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DELIVERABLE N° 7.4

Title: Report on education and training session during the project

Validation of diagnostic tests to support plant health

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Abstract:

The deliverable 7.4 details the practical session “the use of kits: training and demonstration” organised in the framework of the task 7.3. “Organization of dissemination/training workshops on the use of validated tools”. This practical session is part of the series of webinars and training activities “the concept of test validation in Plant Health” organised in the framework of VALITEST (the details are presented in the deliverable 6.8 “Report on the organisation of training activities (webinars, video tutorial, practical training sessions)”.

Partners involved: IPADLAB, BIOREBA, LOEWE, CD, SEDIAG, WUR (Prime Diagnostics), EPPO

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TERMS, ABBREVIATIONS AND DEFINITIONS

CD: ClearDetections
CREA: Council for Agricultural Research and Economics
EPPO: European and Mediterranean Plant Protection Organization
EU: European Union
GIORIN: Main Inspectorate of Plant Health and Seed Inspection
IPADLAB: International Plant Analysis and Diagnostics
NIB: National Institute of Biology
TPS: Test Performance Study
WUR: Wageningen University Research (NL)
WP: Work Package

1 Introduction

Dissemination/training workshops for diagnostics laboratories were initially planned at two different levels to minimise travel costs:

- A large training event organised at the end of the project at EU level.
- At least 3 European and regional workshops gathering VALITEST end-users from different sectors and countries organised towards the end of the project, one for Eastern/Central Europe organised in Poland (GIORIN), one in Italy organised by CREA & NIB, and one in the Netherlands organised by WUR & FERA.

During the European and regional workshops, it was planned that Work Package 7 will contribute to dissemination training activities presenting use of kits validated in the framework of VALITEST project.

Due to the Covid-19 pandemic, the physical workshops planned for diagnostic laboratories could not be organised. All the training activities were held online in the format of webinars, practical training sessions and videos (the details are presented in the deliverable 6.8). The deliverable 7.4 details the practical session “the use of kits: training and demonstration”, organised in the framework of task 7.3. “Organization of dissemination/training workshops on the use of validated tools”. This practical session is part of the series of webinars and training activities “the concept of test validation in Plant Health” organised in the framework of VALITEST.

2 Purpose

The objective of the practical session "the use of kits: training and demonstration" was to discuss the use of kits based on ELISA (enzyme-linked immunosorbent assay) and molecular methods. The practical training session was targeting stakeholders working in Plant Health and interested in the concept of test validation (e.g. technical staff of public and private diagnostic laboratories and of companies producing diagnostic kits, scientific community, policy makers, end-users of kits and on-site tests).

For this training activity, participants had the opportunity to test in advance one kit produced by one of the companies that are VALITEST partners and to provide feedback on the use of this kit. Feedback was asked to be sent before the practical session to be discussed during the webinar. The training session was organised in two sessions: in the first one, companies presented the advantages and limitations of the two different technologies (ELISA and molecular) based on the scope of the analysis and, in the second one, the participants had the opportunity to discuss on the use and the performance of the kits they tested directly with the companies.

3 Organisation

3.1 Registration and technical aspects

In the framework of these practical activities, a maximum number of participants for each activity was defined beforehand by the leader. It was decided that available places would be filled on a first come first served basis. However, if the number of applicants exceeded the limit, priority would have been given to participants from EPPO countries and participation was limited to a given number of applicants per organisation.

Registrations were managed using a specific registration tool of the EPPO Secretariat and participants could register directly on the VALITEST website (www.valitest.eu). The invitation to participate was also communicated through mailing lists of VALITEST partners and published on different Twitter accounts (from VALITEST and the companies) and on some companies' websites.

When the participants registered to the practical training session, they had the possibility to choose 2 kits among the list presented in Table 1 with a first and second preference. When the deadline for registration was passed (8th February 2021), the list of participants was sent to the leader of the Work Package 7 and selection and/or distribution of the participants to the different sessions was made. Based on the registration data, some companies had less participants than others for the first choice, so it was decided to distribute the participants in a homogeneous way among the different kits offered to be tested in order to have a quite similar number of participants for each kit. For this reason, it was decided, if the participants agree, that they could test their both choices. Most of the participants agreed to test their both choices.

Table 1. List of the kits provided by the companies that are VALITEST partners.

COMPANY	TESTED PRODUCT
BIOREBA	Tomato spotted wilt virus (TSWV) ELISA kit
BIOREBA	Citrus tristeza virus (CTV) ELISA kit
CLEARDETECTIONS	*Bursaphelenchus xylophilus*, Real-Time PCR kit, RT-N-D-0401
CLEARDETECTIONS	*Meloidogyne chitwoodi*, Real-Time PCR kit, RT-N-D-1305
IPADLAB	Plum pox virus (PPV) One-Step Real-Time RT-PCR kit
IPADLAB	Citrus tristeza virus (CTV) One-Step Real-Time RT-PCR kit
LOEWE	*Xylophilus ampelinus* End-point PCR kit
LOEWE	Tomato brown rugose fruit virus (ToBRFV) RT-PCR kit
PRIME DIAGNOSTICS	Tomato brown rugose fruit virus (ToBRFV) ELISA kit
PRIME DIAGNOSTICS	Cucumber green mottle mosaic virus (CGMMV) ELISA kit
SEDIAG	*Erwinia amylovora* ELISA kit
SEDIAG	Citrus tristeza virus (CTV) ELISA kit

The kits were sent from mid-February 2021 and the practical session "the use of kits: training and demonstration" was held on 22nd of April 2021. The all-inclusive kits have been supplied for free by the companies, but the shipment costs, depending on the preference of each company, have been billed to the participants.

It was asked to the participants to use the kits only for the purpose of the VALITEST practical training session and to test them before the practical training session. Before the event, it was asked to the participants to send back the results together with the answers to a specific questionnaire prepared for both technologies (ELISA and molecular) (see one example in Annex), depending on the kit tested, in order to give feedback on the use of the kit such as:

- GENERAL INFORMATION (e.g. information about the used samples, the sample preparation, the test procedure, …)
- RESULTS (e.g. information about the expected positive and negative reactions with the positive and negative controls from the kit, opinion on the expected results, any deviation from the test protocol, …)
- CONVENIENCE (e.g. experience on the technique used in the kit, clearness and easiness of the protocol, handling of the assay preparation, timeframe, satisfaction of the use of the kit, suggestions, …)
- KIT AND ASSAY PROBLEMS (e.g. problems encountered, assay procedure, …)

3.2 Description of the activities

The platform WEBEX was used for the practical training session, allowing interactivity with the participants.

First, an introductive session presenting the VALITEST webinar series and the presenters of the practical session was done and then the event was organised in two main sessions (theoretical and practical sessions).

3.2.1 Theoretical session

During this session, a general presentation of both methods (ELISA and molecular) was made, focusing on the advantages and limitations of the methods depending on the scope and context of the analysis to be performed. In addition, a presentation on sample preparation was also held for both methods. Finally, the data analysis with the definition of threshold was also presented and discussed with the participants. During this session, all the companies and all the participants participated. The participation of all companies has been decided to transmit the message that EU companies work together that is the basis for the Quality Charter and the EPDIA establishment.

Table 2. Resume of the theoretical session organization.

Timing	Title	Content	Supporting media	Presenters
20‘	ELISA TECHNOLOGY	- Presentation of the different ELISA methods - Advantages and limitations of ELISA methods (choice of ELISA considering scope and context of the analysis) - Sampling and sample preparation for ELISA analysis - ELISA data analysis (definition of threshold)	Power point presentation Videos	Caroline FREYE (LOEWE) Marco KAISER (BIOREBA)
20’	MOLECULAR TECHNOLOGIES	- Presentation of the different molecular methods - Advantages and limitations of molecular methods (choice of molecular considering scope and context of the analysis) - Sampling and sample preparation for molecular analysis - Molecular data analysis (definition of threshold)	Power point presentation Videos	Marieke BELTMAN (CLEARDETECTIONS) Camilo GIANINAZZI (IPADLAB)

3.2.2 Practical session

During the second part of the event, practical sessions (ELISA and molecular technologies) were organised individually by all companies at the same time. The participants were divided in different virtual rooms according to the kits they tested, and they were able to switch from one room to the other based on their interest and regardless of whether they have tested the kit or not. Due to the number of participants and the fact that some participants tested the kit of two different companies, two rounds of webinars were organised for each room. Furthermore, some participants also participated, for interest, to the webinar of companies although they did not test their kit.

Table 3. Resume of the practical session organization.

Timing	Virtual room	Content	Supporting media	Presenters
45’	ROOM 1 	Presentation by the companies: - Results of TPSs for the kits presented - Use of the kits from the sample preparation to the final data analysis	Power point presentation Videos	LOEWE
		- Discussion on the feedback received in the questionnaire/information form filled in by the users of the kits	Questions and answers	
45’	ROOM 2 	Presentation by the companies: - Results of TPSs for the kits presented - Use of the kits from the sample preparation to the final data analysis	Power point presentation Videos	PRIME DIAGNOSTICS
		- Discussion on the feedback received in the questionnaire/information form filled in by the users of the kits	Questions and answers	
45’	ROOM 3 	Presentation by the companies: - Results of TPSs for the kits presented - Use of the kits from the sample preparation to the final data analysis	Power point presentation Videos	BIOREBA CLEARDETECTIONS IPADLAB
		- Discussion on the feedback received in the questionnaire/information form filled in by the users of the kits	Questions and answers	

A final session was organised with all the participants and companies to close the event. The Work Package 7 partners promoted the launch of EPDIA (European Plant Diagnostic Industry) and Quality Charter and invited the participants to register to the launch of EPDIA on June 2021.

The presentations are available on the website: https://www.valitest.eu/training/activities_and_webinars.

3.3 Feedback of the participants

During the webinars, the participation was very active (see details Section 3.4), and questions were made over the theoretical and the practical sessions.

In the first theoretical session, the participants mainly interacted with companies on general topics for ELISA and molecular methods use such as the validation procedure of commercial kits, the choice of methods for the laboratories and the results analysis. For example, a question was made on how ELISA kits for seed detection are developed and validated and how the threshold is determinate. The presenters explained the difficulties that the companies face sometimes due to the fact of a limited access to large, infected seed lots. The way how some companies deal with this problem is by mixing seed lots (high infected, mild infected and low infected) and by making a dilution. These samples are then used to evaluate the analytical sensitivity (Limit of Detection). Another important topic was on how to define the positive and negative sample based on the interpretation of the Optical Density (OD). The presenters described the different ways of calculation of the threshold and the cut off.

During the practical sessions in the individual rooms, the companies were able to discuss about the feedbacks received and the results obtained by the participants when using their kits. Given the sensitivity of the data received, as they are related to the performance of each individual product, the companies did not share the results and the feedbacks of the questionnaire except at the level of generic information.

Generally, the information acquired were the following:

- GENERAL INFORMATION
    - Information about the samples used: most of the participants worked on fresh and frozen plant material.
    - Information about the sample preparation and the machine used: all the participants used internal or commercial kit for sample preparation. This information will be very useful to the companies for the future development and validation of their products.
    - Components and procedures used: all the participants have used exclusively the components supplied by the companies and they all followed the protocol without any modification.
- RESULTS: most of the participants obtained the expected positive and negative result with the positive and negative control from the kit. When participants have tested an internal sample, they obtained the expected results. All the participants did not apply any deviation from the kit. All the participants sent the basic results to the companies like the OD obtained and the Real-Time raw datafile.
- CONVENIENCE: Most of the participants found convenient the testing procedure, handling and assay preparation.
- KIT AND ASSAY PROBLEMS: the participants did not encounter any particular problems in using the kit, but they gave some feedback for the improvement of the information to be inserted in the manuals such as the positive and negative control description and the methodology to analyze the results data.

3.4 Participation and satisfaction of the participants

The number of registrations and participants were recorded. At the end of the practical training session, a satisfactory survey was sent by email to the participants to evaluate their general satisfaction, the percentage of information that was new to them and when the information they have learnt would be useful to them. Participants could also provide general comments on the activities.

In total, 64 participants registered to the practical training session and 42 attended, which corresponds to an attendance rate of 66%. 45% of the attendees responded to the satisfaction survey. In general, attendees that responded to the satisfaction survey were satisfied by the activities with 90%, answering that the session was good or excellent compared to their expectations. Most of the attendees that responded to the satisfaction survey (42%) considered that 25-50% of the information presented was new to them. Finally, more than 84% of the attendees that responded to the satisfaction survey will use the information they have learnt immediately or in the next year, meaning that the information provided during the practical training session was very useful.

4 Conclusion

The organization of the practical session “the use of kits: training and demonstration” was an enriching exercise for all the companies and the participants. Indeed, companies have been able to develop new communication skills and they have acquired experience for the organization of practical sessions. During the preparation, companies were also able to exchange and share their own experience in interacting and communicating with end users. That has allowed companies to understand that there is a real need in terms of training on the use of kits and active interaction on technical issues between companies and end users.

Finally, as for all the activities of Work Package 7, the organization of these practical sessions allowed companies to learn to no longer see themselves simply as competitors, but also to collaborate for development of a qualitative and competitive European plant diagnostic industry.

For this reason, in the near future, the new EPDIA association (www.epdia.eu) will be the vehicle for the organization of practical sessions that will have a beneficial effect on the whole sector.

ANNEX

VALITEST - Feedback form for molecular kits use

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GENERAL

I. Personal information	
First name	
Last name	
Organization Name	
Organization Country	
Phone	
E-Mail	

II. Information about the used molecular kit	
Supplier name	
Product name	
Part No	
Lot No	
Description	
How did you store the kit before use?	

III. Information about the used samples				
Type of matrix (optional)	☐	Leaves	☐	Seeds
	☐	Others		
Type of tissue (optional)	☐	Fresh tissue	☐	Frozen tissue
Storing conditions if not fresh				
Positive Sample tested (if applicable)				

IV. Information about sample preparation				
Type of extraction	☐	DNA	☐	RNA
Material, equipment and buffers (optional)	Please indicate material, equipment and buffers used for sample homogenization:			

V. Test procedure				
Thermal cycler used				
Have you strictly followed the supplier test procedure as provided with the kit?	☐	YES	☐	NO
If NO, please indicate deviating steps:	Please indicate all the deviating steps			

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RESULTS

PLEASE SEND THE CURVE FILES AND/OR THE GEL PHOTOS WITH THE QUESTIONNAIRE

I. Did you get the expected positive reaction with the positive control from the kit?

☐ YES ☐ NO

II. Did you get a negative result from the negative control from the kit?

☐ YES ☐ NO

II. In the case you have included positive or negative samples from you source, did you get the expected result? ☐

☐ YES ☐ NO

IV. If you did not get the expected results, what in your opinion could be reason?

V. Did you do any deviation from the test protocol?

☐ YES ☐ NO

If YES, please, indicate the reason for the deviation and what you have changed

VI. According to you, did this deviation affect the test outcome?

☐ YES ☐ NO

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CONVENIENCE

I. How is your experience with the assay and /or technique used?

☐ Expert ☐ Intermediate ☐ Beginner

II. In your opinion is the kit manual clear and easy to follow?

☐ Very ☐ Somewhat ☐ Not very

II. How did you find the testing procedure?

☐ Easy ☐ Manageable ☐ Difficult

IV. How was the kit components handling for the assay preparation?

☐ Easy ☐ Manageable ☐ Difficult

V. Did you manage to do the test in a time frame that is for you

☐ Too long ☐ Acceptable ☐ Fast

VI. How is your overall satisfaction with the test kit?

☐ Very good ☐ Fair ☐ Poor

VII. Any suggestions to improve the kit use

Please indicate any suggestions for improvement

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KIT AND ASSAY PROBLEMS

I. What kind of problems did you encounter with the test kit?

II. What kind of problems did you encounter with the assay procedure?

III. Please note all questions that have occurred while using the test kit

IV. Please note all suggestions you have regarding the performance and handling of the kit