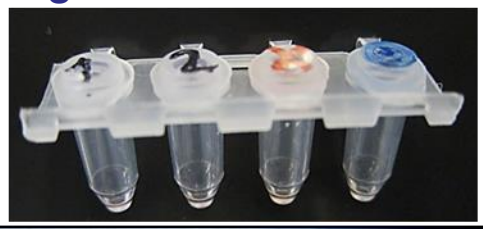


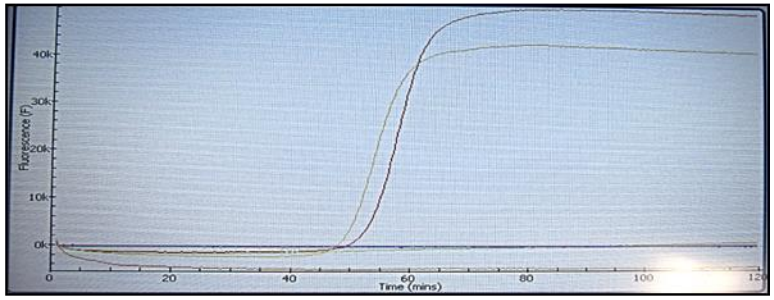


Same picture if test would not work

2. Kit

2x Samples
1x positive control
1x negative control





It is easy to detect for the inspector, that the reaction worked.

3. Conclusions

- LAMP assays are suitable tools for on-site diagnostics
- Allow reliable differentiation between regulated and non-regulated organisms within 1 hour to 2 hours
- LAMP assays were shown to be 100% specific
- DNA amplification based technology → always a risk of false-negative results due to the appearance of previously unknown pest biotypes

4. Outlook

- **Finishing the Validation in Geneva**
- **Developing new LAMP assays for relevant quarantine pests (e.g. *Spodoptera frugiperda, Spodoptera litura, Keiferia lycopersicella)***
- **Increase the knowledge of the inspectors to identify quarantine pests.**
- **Development of a standard Blind- Test for the evaluation of the inspectors.**
- **Strengthen the collaboration with other POE who are using LAMP technology or are interested in using LAMP technology.**

Acknowledgments

Hanspeter Diem[1], **Simon Blaser[2]**, Jürg E. Frey[2], Andreas Bühlmann[3,]

[1]Federal Office for Agriculture, Swiss Federal Plant Protection Service (SPPS)

[2]Agroscope, Method Development and Analytics, Waedenswil

[3]Agroscope, Plant and Plant Products, Waedenswil

Thank you for your Attention