Introduction of the webinar and training activities

The concept of test validation in Plant Health

Webinar 1	What is test validation and why it matters for reliable diagnostics?	Monday 11th January, 2 pm
Webinar 2	**How to adopt a new test in your laboratory?**	**Friday 15th January, 2pm**
Webinar 3	The use and validation of on-site tests	Wednesday 20th January, 2pm
Practical training session 1	Analysis of performance characteristics	Tuesday 26th of January, 2pm to 4:30 pm
Webinar 4	How do companies handle quality control and validation of products and how will the EPDIA charter help in improving this task?	Monday 1st of February, 2pm
Webinar 5	Why is communication on test selection between risk managers and diagnostic laboratories important ?	Monday 15th of February, 2pm
Practical training session 2	The use of kits: training and demonstration	Thursday 22nd of April, 2pm

Valitest

VALITEST webinar series and training activities

How to adopt a new test in your laboratory?

January 15th, 2021

Tanja Dreo, National Institute of Biology

Mathieu Rolland, ANSES

Denise Altenbach, BIOREBA

Camilo Gianinazzi, IpadLab

Marta Santos, ClearDetections

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N° 773139

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Outline

- the **process** and **adopting a real-time PCR** for detection of Stewart‘s wilt in maize seeds
- some adoption **difficulties** identified from the **laboratory**'s point of view
- how to adopt (or re-adopt) **a commercially available ELISA Kit** that was subject to a modification?
- Switch **from a molecular internal method to** the use of a **commercial kit**
- **from morphological to real-time PCR** quantification in nematodes
- Q&A

Poll

Does your laboratory perform tests under ISO 17025 accreditation and have you adopted a new test in the past two years?

select one answer

- we do not test (ISO17025) & have not adopted a test
- we do not test (ISO17025) & have adopted a test
- we test (ISO17025) & have not adopted a test
- we test (ISO17025) & have adopted a test

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The process & adopting a real-time PCR for detection of Stewart‘s wilt in maize seeds

Tanja Dreo

This project has received funding from the European Union’s Horizon 2020 research and innovation program under grant agreement N° 773139

risk of introduction with contaminated seeds

Chaetocnema pulicaria

real-time PCR (Tambong *et al.*, 2008)

real-time PCR (Pal *et al.*, 2019)

- **low concentrations** in seed
- **differentiation from *P. s.* subsp. *indologenes***

Source of distribution map: EPPO Global Database; Image: http://bugguide. net /node/view/3531963.

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PM 7/98 (4) Specific requirements for laboratories preparing accreditation for a plant pest diagnostic activity

identify test:
organism, matrix, test

define intended use

define adequate performace characteristics

review of validation or other data already available

review of possible altered conditions when performing a test with validation data available

risk analysis

NIB

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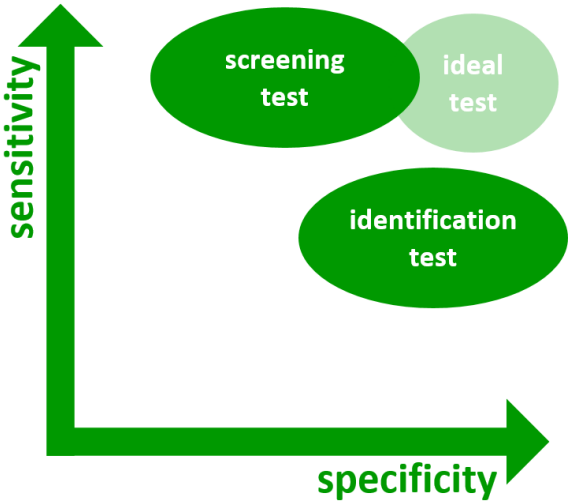
What do I need?
test = HO + method + matrix

- define **diagnostic parameters in advance**



- analytical sensitivity
- analytical specificity
- repeatability
- reproducibility
- diagnostic sensitivity
- diagnostic specificity

- intended use e.g. **zero tolerance**/qualitative pathogens
- **appropriate (fit for purpose)**
- **communication with risk managers**

Find a test
Review its diagnostic parameters

- legislation
- international guidelines (ISPM, EPPO, ISTA)

- literature search (scientific publications, reports)
- web search for kits

https://dc.eppo.int/validation_data/validationlist

?

- time consuming
- low comparability

Review

- to what extent it is validated? *crucial diagnostic parameter not determined?*
- do the diagnostic parameters correspond to my requirements?
- how similar it is to my test? *Δmatrix?*
- what else will I modify?

risk analysis

PM 7/98 (4) Specific requirements for laboratories preparing accreditation for a plant pest diagnostic activity

documentation on conclusions
(what to evaluate and to what extent)

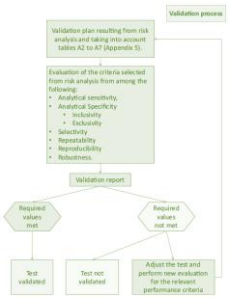
relevant performance characteristics NOT available

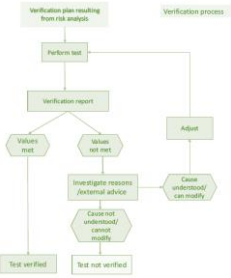
not all relevant performance characteristics available

all relevant performance characteristics available

validation

verification





NIB

Valitest

Poll

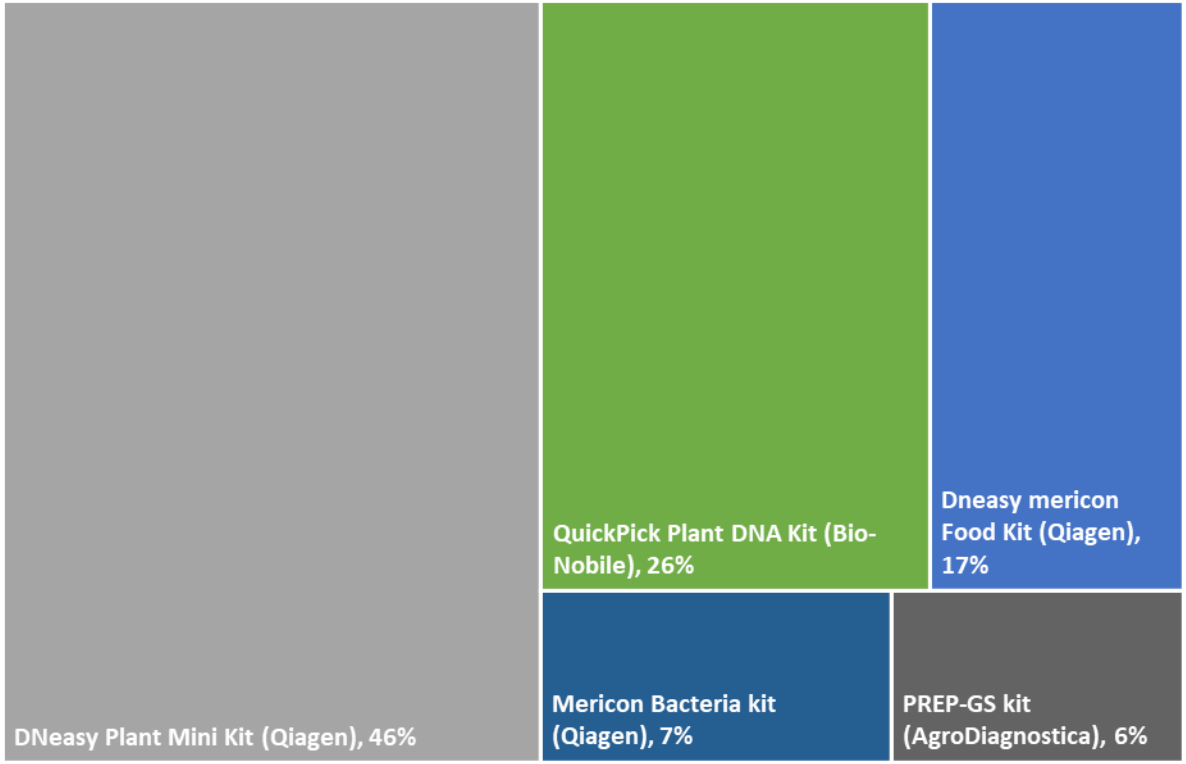
Which sources do you commonly check for validation data?

more answers possible

- international standards (ISPM, EPPO, ISTA)
- EPPO database on validation
- scientific literature
- companies
- colleagues

Common modification: changing DNA extraction

- DNA extraction

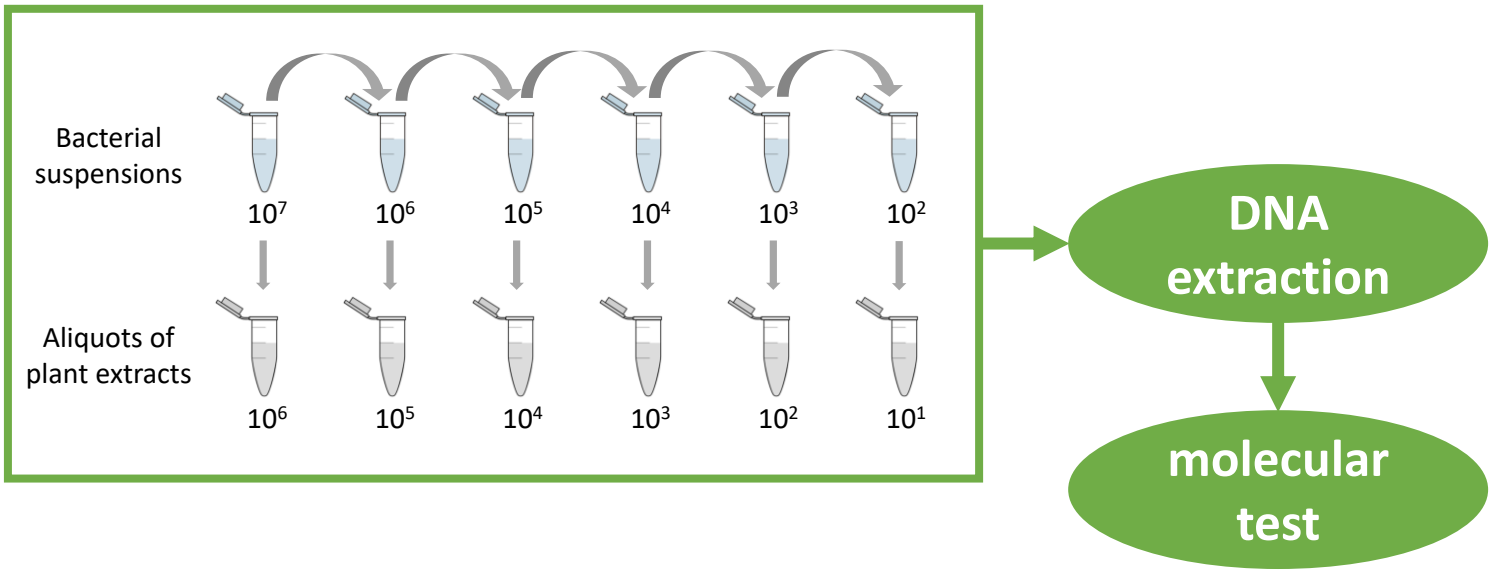


Dreo, T. Report on the results of the test performance study for molecular detection of Pantoea stewartii subsp. stewartii in asymptomatic plant material (maize seeds), v1.0, Valitest, GA n°773139. National Institute of Biology, Ljubljana, 2020.

Example: analytical sensitivity – validation*

- DNA extraction is a critical step however, it can only be assessed in combination with another test

3x



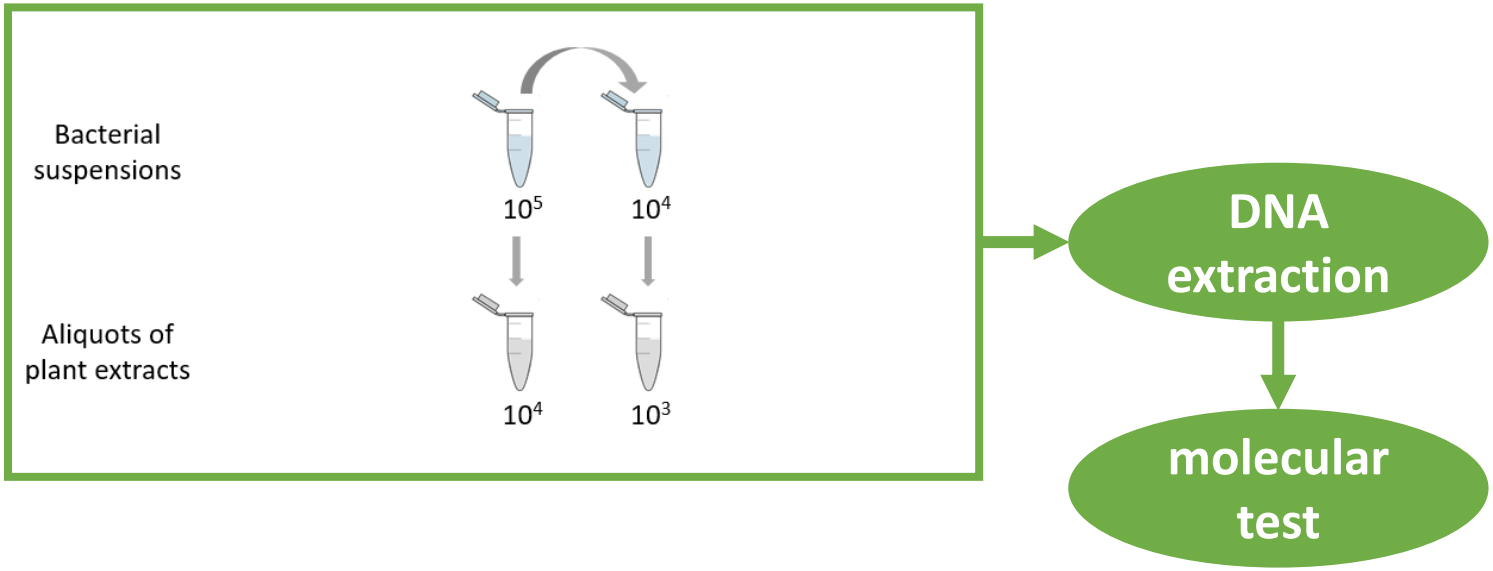
- additional data → verification possible

EPPO PM 7/98: Analyse at least three series of spiked sample extracts with a range of 101–106 cells of the target organism per mL. Preferentially, this is done by making decimal diluted cell suspensions of the target bacterium in the sample extracts. Determine the lowest cell density giving a positive test result. If consistent results are not obtained after three series, then additional series should be prepared and tested.

Example: analytical sensitivity – verification**

- focus on Δ concentrations of interest

8x



***EPPO PM 7/98: Analyse at least eight samples at the established limit of detection (for viruses, viroids and phytoplasma this should be at low level).*

Validation

- QuickPick Plant Mini Kit (Bionobile)
- automated on KingFisher mL

- real-time PCR for detection of *Pantoea stewartii* subsp. *stewartii* on maize seeds (Tambong *et al*., 2008)

Concentration		Method and test		
		real-time PCR		
[cells/mL]	log[cells/mL]	Tambong_2008 _cps		
Analytical sensitivity: DNA				
100000000	8.0	+		
10000000	7.0	+		
1000000	6.0	+		
100000	5.0	+		
50000	4.7	+		
10000	4.0	+		
5000	3.7	+		
1000	3.0	(+)		
500	2.7	(+)		
100	2.0	(+)		
10	1.0	-		
0	0.0	-		
Analytical sensitivity: plant material (3 standard curves)				
10000000	7.0	+	+	+
1000000	6.0	+	+	+
100000	5.0	+	+	+
10000	4.0	+	+	+
5000	3.7	+	+	+
1000	3.0	(+)	(+)	+
500	2.7	(+)	(+)	(+)
100	2.0	-	-	(+)
50	1.7	-	-	-
0	NA	-	-	-

Dreo, T. Report on the results of the test performance study for molecular detection of Pantoea stewartii subsp. stewartii in asymptomatic plant material (maize seeds), v1.0, Valitest, GA n°773139. National Institute of Biology, Ljubljana, 2020.

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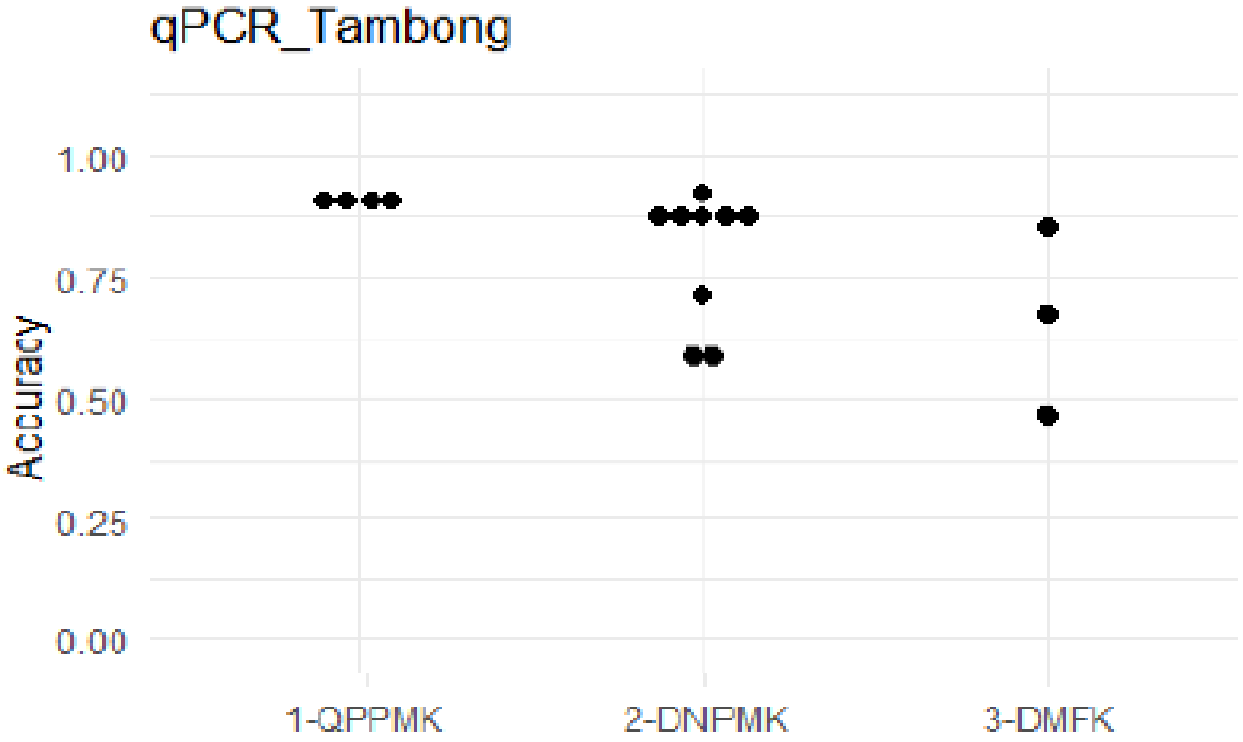


Accuracy achieved in real-time PCR tests based on Tambong et al. (2008), depending on the DNA extractions. Each dot represents one valid data set. Legend: 1-QPPMK = QuickPickTM SML Plant DNA Kit (Bio-Nobile), 2-DNPMK = DNeasy Plant Mini Kit (Qiagen), 3-DMFK = Dneasy mericon Food Kit (Qiagen). Disclaimer: the results only reflect the specific study case and only the results on reagents at the time when they were included in the study.

Dreo, T. Report on the results of the test performance study for molecular detection of Pantoea stewartii subsp. stewartii in asymptomatic plant material (maize seeds), v1.0, Valitest, GA n°773139. National Institute of Biology, Ljubljana, 2020.

Common modification: changing mastermix

- **real-time PCR (Pal *et al*., 2019)**, detection of *P. stewartii* subsp. *stewartii* and its differentiation from subsp. *indologenes*
- **SybrGreen** based

Criteria for acceptable modification

✓differentiation of the two subspecies

✓expected analytical sensitivity, similar to other tests

Dreo, T. Report on the results of the test performance study for molecular detection of *Pantoea stewartii* subsp. *stewartii* in asymptomatic plant material (maize seeds), v1.0, Valitest, GA n°773139. National Institute of Biology, Ljubljana, 2020.

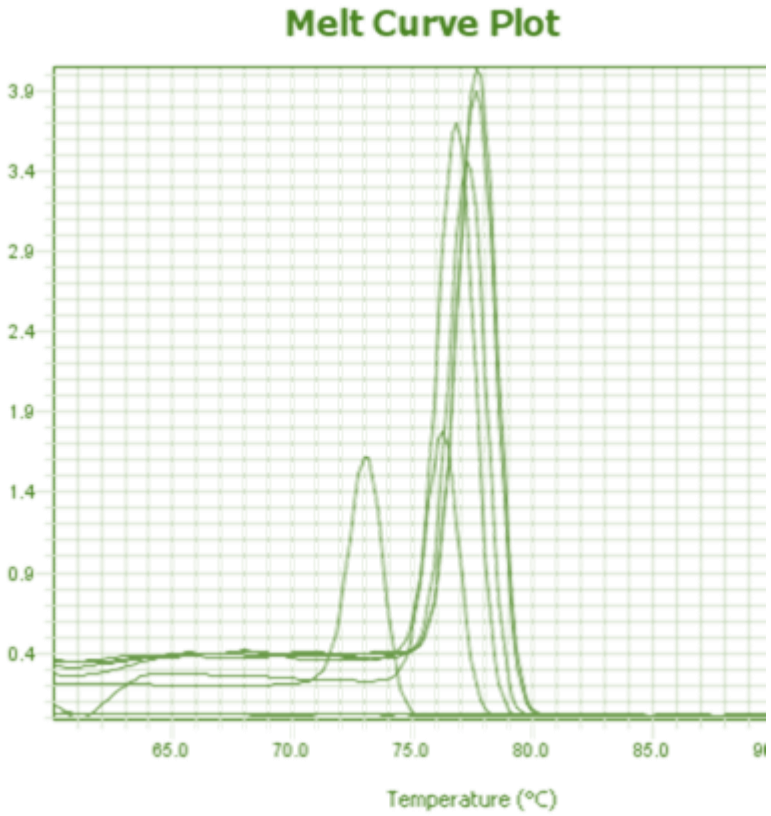
original publication	modifications
B-R SYBR Green Supermix for iQ Systems (Quanta Biosciences, Beverly, MA)	**2xPower SybrGreen PCR MasterMix (ThermoFisher Scientific, 4367659)**
CFX96 (BioRad)	**ViiA7 (Life Technologies)**
Amplification program: **95°C 3 min** 40 cycles: 95°C 15 sec, **68°C** 45 sec **T ramping: ND** Tm (65 °C → 95 °C, 0.5)	Amplification program: **95°C 10 min** 40 cycles: 95 °C 15 sec, **65 °C** 45 sec **T ramping: 1.6 °/sec** Tm (65 °C → 95 °C, 0.5)
400 nM F/R **1 µL DNA/10 µL rxn**	400 nM F/R **4 µL DNA/20 µL rxn**

Dreo, T. Report on the results of the test performance study for molecular detection of *Pantoea stewartii* subsp. *stewartii* in asymptomatic plant material (maize seeds), v1.0, Valitest, GA n°773139. National Institute of Biology, Ljubljana, 2020.

P. stewartii **subsp.** ***stewartii***

P. stewartii **subsp.** ***indologenes***

gradient PCR (60-68 °C) → **Tm**



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Concentration		Method and test			Method and test		
		real-time PCR			real-time PCR		
[cells/mL]	log[cells/mL]	Tambong_2008_cps			Pal_2019		
Analytical sensitivity: DNA							
100000000	8.0	+			+		
10000000	7.0	+			+		
1000000	6.0	+			+		
100000	5.0	+			+		
50000	4.7	+			+		
10000	4.0	+			+		
5000	3.7	+			+		
1000	3.0	(+)			+		
500	2.7	(+)			+		
100	2.0	(+)			-		
10	1.0	-			-		
0	0.0	-			-		
Analytical sensitivity: plant material (3 standard curves)							
10000000	7.0	+	+	+	+	+	+
1000000	6.0	+	+	+	+	+	+
100000	5.0	+	+	+	+	+	+
10000	4.0	+	+	+	+	+	+
5000	3.7	+	+	+	+	+	+
1000	3.0	(+)	(+)	+	+	+	+
500	2.7	(+)	(+)	(+)	+	+	+
100	2.0	-	-	(+)	(+)	(+)	-
50	1.7	-	-	-	-	-	-
0	NA	-	-	-	-	-	-

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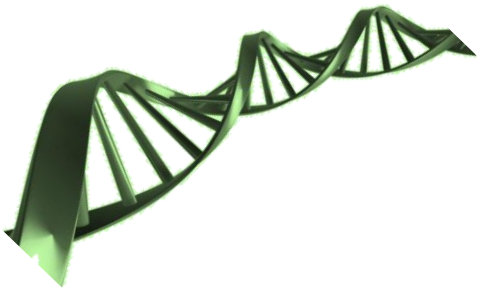
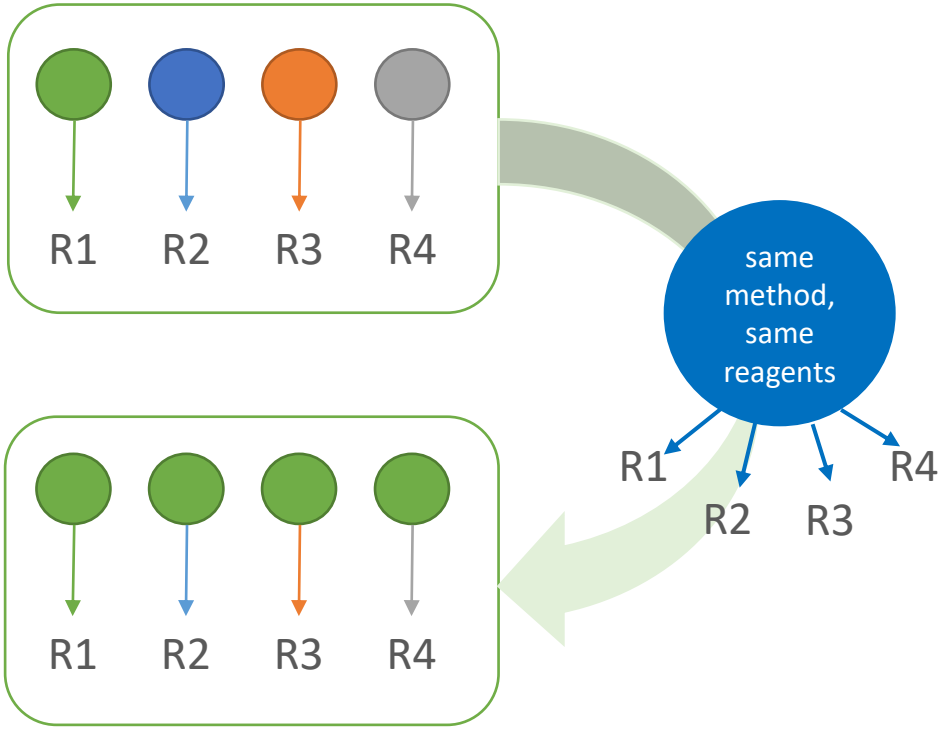
Reporting and making conclusions on the tests

- check **performance against initially set criteria**
- write a report
- **formal decision to adopt a test**
- **(external audit)**
- please, **submit to EPPO validation database**

Reference materials

- samples used in verification/validation
- can be of different complexity
- **synthetic dsDNA**





Some adoption difficulties identified from the laboratory's point of view

Mathieu Rolland

anses
AGENCE NATIONALE DE SÉCURITÉ SANITAIRE de l'alimentation, de l'environnement et du travail

This project has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement N° 773139

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Setting up analyses on *Xylella fastidiosa* by ELISA to reduce the cost of official controls

- OD varies between host species
- requires **negative and positive reference material for all the host species**

Xylella fastidiosa by PCR: CTAB extraction required for oak and olive tree samples

- Move **from automated to manual extraction**
- New products to handle: Health and safety

Training, timing (compliance with deadlines)

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The difficulties / How to overcome them

- Validation/Verification project management
  - Before starting, save time to make sure that you have all you need: protocol / biological materiel / equipment
  - Make sure that the deadline can be met
- Reference Material
  - Commercially available: Companies, Biological resources centers
  - Network
- Validation/Verification:
  - make sure that all the steps are interoperable (sampling, DNA extraction)
  - When difficulties occur, seek assistance from the supplier
- Training of the staff
- Regular quality controls, proficiency tests…

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How to adopt (or re-adopt) a commercially available ELISA Kit that was subject to a modification?

Denise Altenbach

BIOREBA
Your Partner in Agro-Diagnostics

This project has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement N° 773139

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Serological tests -> what to do if a commercial ELISA test kit is subject to a change/modification?

- Manufacturers view
- Testing laboratory view (with accreditation (ISO/IEC 17025))

- Are the performance characteristics provided by the kit producer sufficient for my laboratory / my application?
- Is there a gap? Accreditation requirements? Modification requirements?
- Verification in your laboratory sufficient?
- Validation needed? Collaboration with a kit producer?

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Certified Management System SQS ISO 9001

SWISS ACCREDITATION sas.admin.ch STS 0541

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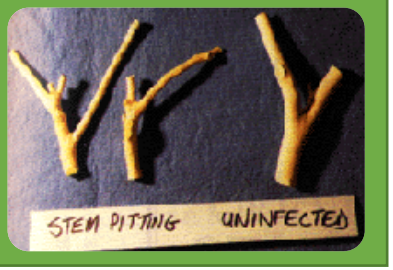
Example: CTV ELISA



- Reason: **Our existing stock of reagents depleted**. Usually stock for many years!
- **New antibody production**: In the frame of a CRADA (Cooperative Research and Development Agreement) project. Development & validated in collaboration with the laboratory of Dr. Richard Lee (USDA, Riverside, CA, USA).
- **Goal: Develop a novel DAS-ELISA reagent for «broad-spectrum» detection of CTV**.
- Approach: New antibodies were raised **against recombinant coat protein** (Iracheta-Cardenas et al., 2008).
- The **validation of the new reagents was done in collaboration with the laboratory** of Manjunath Keremane (National Clonal Germplasm Repository for Citrus and Dates, riverside, CA, USA). They possess the biggest collection of CTV-infected trees in the world (from CCTEA: Central California Tristeza Eradication Agency).

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Iracheta-Cárdenas M., Sandoval-Alejos B.D., Román-Calderón M.E., Manjunath K.L., Lee R.F. and Rocha-Peña M.A., 2008. Production of Polyclonal Antibodies to the Recombinant Coat Protein of *Citrus tristeza virus* and their Effectiveness for Virus Detection. Journal of Phytopathology 156: 243-250.

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The challenge for the manufacutrer

Challenges for the manufacturer:

- Define the **approach**: antibody against rec. coat protein? Classical approach with virus purified from infected host plant?
- Find a **good partner for validation**
- **Reference material**
- **Meet the requirements** of testing laboratories: worldwide
- Participation in **test performance study (TPS) and/or proficiency test (PT)**

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Product Information: DAS-ELISA

Citrus tristeza virus (CTV)

CTV (2) occurs worldwide wherever citrus is growing and is the economically most important viral disease of citrus. It has a narrow range of hosts mostly restricted to citrus species. The virus is transmitted by aphids in a semi-persistent manner. Important aphids are "the brown citrus aphid" (Toxoptera citricida) and "the melon aphid" (Aphis gossypii). There is a great diversity of symptoms; the severity depends on the virus strain and on the host (often also on the scion-rootstock combination). Symptoms include declining (e.g. on sour orange rootstocks), seedling yellows, stem pitting, loss of vigour, wilting, stunting, yellow-brown staining at bud union and on inner surface of bark, etc. up to complete die-back (e.g. of limes irrespective of rootstocks).

Specificity and sampling instruction

Polyclor… in
DAS-EL… m
differen… s,
conscio… ls
and but… or
autumn… nt
locatior… er
«Gener…

The pro… al
Clonal …

Product Information
Version: x – date
Adaptations from last version:
new polyclonal broad-spectrum antibodies

Verification? or **Validation?**

Further challenges for the testing laboratory and how to overcome it

- Access to reliable reference material
- Access / participation to TPS and/or PT
- **Contact your manufacturer for additional results if required**

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Switch from a molecular internal method to the use of a commercial kit

Camilo Gianinazzi

Ipa&d lab
INTERNATIONAL PLANT ANALYSIS AND DIAGNOSTICS

This project has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement N° 773139

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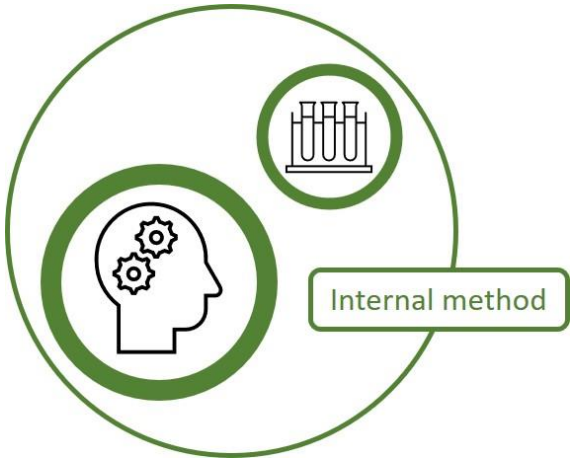
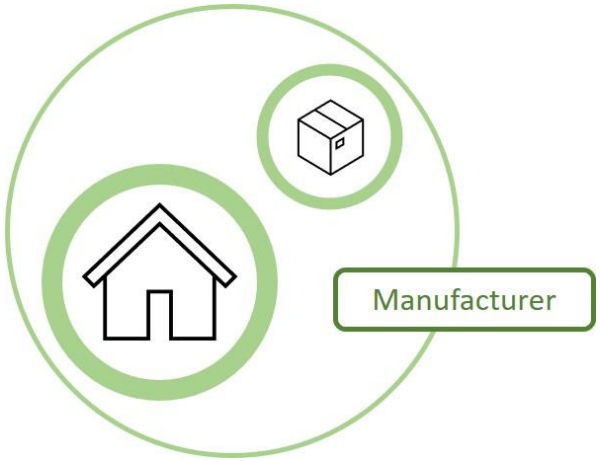


- Reference material
- Multiple suppliers
- Suppliers' selection and monitoring
- Quality control on several reagents

Time consuming
High staff effort



- One supplier
- Quality control on one reagent
- Lot-to-lot consistency

Time saving
Low staff effort

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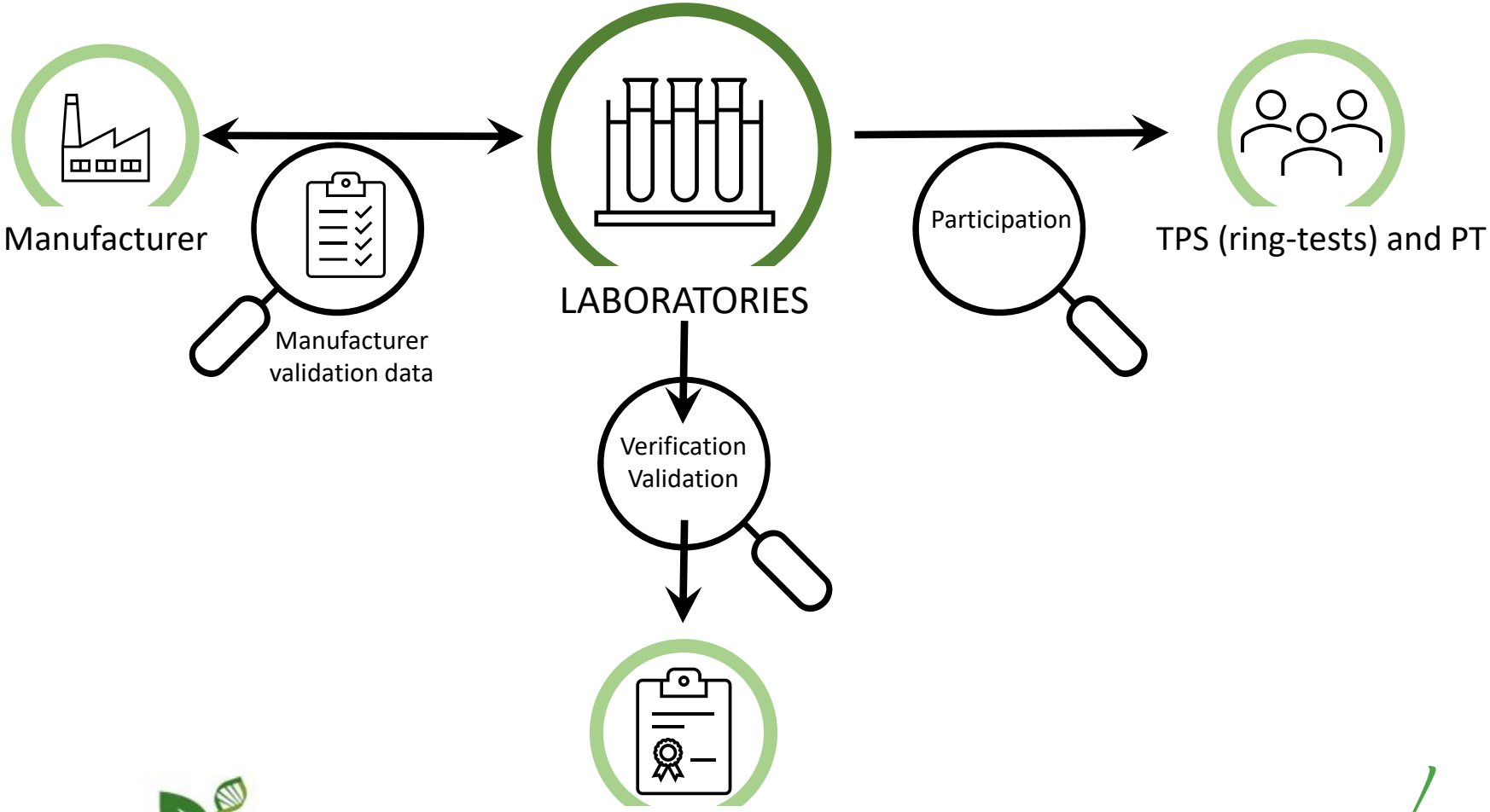
INTERNATIONAL PLANT ANALYSIS AND DIAGNOSTICS

Valitest

The Challenge for Laboratories



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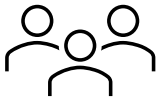
The constrains for Laboratories

Manufacturer validation data

Constrains

1. Sufficient performance data may not be available
2. Industrial secret on some components
3. Reference and pubblication may not be available



TPS (ring-tests) and PT participation

Constrains

1. Costs to participate
2. Get the information
3. Mainly at the national level

Accreditation requirements

Constrains

1. Validation data may not be always available
2. Validation procedures
3. Different requirements from the national accreditation bodies

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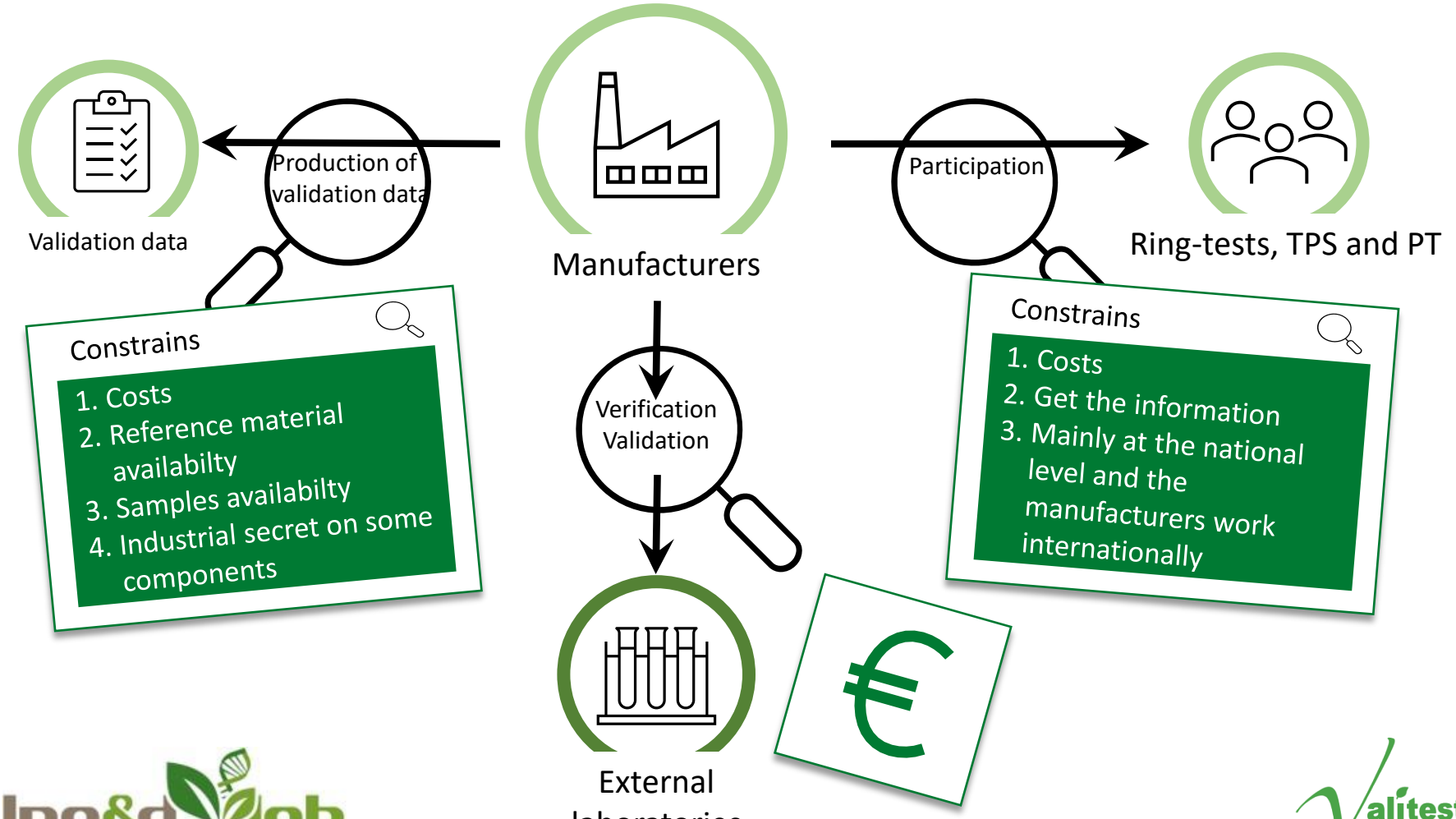
The challenge and constrains for Manufacturers



EXAMPLE: IpadLab kit for Flavescence dorée and Bois noir

qPCR kit for the simultaneous detection of Flavescence dorée, Bois noir and Internal Control (IC)

Triplex Real-Time PCR
(FAM, Vic, Cy5)

Manufacturer validation

Laboratories external validation

Start of the commercialisation

Validation data from Euphresco Grafdepi project TPS

Method mentionned in Appendix 6 of the PM7/079 (2) Grapevine Flavescence dorée phytoplasma, EPPO Bulletin (2016) 46 (1), 78-93

First laboratories accreditation on ISO 17025

2010

2011

2013

2016

2021

Ipa&d Lab
INTERNATIONAL PLANT ANALYSIS AND DIAGNOSTICS

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From morphological to real-time PCR quantification in Nematodes

Marta Santos Paiva

Clear® Detections
revolutionary diagnostics

This project has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement N° 773139

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The question

How to go from visual quantification under the microscope for *D. dipsaci* to an automated qPCR method?

HLB
research and consultancy in agriculture

www.hlbbv.nl

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revolutionary diagnostics

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The challenge

Gather Reference Material

- Enough identified and quantified reference material

Fit the requirements imposed by the diagnostic

- Biological relevance vs practical DNA extraction methods

Standardize molecular analysis

- Intrinsic variation in DNA extraction & PCR reaction

Comply with accreditation criteria

- Information about the validation & implementation
- Quality control

Two methods based on different principles will never generate the exact same result!

Clear® Detections revolutionary diagnostics

HLB research and consultancy in agriculture

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The approach

Comparison studies between the laboratories

Customization of ClearDetections DNA extraction method fit to purpose

Use of ClearDetections validated species specific primers

Implementation of ClearDetections calibration lines

Generation of the remaining verification / validation by HLB

Supply reagents with quality assurance by ClearDetections

ClearDetections revolutionary diagnostics

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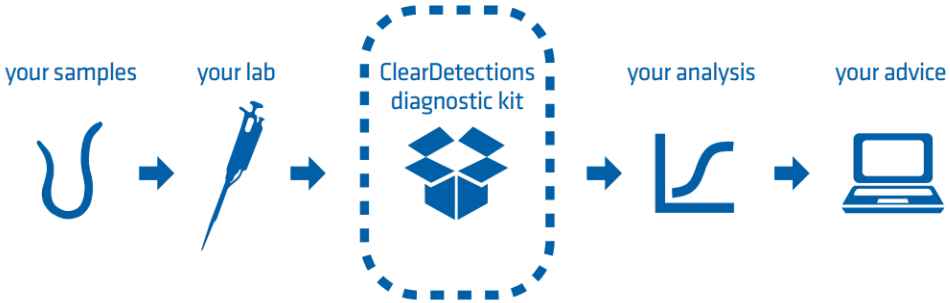
The result

A molecular quantification method for *D. dipsaci* using ClearDetections kits

- Accredited by ISO 17025:2005
- Gives a level of infestation with enough detail to proceed with advice
- Increased the capacity of analysis of the laboratory in at least 3 times



Special Thanks to Phyto laboratory of HLB for the collaboration on this presentation!

ClearDetections revolutionary diagnostics

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Poll

Do you use kits in your accredited activity?

Conclusions

The decision of adopting a new test is complex and should reflect the best compromise between:

- Requirements (Fit for purpose and meeting your critical points)
- Your time and resource constraints
- Necessity to be Verified or even Validated

There are several challenges to the implementation such as:

- Availability of reference material
- Dispersion of information
- Different requirements from accreditation bodies
- Lack of availability of TPS and Proficiency tests and information about them

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Conclusions

Key points to facilitate the implementation of a new method are:

- Collaboration between kit providers & laboratories
- Communication between stakeholders (laboratories, companies, EURL, NPPO, EPPO)
- Use of Reagents with Quality assurance
- Sharing of Validation data with community

Thank you for your attention!

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https://www.valitest.eu/training/activities_and_webinars

Webinar 1	What is test validation and why it matters for reliable diagnostics?	Monday 11th January, 2 pm
Webinar 2	How to adopt a new test in your laboratory?	Friday 15th January, 2pm
Webinar 3	**The use and validation of on-site tests**	**Wednesday 20th January, 2pm**
Practical training session 1	Analysis of performance characteristics	Tuesday 26th of January, 2pm to 4:30 pm
Webinar 4	How do companies handle quality control and validation of products and how will the EPDIA charter help in improving this task?	Monday 1st of February, 2pm
Webinar 5	Why is communication on test selection between risk managers and diagnostic laboratories important ?	Monday 15th of February, 2pm
Practical training session 2	The use of kits: training and demonstration	Thursday 22nd of April, 2pm

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