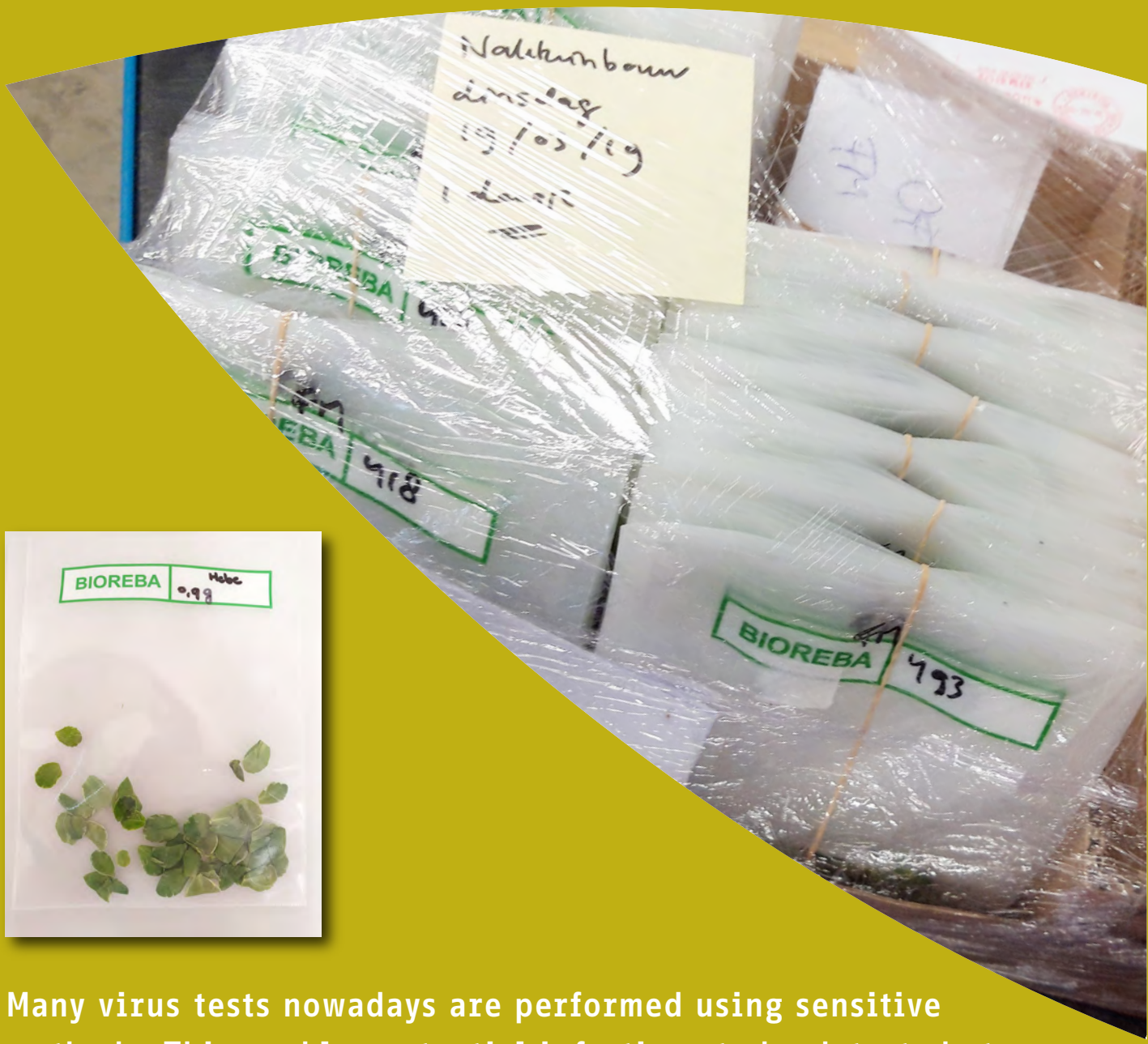
nak tuinbouw

Manual for sampling of plant material for virus tests



Many virus tests nowadays are performed using sensitive methods. This enables potential infections to be detected at an early stage. This manual provides information about hygiene, sampling for PCR and ELISA and the correct way to submit samples.

Hygiene

Why is hygienic sampling so important?
Many virus tests nowadays are performed using sensitive methods. This enables potential infections to be detected at an early stage. Virus testing can also detect any cross-contamination between samples. This is why hygiene during sampling is very important. By using the proper method you can prevent cross-contamination during sampling.

How do you submit sample material?
Always submit samples of plant material in grinding bags measuring 12 x 15 cm, of the brand Bioreba. Collect the material in the grinding bag at the front of the filter (this is the side with the text box). You can order these through the relevant suppliers of these bags. You can find the suppliers on www.bioreba.ch.

Why should sample material be submitted in grinding bags?
In the laboratory, samples are tested in these specific grinding bags because our protocols are validated with these specific bags. The advantage of submitting your samples in grinding bags is that we do not have to repack them. This reduces the risk of cross-contamination and allows a faster processing time at the laboratory. In addition, the grinding bags make it possible to robotize the testing process in the future.

When submitting a sample, make sure the material is suitable for testing. This means that the leaves should not be brown, dehydrated, mouldy or necrotic. Send material that is showing symptoms to Naktuinbouw, Diagnostics Team. On the website you can find more information about submitting material with symptoms.

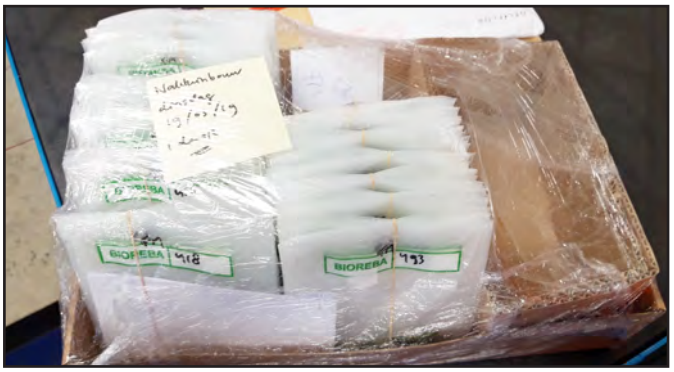
Method

- Wear a new pair of disposable gloves for each sample (each grinding bag) or fold a clean plastic bag around the hand you use to take the sample.
- Collect the material in the grinding bag at the front of the filter (this is the side with the text box).
- Number the grinding bags using the text box or place a sticker in the centre of the front of the bag. Do not use this sticker to seal the bag.
- **Important:** the number of leaf samples should not deviate too much from the specified quantity. Too much (more than 50% of the specified quantity) will interfere with the analysis. Only sample the prescribed quantity.

Submitting samples

Please include a fully completed submission form with your samples. You can find this form on the Naktuinbouw website under: Submission requirements.

Submitting samples correctly: Leave grinding bags open and bundle in number order with an elastic band. The mesh in the bags keep the material in place.



Post address

Post address for samples:
Naktuinbouw
Attn. Laboratories Administration
P.O. Box 40
2370 AA Roelofarendsveen, NL

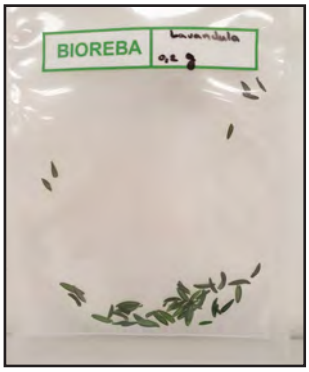
With large quantities of samples (e.g. on a pallet), please use the visiting address:
Naktuinbouw
Attn. Laboratories Administration
Sotaweg 22
2371 GD Roelofarendsveen, NL

Sampling for PCR

The number of leaves varies according to the crop variety for sampling for PCR analysis. The table below indicates the number of leaves you can submit per bag for PCR analysis.

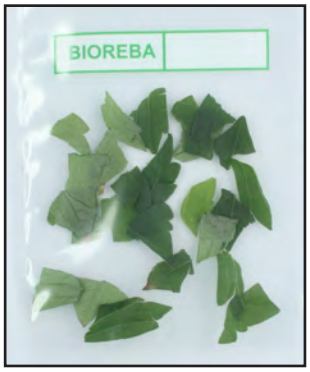
Crop	Sample material	Number of leaves per bag	Min-max weight per bag	Specifics
Ornamental crops	Leaf (pieces)	1 – 25	0.5 – 1 gram	
Phalaenopsis	Leaf or pieces of stem	1 – 25	0.5 – 1 gram	
Tissue culture material	Leaf from material that has grown as much as possible	1 – 25	0.5 – 1 gram	Material must be free of agar.
Freesia 5 x 10	Leaf tips	1 – 10	0.5 – 1 gram	

Examples of samples of ornamentals submitted correctly for PCR analysis:

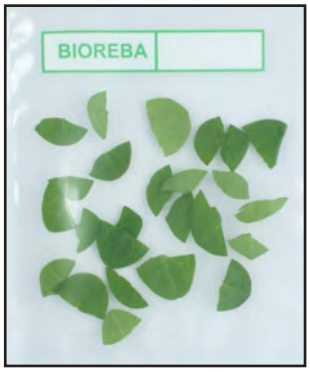


Lavendel

Bacopa Sutera



Dahlia

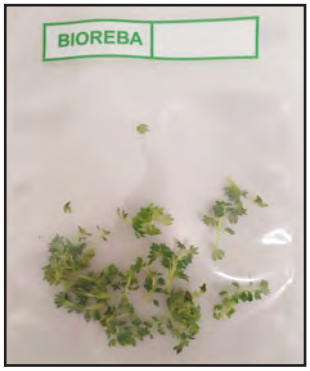


Petunia

Chrysant

Tissue culture samples

Tissue culture samples



Tissue culture samples

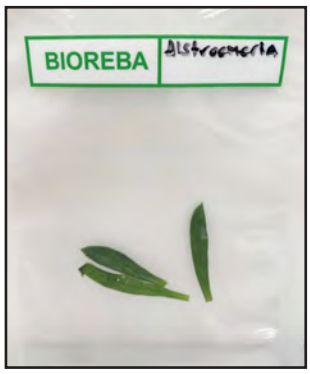
Sampling for ELISA

The number of leaves varies according to the crop variety for sampling for ELISA analysis. The table below indicates the number of leaves you can submit per bag for ELISA analysis.

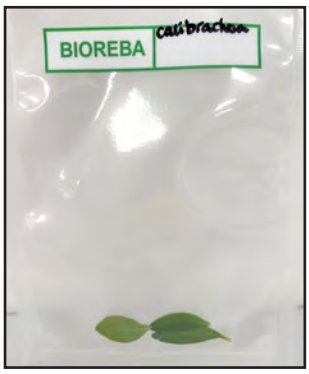
Crops	Sample material	Number of leaves per bag	Sample size per bag	Specifics when submitting tissue culture material
Ornamental crops	Leaf (pieces)	1 – 2	ca. 2 cm^2	Preferably use in vivo (propagating) material or in vitro material that has grown. Do not use material that has just been transferred. With tissue culture material, clones are tested individually. The leaf or the entire plant without the root is used. Material must be free of agar.
Phalaenopsis	Pieces of leaf or stem	1 – 2	ca. 2 cm^2	
Tissue culture material	Leaf from material that has grown as much as possible. One clone per bag	1	ca. 2 cm^2	
Alstroemeria	Leaves	1 – 3	ca. 2 cm^2	

Important: Are you submitting material for potyvirus testing as well as ELISA? Please submit the samples in two separate grinding bags: One bag for the 'standard' ELISA and one bag for the Potyvirus test each containing the number of leaves required.

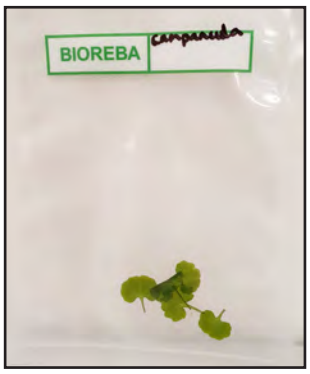
Examples of samples of ornamentals submitted correctly for ELISA analysis:



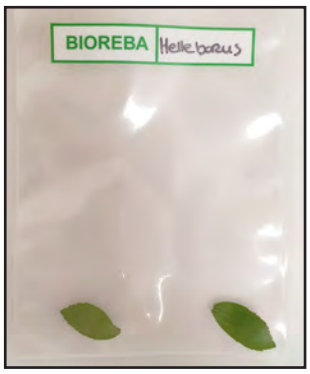
Alstroemeria



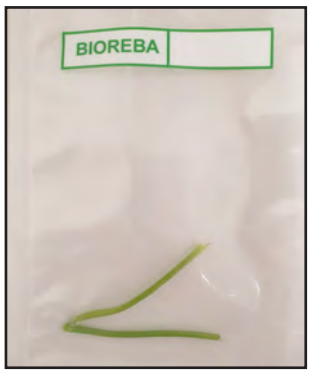
Callibrachoa



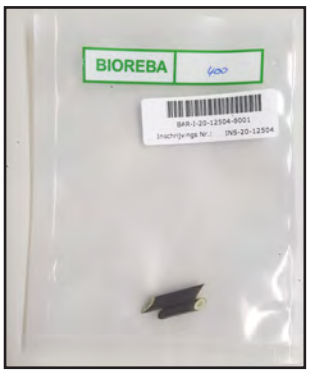
Campanula



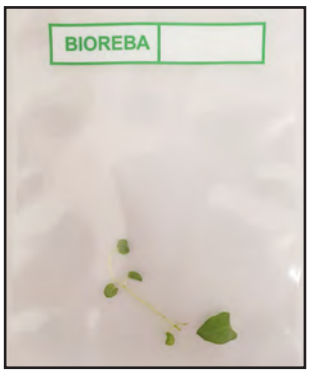
Helleborus



Pelargonium (leaf stem)



Phalaenopsis (flower stem)



Tissue culture samples

Tissue culture samples