Introduction of the webinar and training activities

Test Performance Studies organisation

Videos	What is a TPS?	On the week 02/15
Videos	VALITEST TPS: selection of the pests and of the TPS organizers	On the week 02/15
Webinar 1	Preparing the TPS plan	Friday 19/02, 11am
Webinar 2	Selection of the tests and associated documents	Wednesday 24/02, 2pm
Webinar 3	Selection of participants and contract	Monday 1/03, 2pm
Webinar 4	Preparation and dispatch of samples	Friday 5/03, 11am
Webinar 5	Production of reference material for TPS	Wednesday 10/03, 2pm
Practical training sessions	How to organise Test Performance Studies?	15-18/03 (4 sessions)
Webinar 6	How to tackle the analysis of TPS results?	Monday 22/03, 2pm
Videos	Calculate performance characteristics of a test and get useful information from your validation data by statistical analysis.	On the week 22/03
Webinar 7	Q&A session: the statistical analysis of TPS results	Monday 29/03, 2pm
Practical training sessions	How to analyse the results of Test Performance Studies?	30/03-1/04 (3 sessions)
Webinar 8	**From TPS organisation to analysis of the results: example of the TPS on ToBRFV**	**Wednesday 7/04, 2pm**

Valitest

VALITEST webinar series and training activities
Test Performance Studies organisation

From TPS organisation to analysis of the results: example of the TPS on ToBRFV

7 April 2021

Francesco Faggioli – francesco.faggioli@crea.gov.it

Marta Luigi – marta.luigi@crea.gov.it

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

crea
Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria

Valitest

VALITEST project

VALITEST aims at producing validation data for the detection and identification of plant pests. Two rounds of test performance studies (TPS) have been organized:

- The first round of TPS organized in 2019 targeted six pests identified as important by the consortium before the beginning of the project

Erwinia amylovora

Pantoea stewartii subsp. *stewartii*

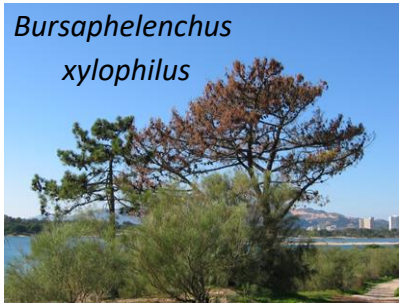
NIB NATIONAL INSTITUTE OF BIOLOGY

Fusarium circinatum

fera

citrus tristeza virus

Bursaphelenchus xylophilus



anses

plum pox virus

Netherlands Food and Consumer Product Safety Authority
Ministry of Agriculture, Nature and Food Quality

Valitest

VALITEST project

- For the second round of TPS organized in 2020, the pests were selected based on the needs expressed by various stakeholders in order to better align validation priorities with stakeholders needs and with the market

Xanthomonas citri pv. *citri*

plum pox virus
(on-site detection)

tomato brown rugose fruit virus

Xylophilus ampelinus

tomato spotted wilt tospovirus

Cryphonectria parasitica

Why to organise a Test Performance Study (TPS)?

TPS is the evaluation of the performance of one or more tests by two or more laboratories using defined samples (EPPO - PM7/76 (5))

According to EPPO Guidelines PM7/122 (1):
Test performance studies provide added value to the validation process.

MAJOR objectives of a TPS

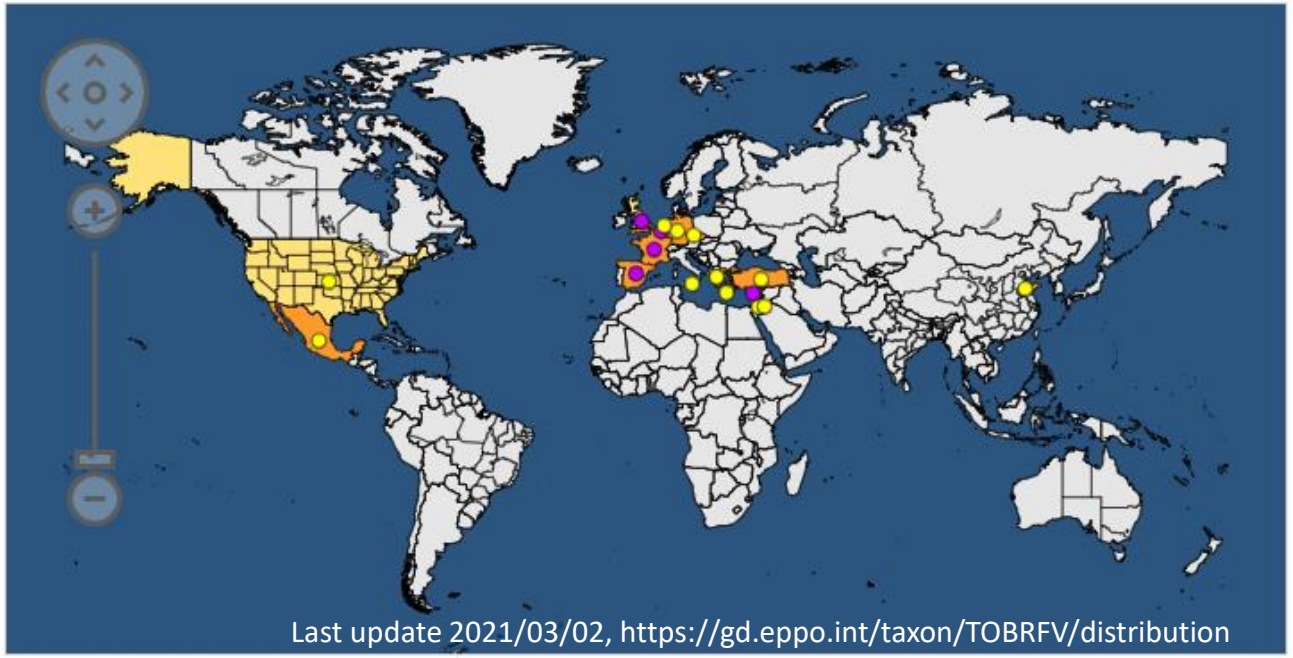
- Establishment of the effectiveness of a test
- Establishment of uncertainty levels
- Evaluation of the performance characteristics of a test
- Establishment of the comparability of tests

MINOR objectives of a TPS

- Evaluation of the accuracy of the results produced by laboratories for specific tests and monitoring laboratories' continuing performance
- Provision of additional confidence to laboratory customers

Why on ToBRFV?

The disease



- Starting from Israel and Jordan in 2014, rapidly spread worldwide
- January 2019: EPPO Alert list - COMMISSION IMPLEMENTING REGULATION (EU) 2019/1615
- August 2020: EPPO A2 list - COMMISSION IMPLEMENTING REGULATION (EU) 2020/1191
- January 2021: EPPO A2 list - COMMISSION IMPLEMENTING REGULATION (EU) 2021/74

Valitest

Symptoms

Taxonomy

Family: *Virgoviridae*

Genus: *Tobamovirus*

Host

- *Solanum lycopersicum*
- *Capsicum annuum*

Trasmission

- Seeds
- Contact
- *Bombus terrestris*

Valitest

A TPS on an emerging pathogen

PROS

- Early establishment of the performance of tests
- Provision of expertise to the participants
- Early establishment of containment measures

CONS

- Participants have small expertise on the specific pathogen
- Low number of tests developed
- Years of use provide evaluation of robustness of a test

Main workflow for TPS organization

1 - Scope definition

2 - Test selection

3- Participants selection

4 - Samples preparation and dispatch

5 - Analysis of the results

1 - Scope definition

- Sample type:
  - Plant extract
  - Infected/non-infected plant material (Fresh/Freeze-dried)
  - Plant material with deactivated pathogen
  - DNA and RNA extract
- Matrix:
  - Leaves, fruits, wood, seeds, shoots, stem material
  - Plant species
  - Culture
- Suitable for:
  - Symptomatic and/or Asymptomatic samples
- Purpose:
  - Detection and/or Identification

Valitest

1 - Scope definition

	DAS-ELISA	RT-PCR	REAL-TIME PCR	OTHER METHODS APPLICABLE FOR ON-SITE:
SAMPLE TYPE	Freeze dried plant material	Freeze dried plant material	Freeze dried plant material	Freeze dried leaves
MATRIX	Leaves and fruits (tomato and pepper)	Leaves and fruits (tomato and pepper)	Leaves and fruits (tomato and pepper)	Leaves (tomato and pepper)
SUITABLE FOR:	symptomatic / asymptomatic	symptomatic / asymptomatic	symptomatic / asymptomatic	symptomatic / asymptomatic
PURPOSE:	detection	detection	detection	Early warning

SEEDS - Euphresco 2019-A-327

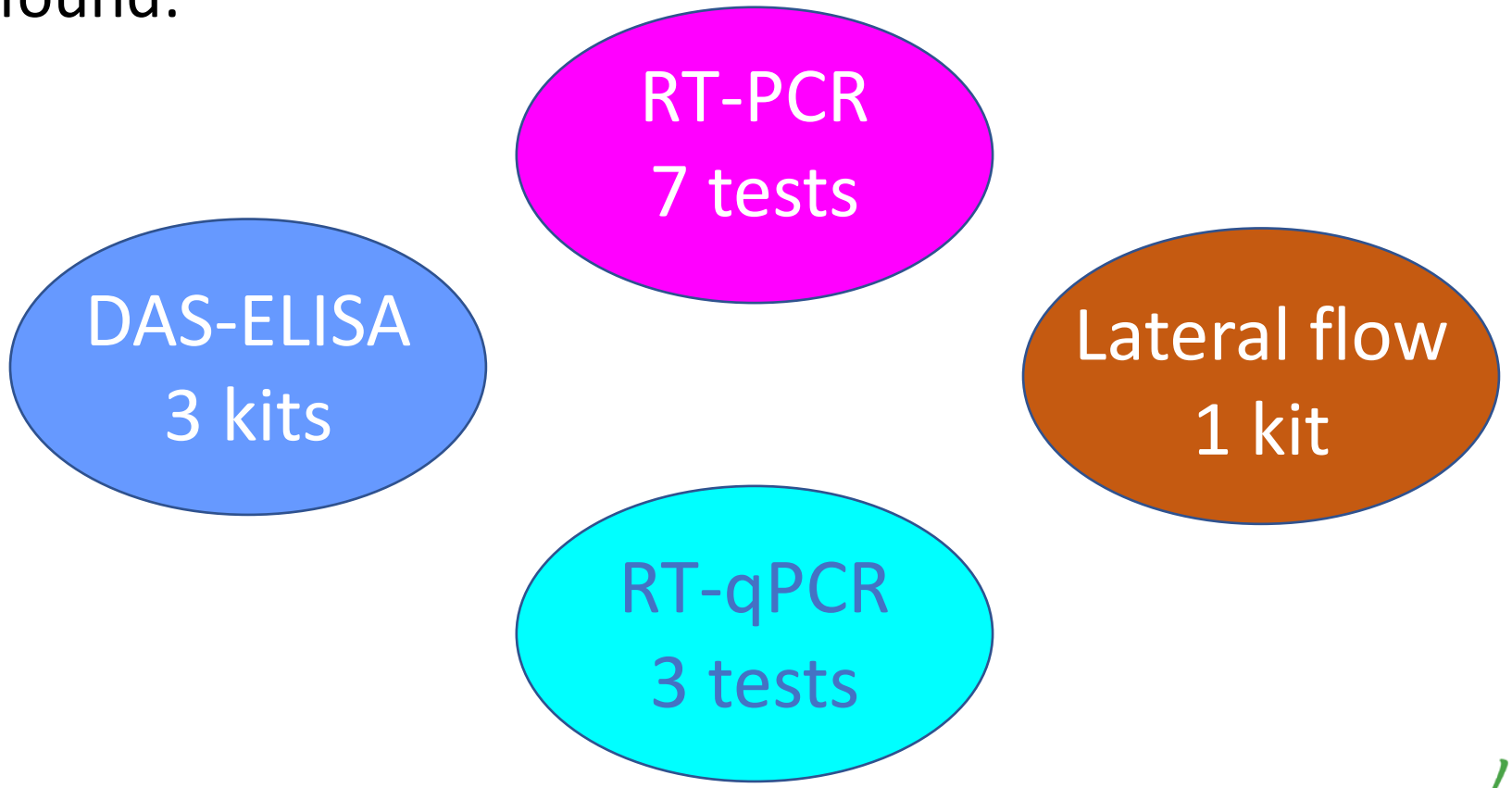
Valitest

2 - Test selection

- Collection of available data **Emerging pathogens**
  - EPPO standards None
  - EPPO database (validation data) None
  - EPPO/dedicated surveys None
  - EUPHRESCO final reports None
  - Research articles Few and with few validation data
  - Kit's providers websites (kits instructions) Few

2 - Test selection

After literature and website search 14 tests/kits were found:



2 - Test selection

DAS-ELISA
3 kits

After acquiring information from the companies producing Elisa Kits, it emerged, at that moment, that all showed a cross-reaction with other Tobamoviruses

None of the kits was included in the preliminary study

2 - Test selection

After literature and website search:

RT-PCR
7 tests

RT-qPCR
3 tests

Primers and probes were tested *in silico* for their inclusivity and exclusivity

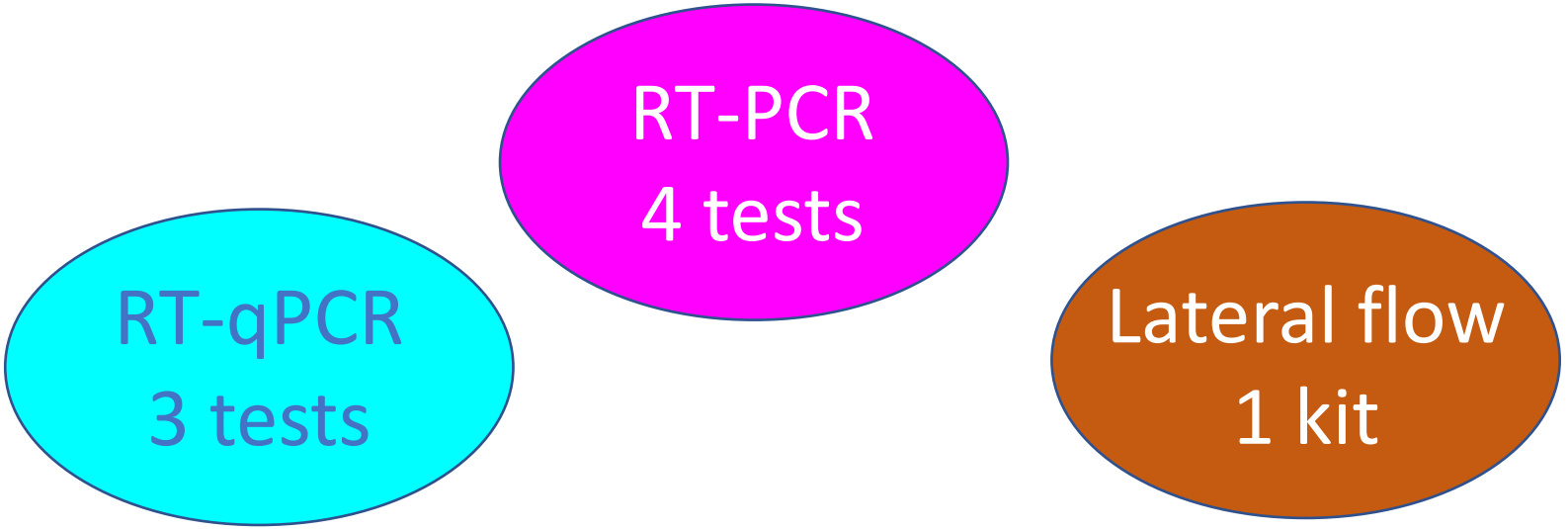
Two tests were discarded:

- Luria et al., 2017 – amplification product too long
- Levitzky et al., 2019 – cross reaction with other tobamoviruses
- Kit by Loewe and test by Rodriguez-Mendoza et al., 2019 used the same primers → SINGLE TEST

Valitest

2 - Test selection

8 tests were selected for preliminary study:



Preliminarily the molecular tests need to be harmonized due to:

- Difficulties in standardization
- Long and laborious procedures
- Developed for different matrix

2 - Test selection

Harmonization:

- RNA extraction – RNeasy Plant Mini kit Qiagen
- Amplification kit (Master mixes for conventional RT-PCR and real time RT-PCR)

The annealing temperature and the primers or primers and probes concentrations from the original protocols were generally maintained (except for ISHI-Veg 2019)

↓

Validation of the tests according to PM 7/98

2 - Test selection

		conventional RT-PCR				real-time RT-PCR			lateral flow
		Alkowni et al., (ALK)	Loewe (Rodriguez-Mendoza et al.) LOE	Ling et al.,	Panno et al., a	ISHI-Veg (ISH)	Menzel and Winter (M&W)	Panno et al., b (PAN)	Agdia
inclusivity	Sicily isolate	+	+	+	+	+	+	+	not tested
	Piemonte isolate	+	+	+	+	+	+	+	not tested
	PV-1236	+	+	+	+	+	+	+	not tested
	PV-1241	+	+	+	+	+	+	+	not tested
exclusivity	ToMV PV-0141	-	-	-	-	-	-	-	not tested
	TMV PV-1252	-	-	+	+	-	-	-	not tested
	PMMoV PV-0165	-	-	+	-	-	-	-	not tested
	BPeMV PV-0170	-	-	-	-	-	-	-	not tested
	TMGMV PV-0124	-	-	-	-	-	-	-	not tested
analytical sensitivity (tomato)	10^{0}	+	+	not tested	not tested	+	+	+	+
	10^{-1}	+	+	not tested	not tested	+	+	+	-
	10^{-2}	+	+	not tested	not tested	+	+	+	not tested
	10^{-3}	+	+	not tested	not tested	+	+	+	not tested
	10^{-4}	10^{-3}	+	not tested	not tested	+	+	+	not tested
	10^{-5}	-	+	not tested	not tested	+	+	+	not tested
	10^{-6}	-	10^{-5}	not tested	not tested	+	+	+	not tested
	10^{-7}	-	-	not tested	not tested	+	+	+	not tested
	10^{-8}	-	-	not tested	not tested	+/-	+/-	+/-	not tested
	10^{-9}	-	-	not tested	not tested	-	10^{-7}	-	not tested
	10^{-10}	-	-	not tested	not tested	-	-	-	not tested
analytical sensitivity (pepper*)	10^{0}	+	+	not tested	not tested	+	+	+	-
	10^{-1}	+	+	not tested	not tested	+	+	+	not tested
	10^{-2}	-	+	not tested	not tested	+	+	+	not tested
	10^{-3}	10^{-1}	+	not tested	not tested	+	+	+	not tested
	10^{-4}	-	-	not tested	not tested	+	+	+	not tested
	10^{-5}	-	10^{-3}	not tested	not tested	+/-	+/-	+/-	not tested
	10^{-6}	-	-	not tested	not tested	-	-	-	not tested
	10^{-7}	-	-	not tested	not tested	-	10^{-5}	-	not tested
	10^{-8}	-	-	not tested	not tested	-	-	-	not tested
	10^{-9}	-	-	not tested	not tested	-	-	-	not tested
	10^{-10}	-	-	not tested	not tested	-	-	-	not tested

* experimentally inoculated plants

Valitest

2 - Test selection

Method	Tests selected for final selection	Final selection (Yes/No)	Comment
DAS-ELISA	Commercial kits (Agdia, DSMZ, Loewe)	No	According to information provided by the companies, at the date, all the ELISA kits cross reacted with other tobamoviruses
RT-PCR	Levitzky et al., 2019	No	In silico tests highlighted a cross reaction with other tobamoviruses
	Luria et al., 2017	No	Too long amplification product, suitable for virus characterization
	Alkowni et al., 2019 Rodriguez-Mendoza et al., 2019 Commercial kit (Loewe)	Yes	Satisfactory results after preliminary studies. Primers from Rodriguez-Mendoza et al., (2019) used in the Loewe kit
	Ling et al., 2019 Panno et al., 2019a	No	In the preliminary tests cross reaction with other tobamoviruses has been highlighted
LATERAL FLOW	Commercial kits (Agdia)	No	In the preliminary test the kit showed a lack in sensitivity
Real Time RT-PCR	ISHI-Veg 2019 Menzel and Winter 2019 Panno et al 2019 b	Yes	Satisfactory results after preliminary studies.

Valitest

3- Participants' selection

Participant: laboratory, organization or individual that receives samples for interlaboratory comparison and submits results for review by the organizer of the interlaboratory comparison (EPPO PM7/98)

Participants were identified by checking:

- EPPO database on diagnostic expertise
- Laboratory networks
- Universities and research centres
- NPPOs
- Kits providers
- …

Before sending invitation, criteria for selecting participants must be set

↓

Common for all the Valitest TPS organizers

N° of participants must be balanced between:
MINIMUM: 10 laboratory (PM 7/122)
MAXIMUM: depending on different factors; i.e., availability of plant material and time and budget constraints

3- Participants' selection

- **Number of invited potential participants: 96**
- **Number of participants who responded: 37 (38%)**
- **Number of selected participants: 36 (97%)**

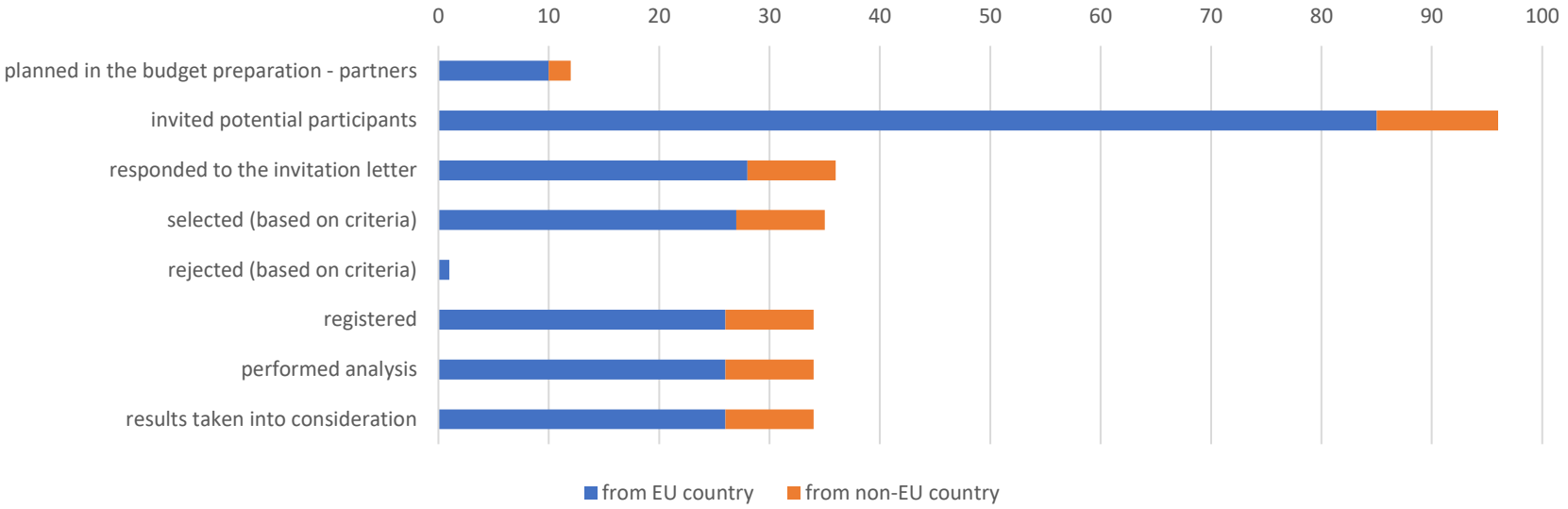
- **Number of participants who performed analysis: 34**

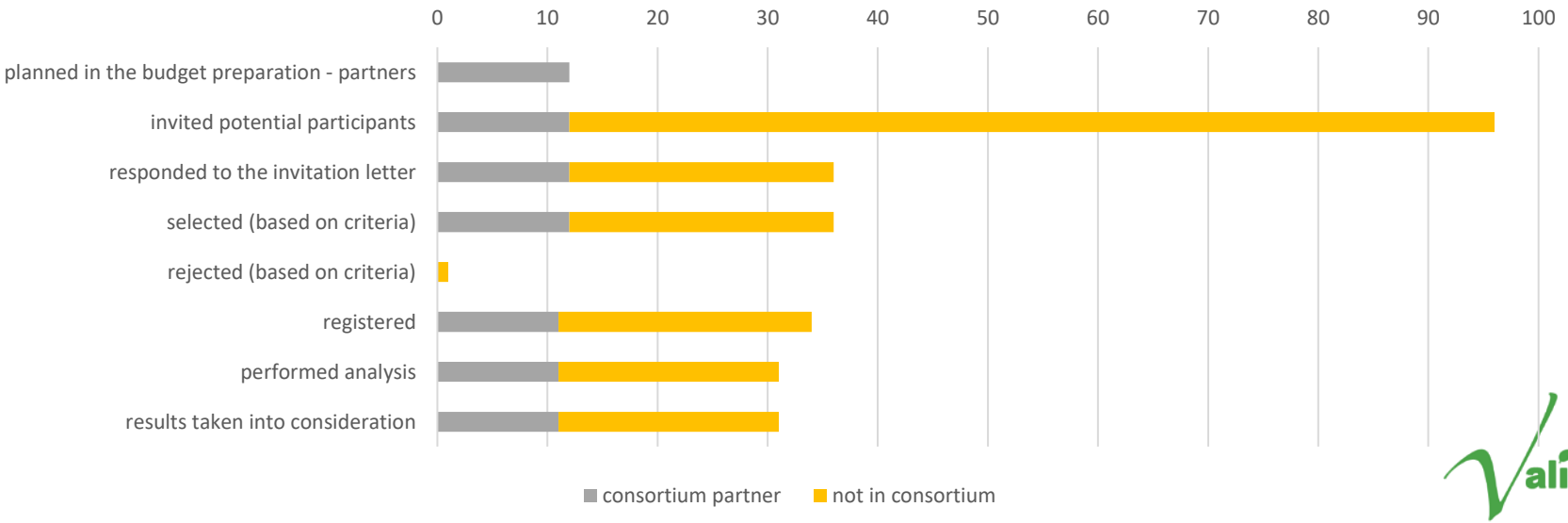
2 participants failed to participate in the TPS due to COVID-19 emergency

- **Number of participants who submitted results: 34 (100%)**

- Valitest consortium
- Euphresco project
- TPS Round 1 participants

3- Participants' selection





4 - Samples preparation and dispatch

According to EPPO PM 7/122:

- Samples must be as close as possible to the materials of the routine testing
- Samples must fulfill the statistical requirements needed to evaluate the different performance criteria
- Samples must be chosen considering the characteristics of the pathogen (survival, detectability) and technical difficulties (availability of reference material).

The amount of material should be prepared considering:

- The number of participants
- Performing of homogeneity and stability testing
- Replacement of materials that are lost or damaged during distribution/handling.

4 - Samples preparation and dispatch

Characteristics of the sample items match the requirements of Reference Materials defined by ISO 17034; EPPO PM7/76 and WP3:

- Traceability
- Homogeneity
- Stability
- Commutability level
- Target quantity
- Purity

4 - Samples preparation and dispatch

Sample panel prepared according to the recommendations of WP2

ID	REPETITION	TRACEABILITY	TYPE OF SAMPLE	PLANT	DILUTION	EXPECTED OUTCOME (RT-PCR)	EXPECTED OUTCOME (RT-qPCR)
SAMPLE 1	2	-	Healthy	S. lycopersicum		Neg	Neg
SAMPLE 2	2	-	Healthy	C. annum		Neg	Neg
SAMPLE 3	3	ToB-SIC 21/19	Serial dilution	S. lycopersicum	10^{-8}	Neg	Pos (below the LOD)
SAMPLE 4	3				10^{-6}	Pos (below the LOD)	Pos
SAMPLE 5	3				10^{-4}	Pos (LOD)	Pos
SAMPLE 6	3				10^{-2}	Pos	Pos
SAMPLE 7	2				10^{0}	Pos	Pos
SAMPLE 8	2	ToB-SIC 23/19	Low concentration	S. lycopersicum	10^{-6}	Pos (below the LOD)	Pos
SAMPLE 9	2	ToB-SIC 25/19	Medium concentration	S. lycopersicum	10^{-4}	Pos (LOD)	Pos
TOBRFV-M-NIC	1	-	Healthy	S. lycopersicum	10^{0}	Neg	Neg
TOBRFV-M-PIC	1	ToB-SIC 24/19	PIC	S. lycopersicum (fruit)	10^{0}	Pos	Pos
TOBRFV-M-PAC	1	ToB-SIC 22/19	PAC	S. lycopersicum	10^{-2}	Pos	Pos

Valitest

4 - Samples preparation and dispatch

Homogeneity

"Tested after the samples have been packaged in the final form and before distribution to participants" (EPPO PM7/122)

- 9 randomly chosen test items + PIC and NIC
- 3 technical repetitions
- Real Time RT-PCR (Menzel and Winter, 2019)
- Comparison of the Cq values obtained

Stability

"Tested after the deadline for performing analyses by the participants" (EPPO PM7/122)

- 20 weeks after preparation of samples
- Comparison of the Cq values obtained for homogeneity

Stability of PAC was checked storing it at room temperature for 3 days (to simulate extreme conditions of shipping)

4 - Samples preparation and dispatch

Samples were randomized to ensure a fully blind testing of them

Participants' names were coded

Label:

Valitest ToBRFV LXX Sample MXX

L: Laboratory from 1 to 35
M: samples from 1 to 22
+ NIC, PIC and PAC

4 - Samples preparation and dispatch

Ready-to-use mixtures of primers and primers /probes were provided by the organizers and dispatched with the panel of samples thus lowering the possibility of variations and errors in performing tests

Checked before shipping for their homogeneity and stability

4 - Samples preparation and dispatch

1-2 DAYS

TROUBLES:

- Two-month delay due to Covid-19 pandemic;
- Window of 2-3 months for receiving the LoA;
- Shipping and custom troubles for extra-EU countries (New Zealand, Israel...);
- Material to be sent twice in a couple of countries: importance to have extra-material (samples and reagents)

5 - Analysis of the results

According to PM 7/122:
Participant(s) submitting results that fall outside expected ranges should be identified; this should be documented. Consideration should be given to exclude this/these participant(s) from the analysis of the interlaboratory comparison.

Prior to analysis of results, the assessment of the valid dataset with the definition of outliers is needed.

Balance between:

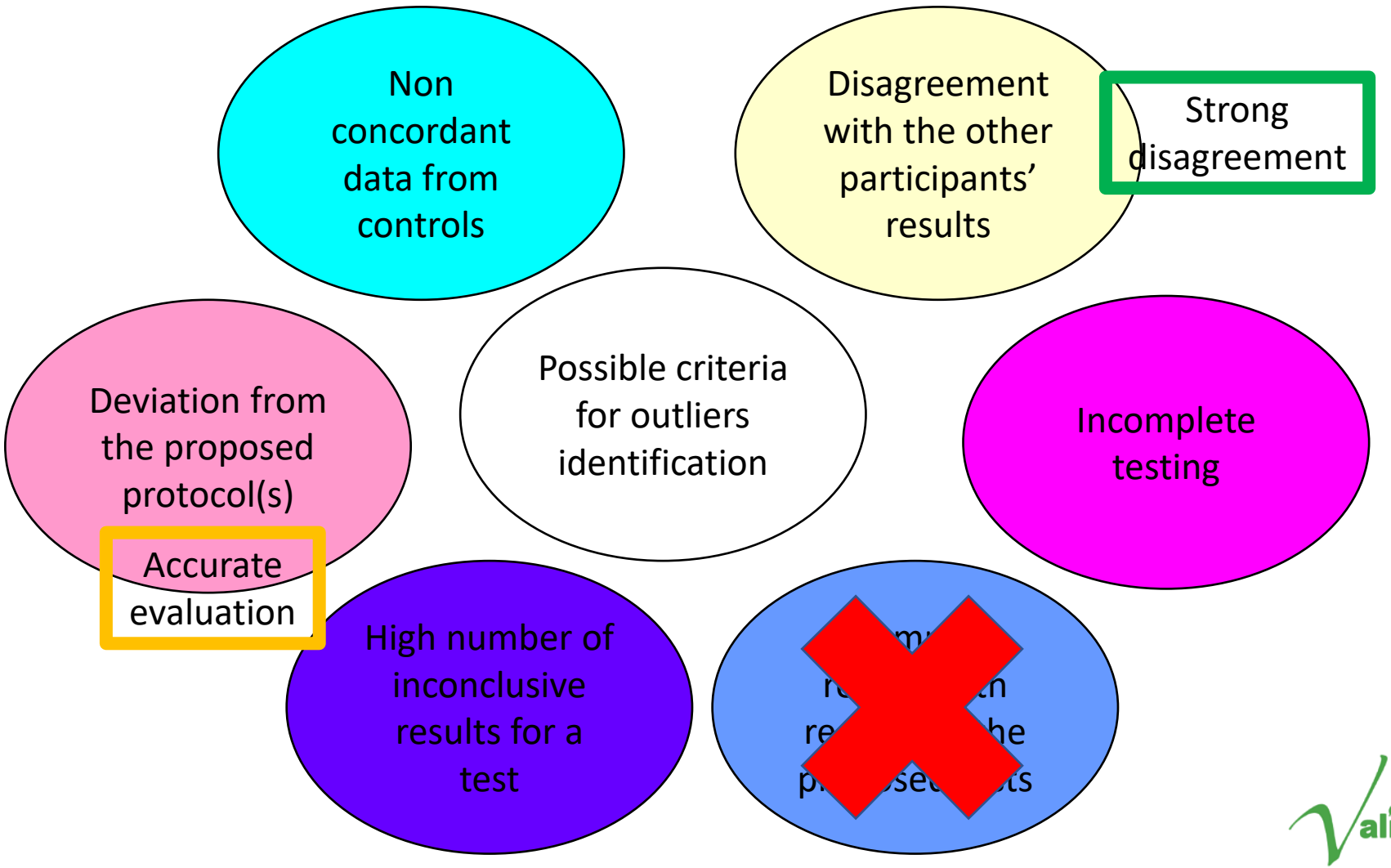
STRICT CRITERIA DEFINITION

PROS:
High values for all the performance criteria
CONS:
Loss of information about robustness of the tests
Minimum number of participants (i.e., valid datasets) not met

WIDE CRITERIA DEFINITION

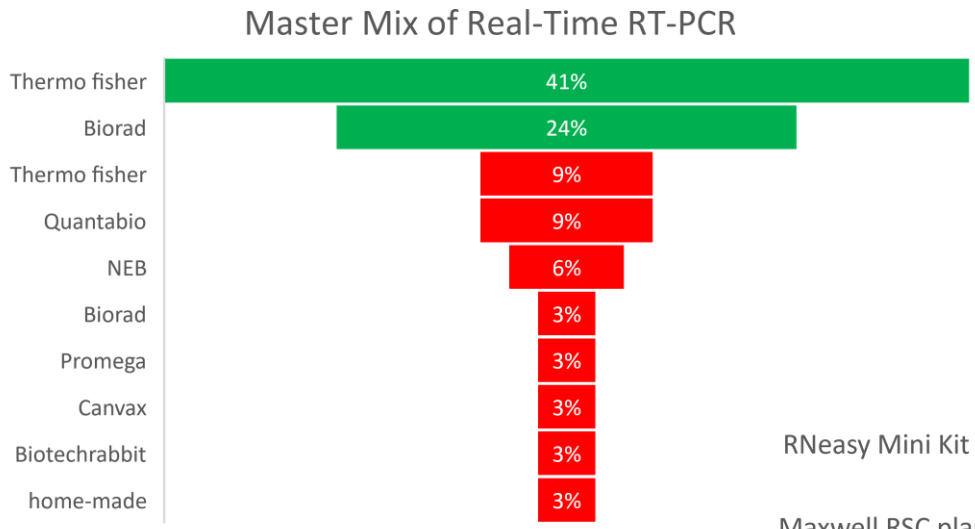
PROS:
Results as a picture of the 'real diagnostic situation'
CONS:
Decrease of all the performance criteria

5 - Analysis of the results

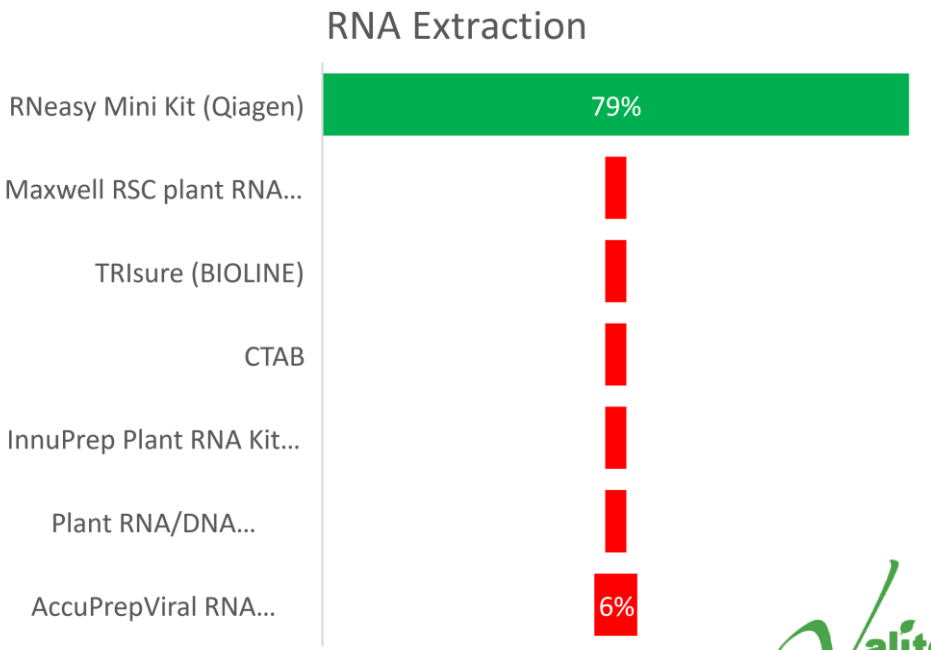


5 - Analysis of the results

Information reported by participants



15/34 (44%) of the labs made at least one deviation from the proposed protocols (12/34 – 35% used different Master mixes for Real Time RT-PCR).



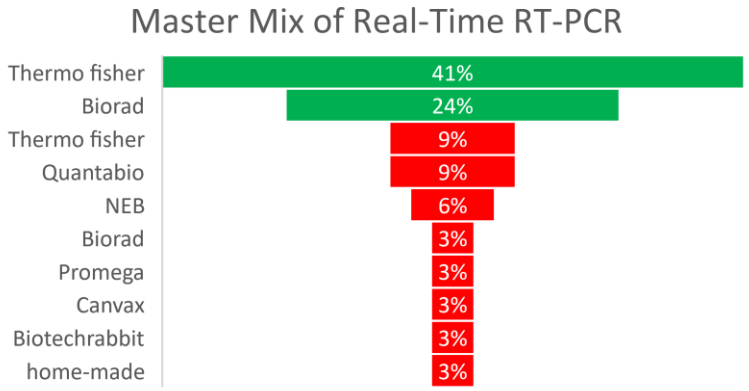
Preliminary evaluation of the deviations

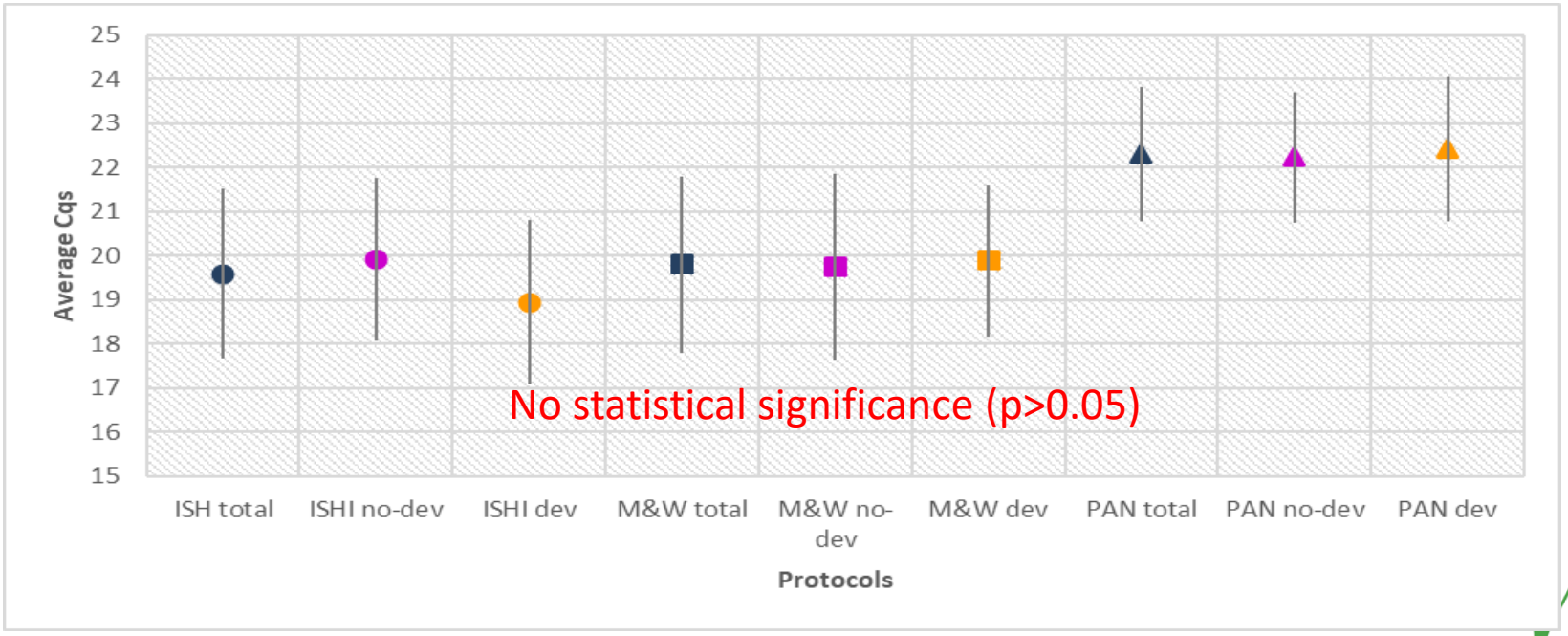
Valitest

5 - Analysis of the results

Analysis of deviation

- Comparison of PAC Cq values

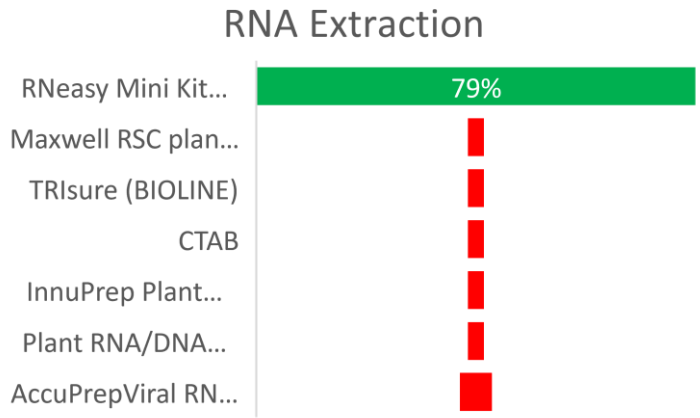


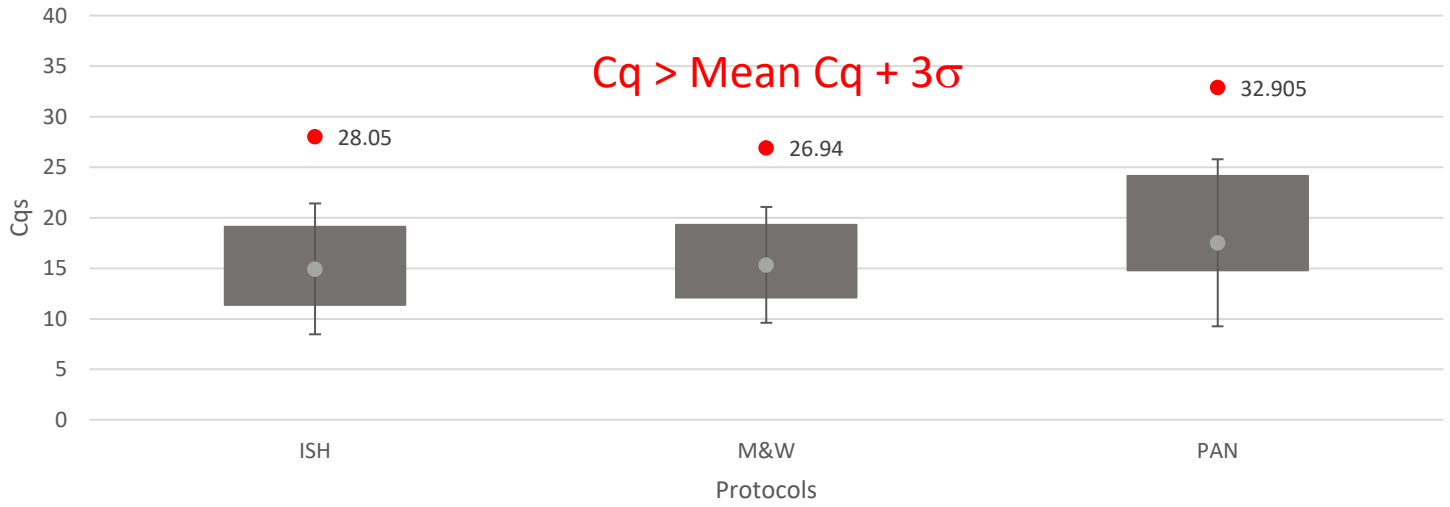


5 - Analysis of the results

Analysis of deviation

- Comparison of PIC Cq values

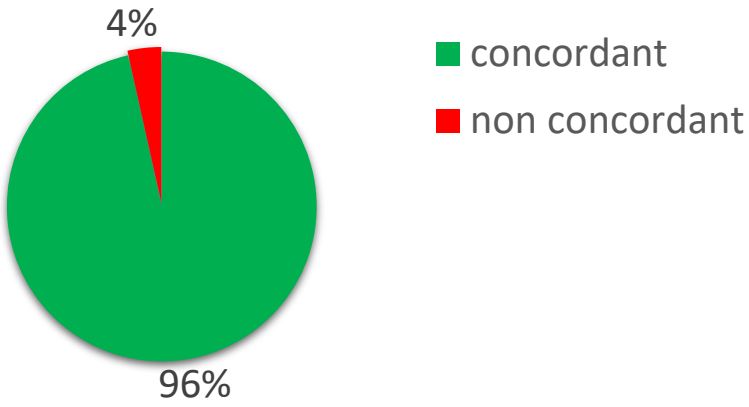




It is not the «same test»

5 - Analysis of the results

- Data from controls
- Number of results in term of negative/positive sample items which differ greatly from other laboratories
- Incomplete test
- High number of undetermined results



Protocols	number of data set	number of valid data set	percentage
Alkowani et al., - ALK	27	22	81%
Loewe (Rodriguez-Mendoza et al.,) - Loe	26	21	81%
Conventional RT-PCR	**53**	**43**	**81%**
ISHI-Veg - ISH	34	24	71%
Menzel and Winter - M&W	34	25	74%
Panno et al., - PAN	30	22	73%
Real Time RT-PCR	**98**	**71**	**72%**
Total	**151**	**114**	**75%**

5 - Analysis of the results

Repeatability (DA)

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	Total	Legend
ALK	100%	95%	97%	72%	76%	100%	100%	91%	61%	88%	100%
LOE	95%	79%	91%	50%	75%	100%	100%	91%	49%	81%	80%
ISH	80%	71%	50%	72%	100%	100%	100%	54%	75%	78%	60%
M&W	88%	73%	49%	71%	100%	100%	100%	53%	78%	79%	40%
PAN	95%	70%	63%	65%	100%	100%	90%	72%	79%	82%	20%

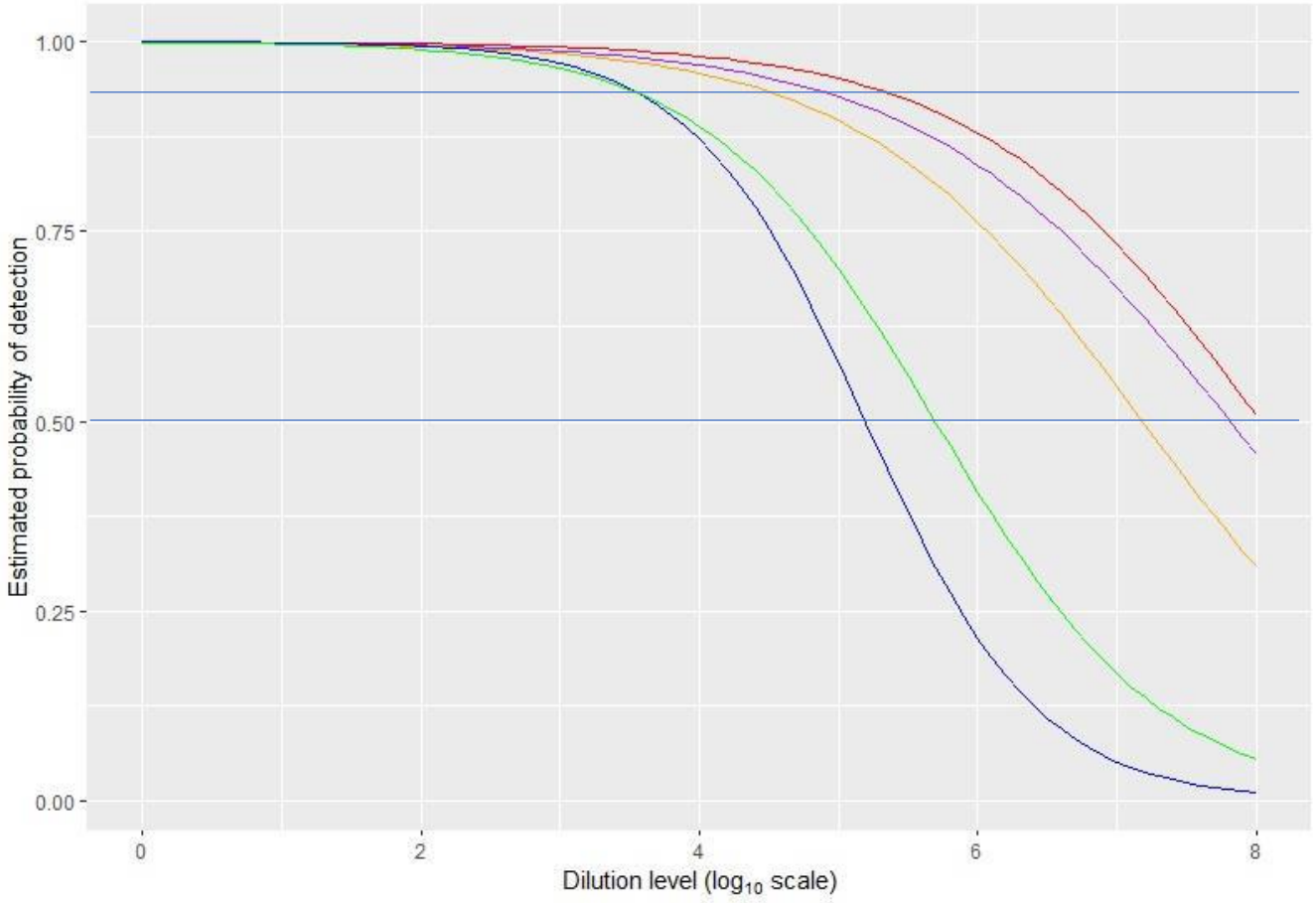
Reproducibility (CO)

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	Total	Legend
ALK	100%	95%	97%	69%	76%	100%	100%	91%	57%	87%	100%
LOE	95%	79%	91%	47%	75%	100%	100%	91%	49%	81%	80%
ISH	79%	71%	49%	63%	88%	88%	96%	54%	66%	73%	60%
M&W	88%	73%	48%	65%	92%	92%	100%	52%	72%	76%	40%
PAN	95%	70%	62%	65%	100%	100%	90%	70%	79%	81%	20%

Valitest

5 - Analysis of the results

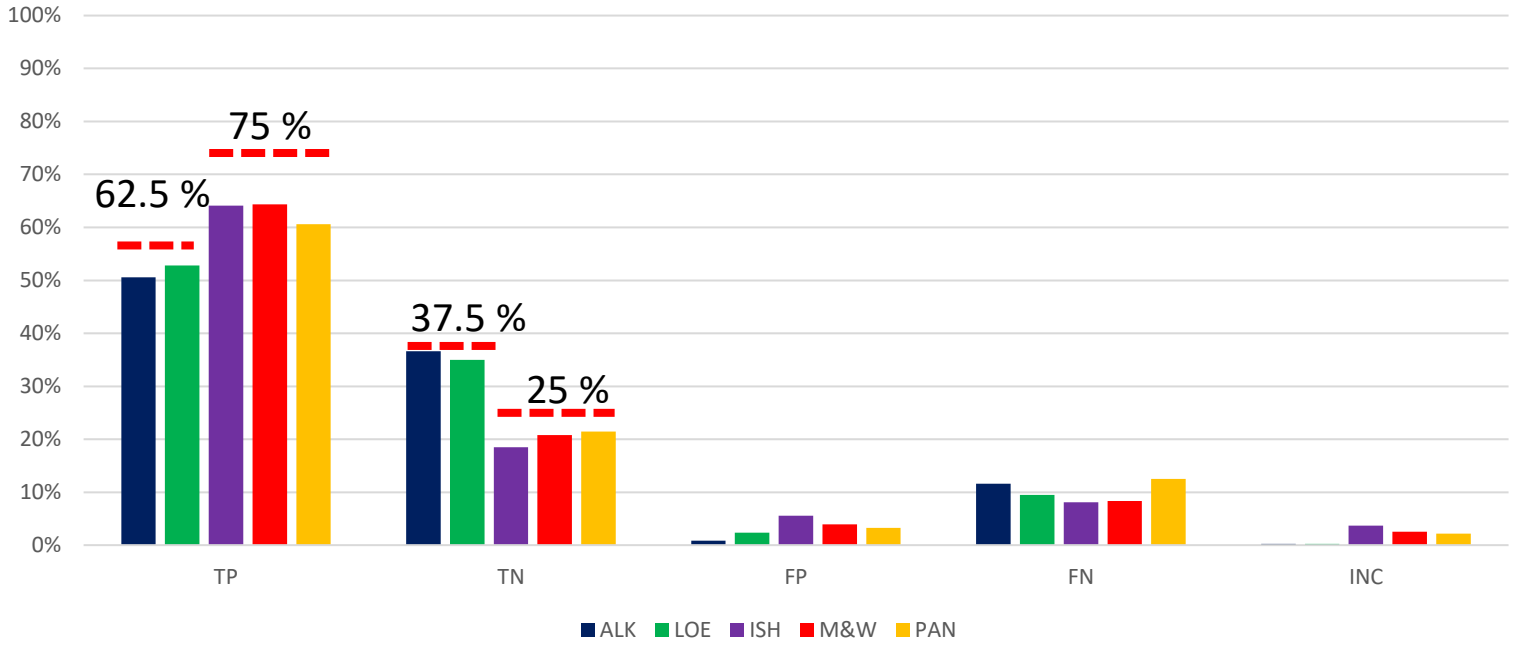
		ALK	LOE	ISH	M&W	PAN
Detection limit at 50 %	bGLM	5.2	5.7	7.8	N.A.	7.2
Detection limit at 95%	bGLM	3.4	3.3	4.6	5.0	4.2



Analysis made by the Valitest WP2 teams

5 - Analysis of the results

		ALK	LOE	ISH	M&W	PAN
Total data set		22	21	27	27	23
Total data points		352	337	432	432	368
TP %	TP/N %	51%	53%	64%	64%	61%
TN %	TN/N %	37%	35%	19%	21%	21%
FP %	FP/N %	1%	2%	6%	4%	3%
FN %	FN/N %	12%	9%	8%	8%	13%
INC %	INC/N %	0%	0%	4%	3%	2%

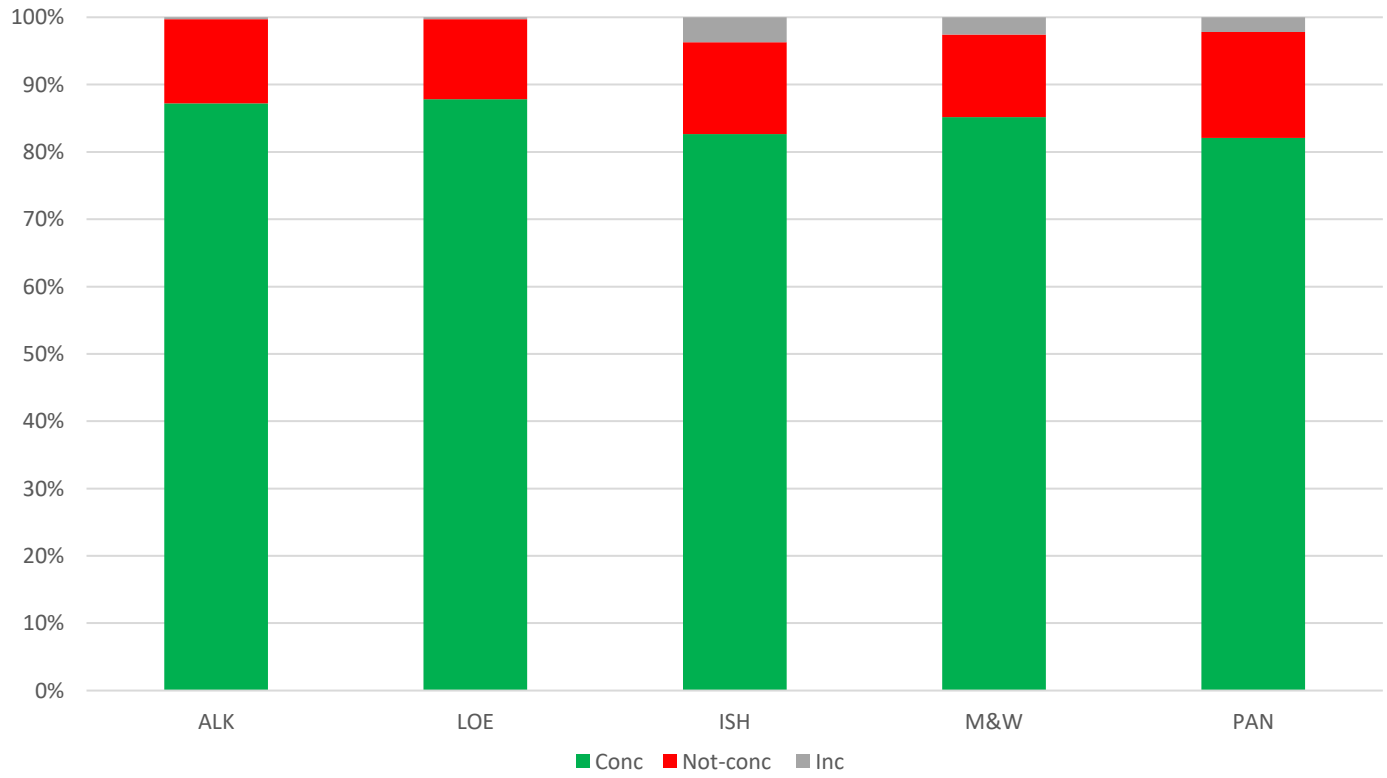


Dotted line – theoretical value of TN % and TP % according to the expected result

Valitest

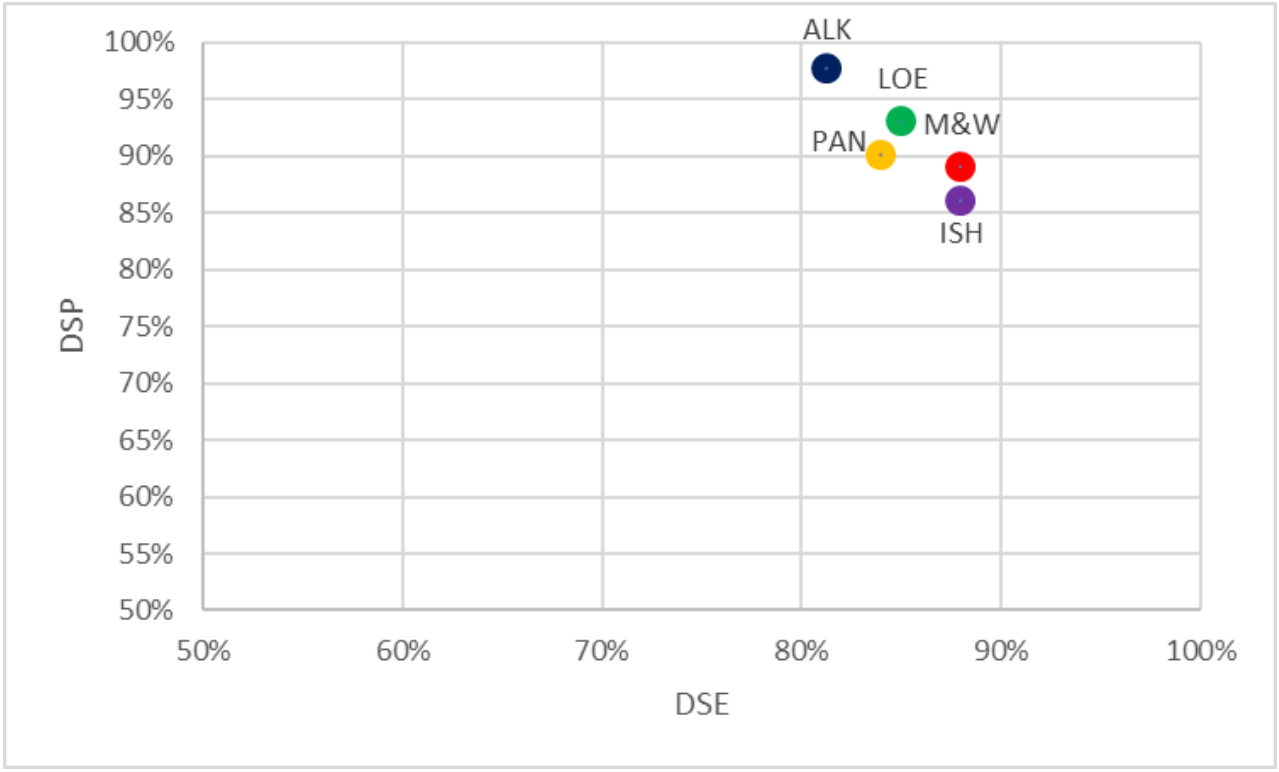
5 - Analysis of the results

		ALK	LOE	ISH	M&W	PAN
Concordant	TP+TN	307	296	357	368	302
Not-concordant	FP+FN	44	40	59	53	58
Concordant %	TP+TN/N	87%	88%	83%	85%	82%
CI_{asp} 95%		66-100 %	73-100 %	67-98 %	68-100 %	59-100 %
Not-concordant %	FP+FN/N	13%	12%	14%	12%	16%



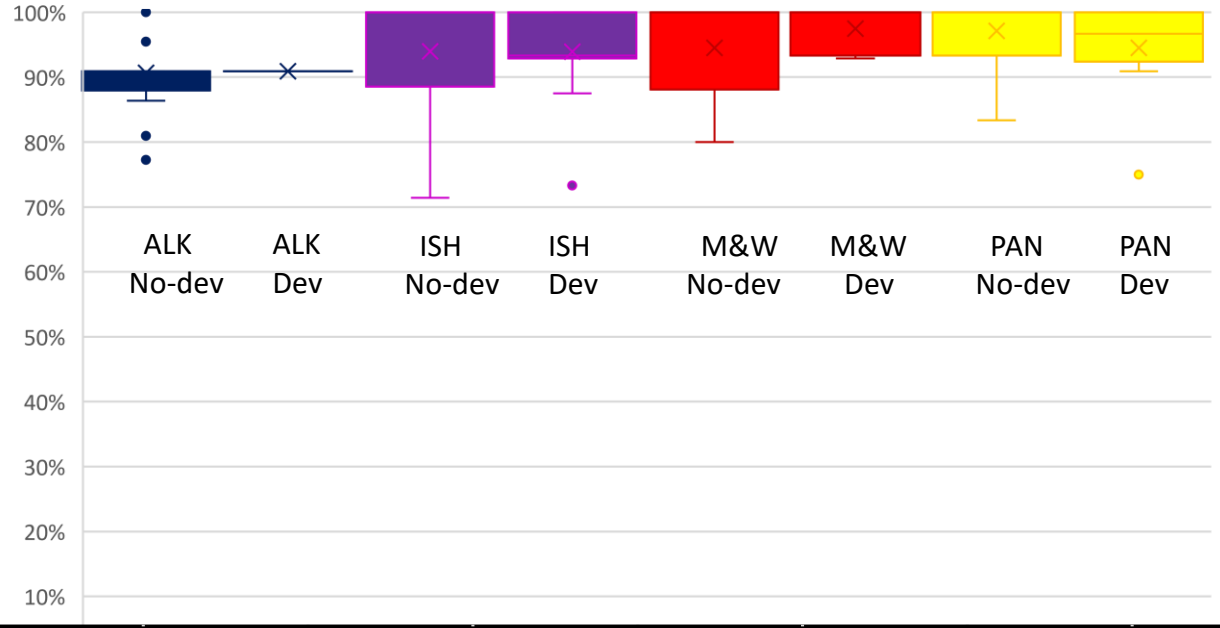
5 - Analysis of the results

		Alk	Loe	ISH	M&W	Pan
Diagnostic sensitivity (=DSE)	TP/TP+FN	81%	85%	88%	88%	84%
CI_{DSE} 95 %		43-100 %	57-100%	59-100%	56-100 %	48-100 %
p-Value Fisher DSE		NS	NS	NS	NS	NS
Diagnostic specificity (=DSP)	TN/TN+FP	98%	93%	86%	89%	90%
CI_{DSP} 95 %		95-100 %	88-99 %	80-92 %	79-99 %	74-100 %
p-Value Fisher DSP		NS	NS	NS	NS	NS



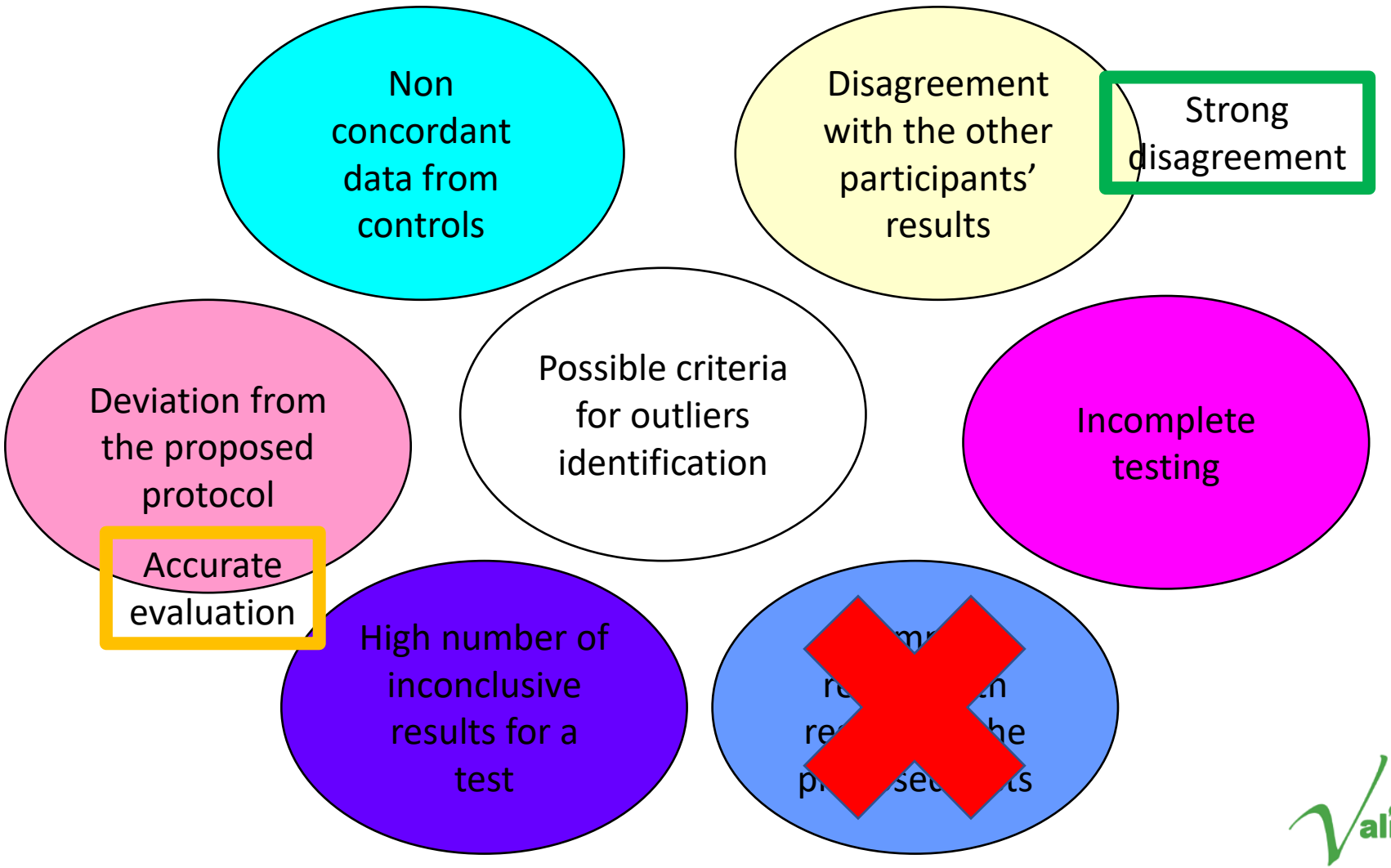
5 - Analysis of the results

Robustness



	ALK		ISH		M&W		PAN	
	no-dev	dev	no-dev	dev	no-dev	dev	no-dev	dev
Lab	20	2	16	11	16	11	13	10
Acc % Av	91%	91%	94%	94%	94%	97%	97%	94%
Acc % Min	77%		71%	73%	80%	93%	83%	75%
Acc % Max	100%		100%	100%	100%	100%	100%	100%
CI % acc	88-93%		88-98 %	89-98 %	90-98 %	95-99 %	94-99 %	89-99 %

5 - Analysis of the results



5 - Analysis of the results

*if a strict evaluation is applied

- Data from controls
- Number of results in term of negative/positive item samples which differ greatly from other laboratories
- Incomplete test
- High number of undetermined results

- Few disagreement results with the other participants
- Deviations from the proposed protocol

Protocols	number of data set	number of valid data set	percentage
Alkowani et al., - ALK	27	22	81%
Loewe (Rodriguez-Mendoza et al.,) - Loe	26	21	81%
Conventional RT-PCR	**53**	**43**	**81%**
ISHI-Veg - ISH	34	24	71%
Menzel and Winter - M&W	34	25	74%
Panno et al., - PAN	30	22	73%
Real Time RT-PCR	**98**	**71**	**72%**
Total	**151**	**114**	**75%**

Valitest

5 - Analysis of the results

*if a strict evaluation is applied

Repeatability (DA):

*Small number of valid dataset

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	tot
ISH	100%	100%	49%	74%	100%	100%	100%	58%	71%	83%
M&W	100%	100%	56%	74%	100%	100%	100%	45%	79%	84%
PAN	100%	100%	52%	74%	100%	100%	100%	69%	58%	84%

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	tot
ISH	80%	71%	50%	72%	100%	100%	100%	54%	75%	78%
M&W	88%	73%	49%	71%	100%	100%	100%	53%	78%	79%
PAN	95%	70%	63%	65%	100%	100%	90%	72%	79%	82%

Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	tot

Large number of valid dataset

60%
70%
80%
90%
100%

Valitest

5 - Analysis of the results

*if a strict evaluation is applied

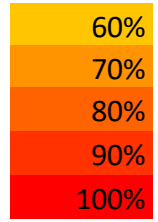
Reproducibility (CO):

*Small number of valid dataset

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	tot
ISH	89%	89%	78%	93%	100%	100%	100%	89%	89%	92%
M&W	100%	100%	85%	93%	100%	100%	100%	56%	100%	93%
PAN	89%	100%	93%	93%	100%	100%	100%	89%	89%	95%

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	tot
ISH	79%	71%	49%	63%	88%	88%	96%	54%	66%	73%
M&W	88%	73%	48%	65%	92%	92%	100%	52%	72%	76%
PAN	95%	70%	62%	65%	100%	100%	90%	70%	79%	81%

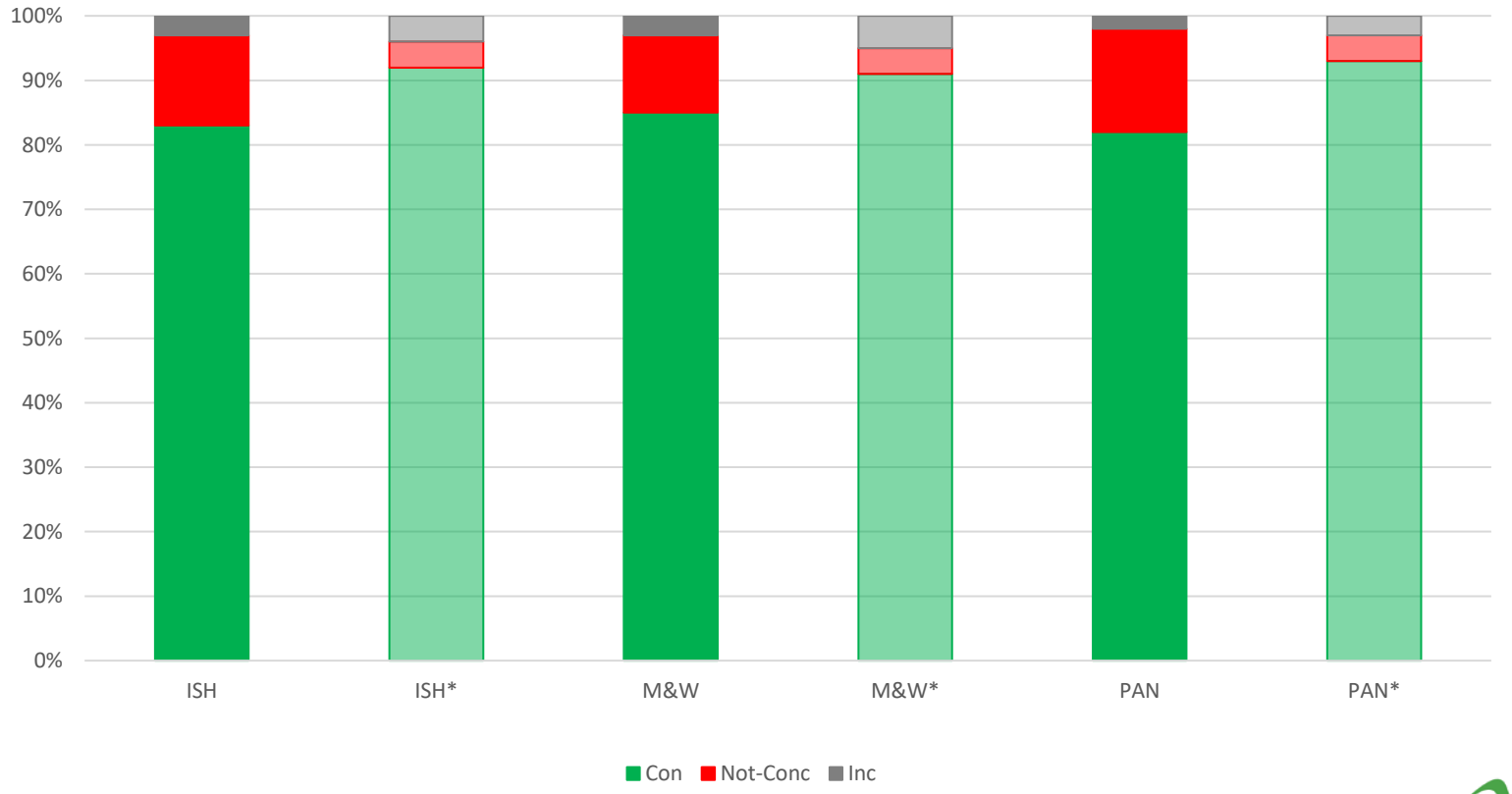
Large number of valid dataset



5 - Analysis of the results

*if a strict evaluation is applied

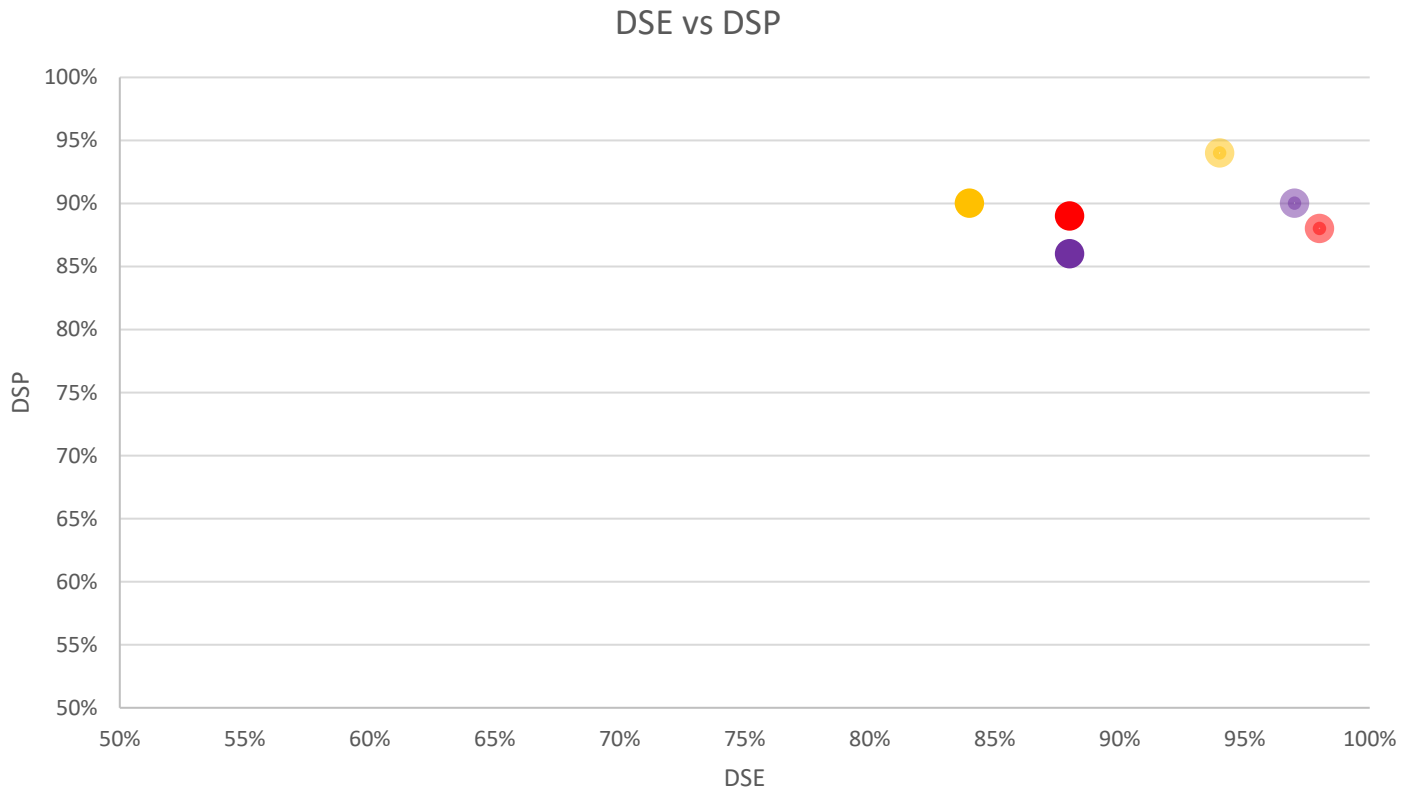
	ISH	ISH*	M&W	M&W*	PAN	PAN*
Concordance %	83%	92%	85%	91%	82%	93%
CI 95%	67 - 98 %	85 - 98 %	68 - 100 %	75 - 100 %	59 - 100 %	85 - 100 %



5 - Analysis of the results

*if a strict evaluation is applied

	ISH	ISH*	M&W	M&W*	PAN	PAN
DSE	88%	97%	88%	98%	84%	94%
CI_{DSE} 95 %	59-100%	88 - 100 %	56-100 %	91 - 100 %	48-100 %	79 - 100 %
DSP	86%	90%	89%	88%	90%	94%
CI_{DSP} 95 %	80-92 %	82-100 %	79-99 %	68 - 100 %	74-100 %	88 - 100%



Summarizing

1 - Scope definition

Definition of a clear scope can help not only to design and prepare the TPS but also to define all the needs for a proper organization.

- It helps in defining the criteria for the tests selection
- Defines the number and the typology of samples to be sent
- May influence treatment of outliers

Summarizing

2 - Test selection

- Defining in advance the criteria for tests selection subsequently supports the analysis of the validation data
- The help of *in silico* data-analysis and the expertise of the organizer in critically evaluating a tests, are crucial when a lot of diagnostic protocols are available
- Inputs from the developer of a test or from the kit providers greatly help in the evaluation of the tests
- Often adaptation of the protocols should be needed by evaluating in advance which parameters must not be modified

Valitest

Summarizing

3- Participants selection

The number of participants depends on many factors: availability of material to prepare samples, interest in the organism of the TPS…
Selection of competent participants is critical to obtain accurate results in TPS but a small number of participants (i.e., top expertise labs) could increase the values of the performance criteria of a test without giving any information on its robustness.

Summarizing

4 - Samples preparation and dispatch

A lot of efforts must be made in preparing samples. Guaranteeing the homogeneity and the stability of all the panels helps to remove the bias of not-accurate samples preparation during the analysis of results.

Randomization of samples ensures the blindness in performing the tests and the anonymizing of participants ensures the confidentiality of the information obtained.

Summarizing

5 - Analysis of the results

Many factors can affect the definition of outlier datasets:

- Accepting all the dataset could lead to a drastic decrease of the performance criteria values obtained for each test
- Stringent approach of the parameters for the selection of valid dataset increase the performance results of each test but does not allow to understand the real robustness of each test

Valitest

Conclusion on ToBRFV TPS

Even if ToBRFV is an emerging pathogen whose manipulation entails high risks of contamination:

- All the tests highlighted good results for all the performance criteria evaluated, both if a more or less stringent approach (to establish valid data set) was applied
- According to that, all the tests resulted easy to interpret, robust and reliable, even if developed recently.
- The real-time RT-PCR tests confirmed their highest analytical sensitivity even if in the TPS a slight reduction of those values was highlighted

Valitest

Thank you for your attention!

E-mail:

francesco.faggioli@crea.gov.it

marta.luigi@crea.gov.it

Valitest