VALITEST

ELISA technology:
Sampling, sample preparation and data analysis

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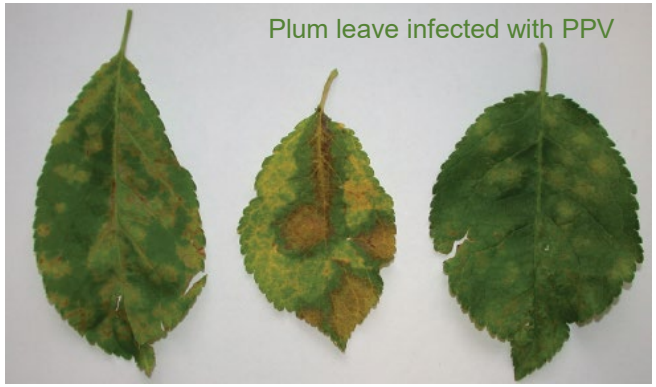
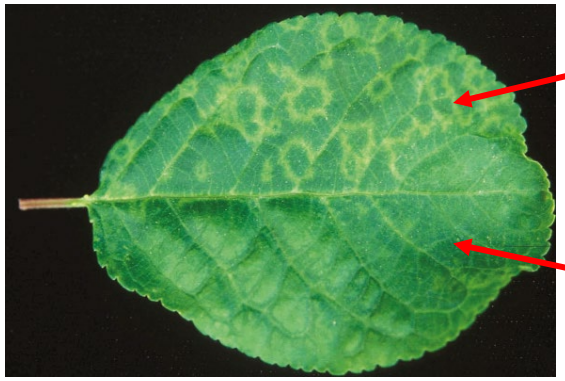
This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N° 773139

Valitest

Sampling for ELISA analysis

- Asymptomatic vs. Symptomatic plant material:





- Optimal tissue for sampling (e.g. leaves vs. seeds, seed transmission?)
- Optimal season/area for sampling (e.g. sampling for viruses on grapevine in Switzerland):

Nepo-, Tricho-, and Sadwaviruses	Ampelo-, Clostero-, Viti- and Maculaviruses
Leaves from young shoots and juicy bark early in the growing season as well as bark (phloem) scrapings from mature canes during dormancy.	Well-developed mature leaves, especially petioles and veins, from the lower part of the plant late in the season as well as bark (phloem) scrapings from mature canes during dormancy

- Storage / freezing of plant material (not all plant material and not all pathogens are the same!)

Equipment for sample preparation for ELISA analysis

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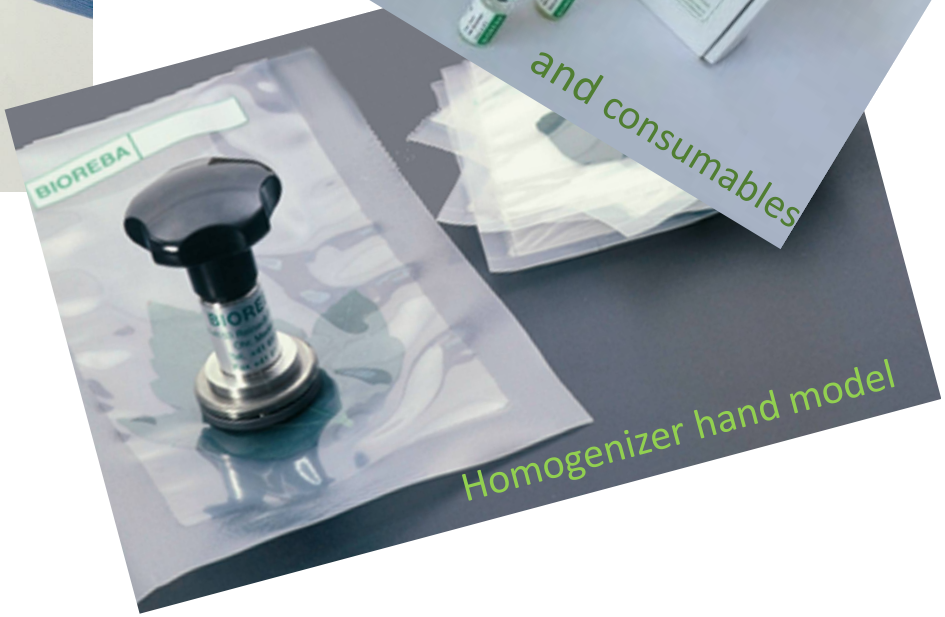

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Video: Sample preparation with HOMEX 6

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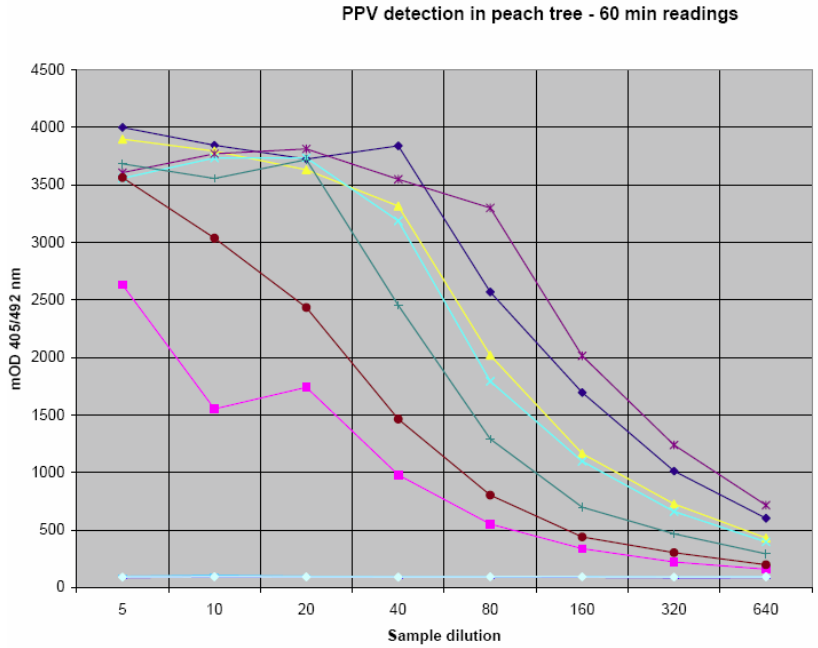
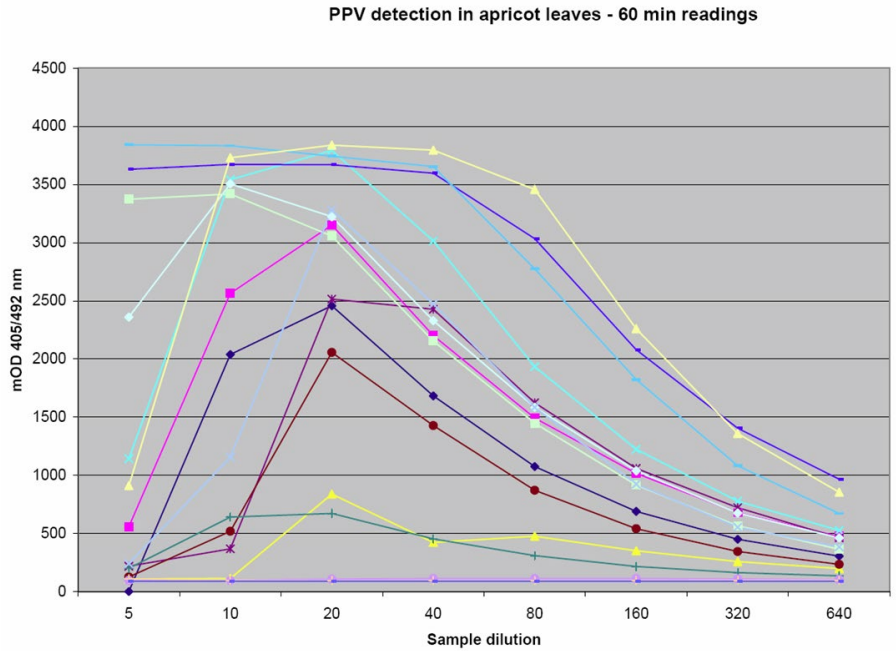
Sample preparation for ELISA analysis

- Usage of the optimal buffer composition (not all plant material is the same!)
- Usage of the optimal sample dilution:





- We optimize and validate our products and the product information accordingly. Therefore, optimal results are obtained using the buffer and dilutions we recommend in our product information.

Troubleshooting

If something goes wrong in ELISA and the test did not work:

- Type of microtiter plate which was used (big differences)
- Handling error (wells drying out, washing steps)
- Buffer composition
- Wrong concentration of reagents
- Wrong dilution of plant material
- Wrong storage of reagents or plant material
- No positive control used, no infected sample
- Wrong tissue for detection
- Wrong season for detection
- Quality of substrate (purity)
- Contamination
- ELISA reader malfunctioning
- …

! Always contact your supplier of the kit as your first option !

ELISA data analysis

- Usage of dual-wavelength (dual filter) extinction measurement (e.g. OD 405 nm / 492 nm)
  - 405 nm: absorbance peak of substrate pNPP (para-nitrophenylphosphate)
  - 492 nm: non-specific emission from other materials (e.g. polysterene).
- Define threshold value, which discriminates positive results from background readings
- The background or the reaction of healthy samples in ELISA-tests depends on:
  - Reagents
  - Chemicals (e.g. purity of pNPP substrate or buffer composition)
  - Type of microtiter plate
  - Incubation conditions
  - Kind of plant tissue
  - Extinction time
  - Handling (especially washing steps!)
  - …
- Even if all these parameters are kept as constant as possible, there are differences from plate to plate.

ELISA data analysis – definition of threshold

- Therefore, it is not advisable to work with a fix OD value as threshold but to calculate the threshold for each microtiter plate.
- Any method of setting the threshold of OD values is arbitrary.
- Any method of setting the threshold of OD values gets as more precise as more healthy / negative samples are measured on a plate.
- BIOREBA can suggest two formulas for the calculation of the threshold of OD values:

Threshold = mean value ("Healthy") * 3	Threshold = mean value ("Healthy") + 3 * Standard deviation ("Healthy") + 10%

➢ "Healthy": OD-values of all different healthy (negative) samples.

ELISA data analysis – definition of threshold

Threshold = mean value (“Healthy”) * 3

- Satisfactory results in most cases
- Suited to be programmed in microtiter plate readers
- Less stringent method
- Restriction: The “healthy” (negative) samples should produce typically OD values in the range of 0.080-0.150 (60 min extinction)

Threshold = mean value (“Healthy”) + 3 * Standard deviation (“Healthy”) + 10%

- Discrimination of potential positive samples with relative low OD value from evenly distributed negative / background OD values.
- Statistically more stringent method
- Laborious and relatively complicated
- Not applicable for programmable microtiter plate readers.

In any case and regardless which method you are ever choosing, we strongly suggest to repeat samples that are close to the cut-off.

ELISA data analysis – definition of threshold

Example:

- 40 duplicate samples in a 96-well ELISA plate
- 1 duplicate positive control (PC)
- 1 duplicate negative control (NC)
- 1 column extraction buffer blank (EB)

Fig. 1. Distribution of 40 duplicate samples in a 96-well ELISA plate.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Extraction buffer	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample	positive
B		1	5	9	13	17	21	25	29	33	37	control
C		sample	sample	sample	sample	sample	sample	sample	sample	sample	sample	negative
D		2	6	10	14	18	22	26	30	34	38	control
E		sample	sample	sample	sample	sample	sample	sample	sample	sample	sample	
F		3	7	11	15	19	23	27	31	35	39	
G		sample	sample	sample	sample	sample	sample	sample	sample	sample	sample	
H		4	8	12	16	20	24	28	32	36	40	

ELISA data analysis – definition of threshold

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E		sample	sample	sample	sample	sample	sample	sample	sample	sample	sample	
F		3	7	11	15	19	23	27	31	35	39	
G		sample	sample	sample	sample	sample	sample	sample	sample	sample	sample	
H		4	8	12	16	20	24	28	32	36	40	

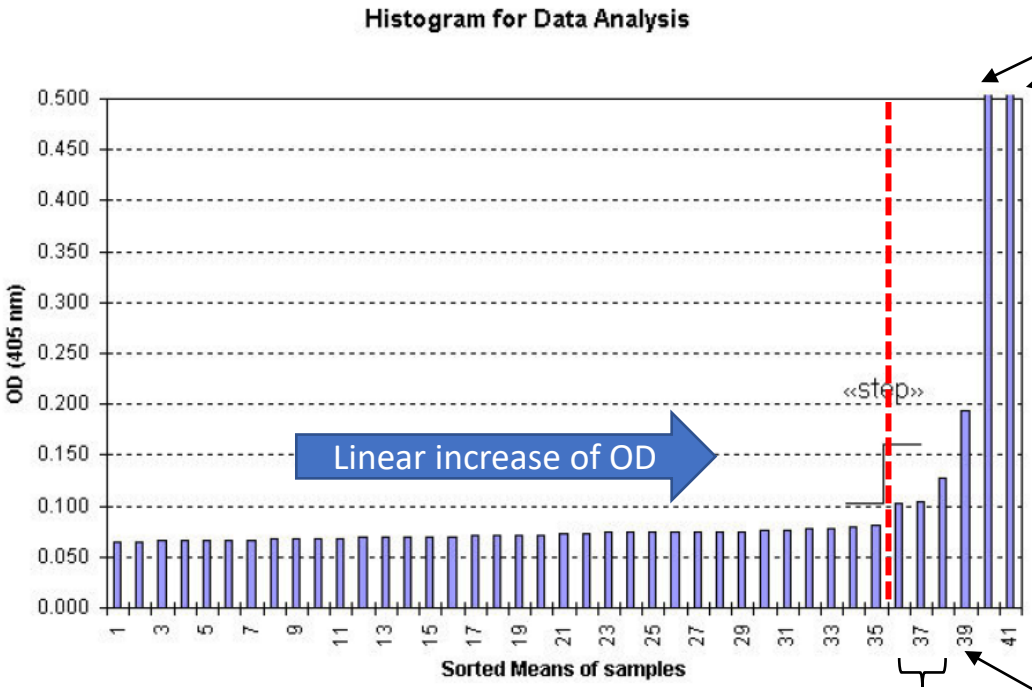
	1	2	3	4	5	6	7	8	9	10	11	12
A	0.069	0.064	0.067	0.068	0.068	0.102	0.071	0.072	0.068	0.065	0.063	2.098
B	0.070	0.064	0.067	0.066	0.068	0.102	0.066	0.068	0.065	0.070	0.067	2.026
C	0.073	0.069	0.073	0.068	0.077	0.070	0.068	0.071	0.072	0.557	0.068	0.069
D	0.075	0.074	0.077	0.071	0.082	0.072	0.070	0.074	0.076	0.592	0.071	0.074
E	0.078	0.200	0.082	0.074	0.076	0.072	0.134	0.075	0.075	0.076	0.089	0.071
F	0.075	0.188	0.075	0.071	0.075	0.071	0.120	0.073	0.073	0.078	0.074	0.073
G	0.073	0.065	0.070	0.071	0.073	0.073	0.104	0.074	0.076	0.067	0.067	0.069
H	0.073	0.069	0.070	0.071	0.075	0.077	0.106	0.076	0.077	0.069	0.064	0.071

By eye: quite even distribution (~ 0.070 OD 60min) but some values are slightly increased. Decision if positive → Threshold determination

ELISA data analysis – definition of threshold

Example:

- Calculate the mean of the duplicates
- Sort the means ascending and make a histogram:



34

PC

17, 23, 24

3

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E		sample	sample	sample	sample	sample	sample	sample	sample	sample	sample	
F		3	7	11	15	19	23	27	31	35	39	
G		sample	sample	sample	sample	sample	sample	sample	sample	sample	sample	
H		4	8	12	16	20	24	28	32	36	40	

Means 1-35 show linear increase of OD values. The 36th OD makes a clear “step”

ELISA data analysis – definition of threshold

Example:

- This “step” has to be determined for each microtiter plate
- The calculation of the threshold indicates if the next values on the right side of the step can be considered as positive or not (these OD values are significantly different from the preceding «background»)

Threshold = mean value (1-35) * 3	Threshold = mean value (1-35) + 3 * Standard deviation (1-35) + 10%
Mean (1-35) = 0.072	Mean (1-35) = 0.072 Standard deviation (1-35) = 0.004
Threshold = 0.072 * 3 = **0.216**	Threshold = 0.072 + 3*0.004 * 1.1 = **0.092**
Samples 34 positive	**Samples 3, 17, 23, 24 and 34 positive!**

For this sample OD values we would recommend to use the more stringent statistical method since it helps to avoid false negatives in case of weak positive samples.

Thank you for your attention!

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