



Fruit sample collection procedure

In accordance with guidelines set out by the Deciduous Fruit Plant Improvement Association – Sampling Procedures

Supplies Required:

- Plastic bags (transparent, self-adhesive/zip-lock plastic bags work well)
- Permanent markers
- Cooler bag/box
- Ice packs (frozen)

Sampling in tunnels/ gauze houses / glass houses:

Most of the viruses that we test for can only be reliably traced if the plants (that will be sampled) have not been subjected to high temperatures (>25°C) for long periods of time.

Sampling under field conditions:

Leaves should be sampled on overcast/cooler days, or in the early morning (during the cooler part of the day).

Sampling Procedure

1. Identify the trees to be sampled and create a name for each sample (based on essential identifiers, like variety/clone, block number and tree number) and label a bag for each sample with a permanent marker.
 - The sample name should enable you to trace the result obtained by ELISA/PCR back to the specific tree from which the sample was obtained.
2. Identify and take two to three developing, young, small and soft leaflets from the tree. Leaf samples can be collected from shoots that are proportionally spread over the tree and are at least 100mm long.
3. Pack the sample into the relevant sampling bag immediately.
 - Ensure that the sample bags are closed and that leaves are unable to fall out.
4. Immediately place the sample into a cooler bag (or similar cool storage at 4°C).
5. Keep samples cool (refrigerate/ fresh ice bricks daily) and deliver it to Patho Solutions within 72 hours after sampling.
 - All samples have to be clearly labelled (variety/clone, block number, row number and tree number).
 - Sample submissions should be accompanied by information regarding the sampling date, owner and farm names.

Sampling Specifications:

- I. Foundation material:
 - Test the site on a tree by tree basis.
 - Collect leaf samples from every tree in the unit.
 - Combine the leaf samples of every ten trees and submit it as one (pooled) laboratory sample.
- II. Mother material:
 - If the unit has less than 60 trees, treat sample set like foundation material.
 - In units that consist of more than 60 trees, Graph A (p.3) can be used to plan representative sampling.
 - Combine the leaf samples of every ten trees and submit it as one (pooled) laboratory sample.
 - Any recognised sampling procedure may be used (e.g. the K, W of S biometry methods)
- III. Suspect material:
 - Take leaf samples from trees that show suspicious visual symptoms.
 - Each tree should be sampled and tested as an individual unit (no pooling).



Pooling Samples:

- 1. When pooling samples, it is important to include a consistent number of leaves for each plant in the pool as with the individual sampling, in order to ensure that all plants are equally represented in the test.
- 2. If a pooled sample returns a positive result after testing (which indicates that at least one of the plants in the pool is infected), we will contact you regarding your requirements for de-pooling (re-testing the relevant plants in smaller pools, or as individual samples).

Sample Size:

Please consider the following tables when preparing for sampling. These tables were obtained from the Deciduous Fruit Plant Improvement Association – Sampling Procedures (Page 5).

4.1 Pome Fruit

Unit	Sample	Test
Nucleus Block	Tree by tree	Pool 10 trees
Foundation: Glass house	Tree by tree	Pool 10 trees
Foundation: Open field	Tree by tree	Pool 10 trees
Foundation: Layer beds	Statistical sampling model according to Graph A	Pool 10 trees
Mother: less than 60 trees	Tree by tree	Pool 10 trees
Mother: more than 60 trees	Statistical sampling model according to Graph A	Pool 10 trees

4.2 Stone Fruit

Unit	Sample	Test
Nucleus block	Tree for tree	Pool 10 trees
Foundation: Glass house	Tree for tree	Pool 10 trees
Foundation: open field	Tree for tree	Pool 10 trees
Mother: less than 60 trees	Tree for tree	Pool 10 trees
Mother: more than 60 trees	Statistical sampling model according to Graph A	Pool 10 trees

Submission:

Submit samples within 72 hours of sampling, accompanied by a completed sample submission form and shipped in a box with ice bricks. Samples can be delivered/couriered to our office:

Patho Solutions
143 Olifantskop Road
Soetendal
Wellington
7654

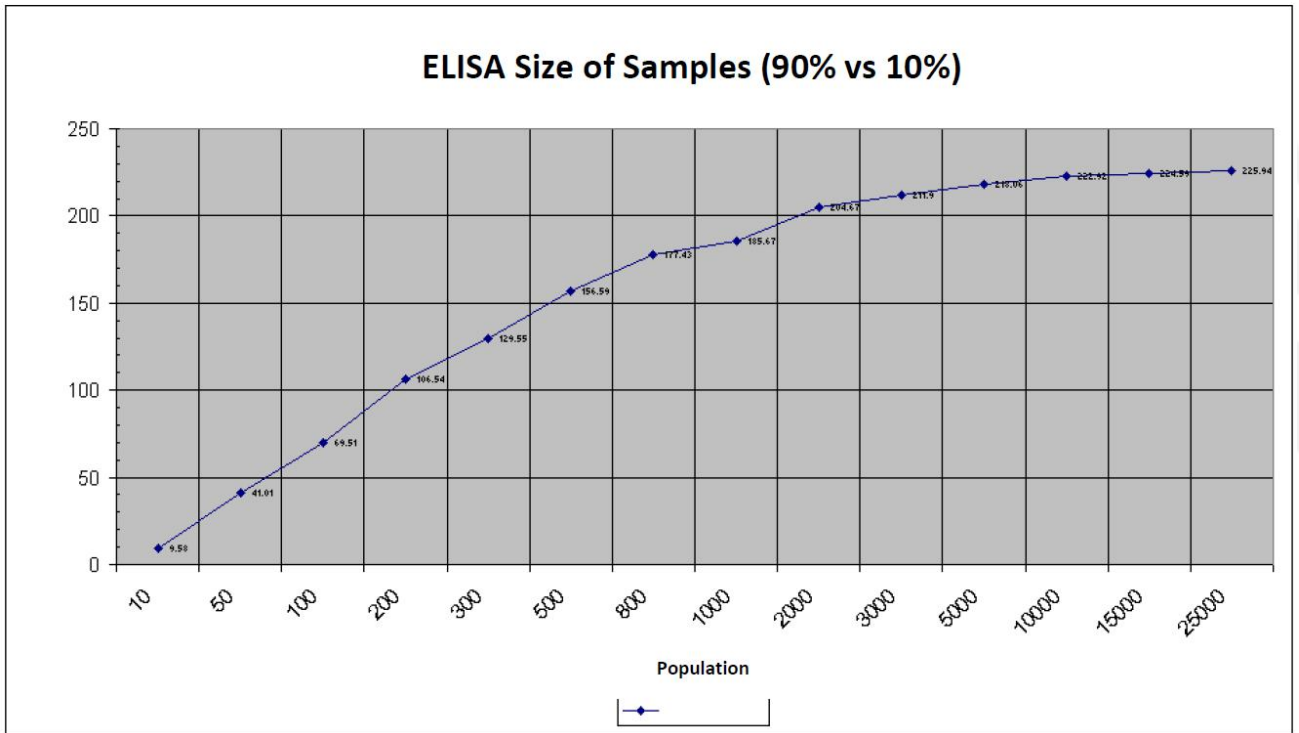
Please confirm sample delivery dates with our ELISA Leader, Tarien- 0718929322 or tarien@pathsol.co.za.

Turnaround time:

We are generally able to give feedback on testing results within ten working days after receiving samples. Larger sample assignments (300 or more) may require more time for processing and reporting.



Graph A



Graph A:

Please consider Graph A when preparing for sampling of a mother block with more than 60 plants. This Graph was obtained from the Deciduous Fruit Plant Improvement Association – Sampling Procedures (Page 6).

DISCLAIMER:

1. In any diagnostic assay there is a lower limit of detection, below which a pathogen cannot be detected, and ELISA assays will only detect the specified pathogen only above a certain threshold value. Pathogen levels may vary due to climatic conditions, host plant genotype, pathogen strain or plant tissue assayed. It is important to understand that the failure to detect a specific pathogen does not guarantee the complete absence of that pathogen from the sample analysed.
2. The ELISA, PCR, Sequencing, LAMP and Next Generation Sequencing testing methods used by Patho Solutions have been developed and tested against specific local and/or international pathogen isolates. However, the possibility remains that there may be variants of the pathogen which cannot be detected in the assay.
3. Due to points 1 and 2, no absolute guarantee can be provided that a given sample yielding a negative assay result will be completely free of the pathogen tested for.
4. Results refer strictly to the actual samples referred to in the list provided at the time of sampling. It should not be assumed that the same results apply to any other plants/progeny/ predecessors of the sample, or of the same sample at another time. Clients should not represent or imply that the results apply other than to the actual samples listed. The client releases and indemnifies Patho Solutions (Pty) Ltd in respect of any loss or liability incurred as a consequence of such representations, which remain the sole responsibility of the client.
5. All data produced from surveys and testing is the property of Patho Solutions, and may be used along with meta-data for research and development purposes. This data is kept private for clients.
6. Information regarding the disease status of the farm will be kept confidential and shared only between Patho Solutions and selected staff of the client.

It is our aim to provide reliable, open, and efficient services. If you have any questions, recommendations, or grievances, please do not hesitate to communicate these to Patho Solutions.

