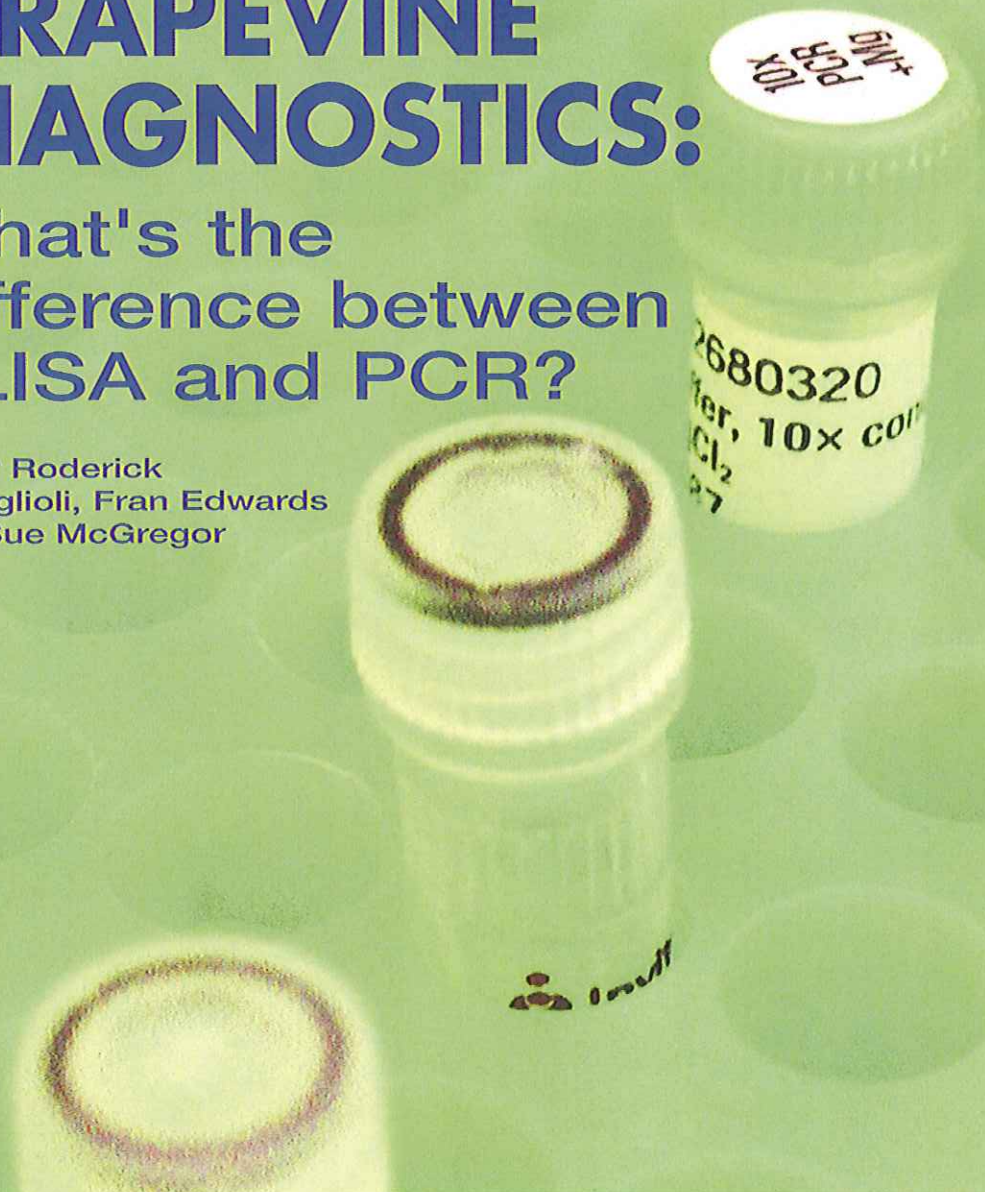


# GRAPEVINE DIAGNOSTICS:

## What's the difference between ELISA and PCR?

By Dr Roderick  
Bonfiglioli, Fran Edwards  
and Sue McGregor



**G**rapevines have few methods to indicate disease or stress.

Similar symptoms are expressed by any number of problems: virus or fungal diseases, nutritional deficiency, physical damage or the presence of insects and mites.

To complicate matters, the symptoms may show up at only certain times of the year - typically in late summer and fall - the rest of the time, vines may display no overt symptoms at all.

Although visual clues can serve

as an "early-warning bell", it's difficult to diagnose diseases based only on physical symptoms.

Just as your family physician relies on laboratory tests to make an accurate diagnosis, so plant scientists require sophisticated laboratory techniques. The purpose of testing is twofold: to verify the absence of pathogens and/or to diagnose diseases.

At Linnaeus Laboratory, we can and do perform a broad range of testing procedures, but the

bulk of our work by far relies on the two most common diagnostic tools: ELISA (Enzyme Linked Immunosorbent Assay) and PCR (Polymerase Chain Reaction).

These tests are vital not only for identifying many of the major grapevine diseases, they are also used by healthcare professionals worldwide to diagnose a myriad of human diseases, including tuberculosis, cancer and HIV/AIDS.

Elisa and PCR have proven worthwhile not only for their accuracy, but also for their speed, (relative) ease of use, low cost and suitability for testing in large volumes.

In the field of viticulture, both techniques have proven invaluable for nursery propagation. For example, many New Zealand nurseries use Linnaeus on an annual basis to test the materials used in their production of grafted vines.

Recently, a growing number of vineyard owners and winemakers have incorporated some level of testing in their operations, sending samples to Linnaeus when they want to propagate their own materials or when vines are showing symptoms of decline.

Increasingly, major buyers of existing vineyards depend on what's known as a "warrant of fitness" — a customised report that assesses a vineyard's health status based on a comprehensive virus-testing programme.

Nonetheless, many growers and viticulturists continue to express confusion when talk turns to ELISA and PCR, asking questions such as:

- Which test is better?
- Why does PCR cost more than ELISA?
- Shouldn't my nursery be doing all the testing?

Both techniques have their place and

not only at the nursery level.

Certainly, extra care is needed when vines are going to be used for propagation. That's why testing is such an integral component of any nursery certification programme, including the one instituted by our parent company, Riversun.

However, ELISA and PCR testing should also be your first action whenever suspicious symptoms appear. Thanks to these techniques, the diagnosis of vine diseases is more accurate today than ever before.

Read on for a brief discussion of the differences between PCR and ELISA and their appropriate uses.

#### WHAT IS ELISA?

It's fair to describe the Enzyme-Linked Immunosorbent Assay as the "workhorse" of grapevine diagnostics.

Accurate, inexpensive and easy to run, ELISA tests can usually produce results in just a few days. The technique is also suitable for commercial volumes, during the peak testing season, for example. Linnaeus typically processes up to 2000 samples daily with ELISA.

This type of test is known as a "serological" test, because it uses the reaction of an "antiserum" (antibody) to verify the presence of the disease.

Antiserum is created by injecting an

animal, usually a rabbit, with a purified preparation of a plant pathogen, such as a virus.

The animal produces antibodies in a direct reaction to the specific pathogen injected. Once the antibodies have been purified from blood serum, the "antiserum" is used to manufacture an ELISA test designed for that particular disease.

Many grapevine diseases now have specific ELISA tests available, including grapevine leafroll-associated viruses (GLRaV) 1 and 3, two of the most critical factors that adversely affect wine quality in New Zealand.

Because minimal equipment, materials and training are involved, costs are substantially lower than those associated with PCR, yet ELISA testing still offers a high degree of reliability, provided the following conditions are met:

- Samples must be selected from the tissue appropriate for the specific test — ELISA can be performed on shoot, cane and/or leaf samples.
- Testing must occur at the correct time of year. Depending on the disease under investigation, ELISA may be reliable for only one or two seasons of the year.
- Samples must be collected from the appropriate section of the vine and arrive at the lab in excellent physical condition.

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# Bio-Start DIGESTER

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Linnaeus has sampling instructions for cane and leaf submissions, including the optimum times for testing and appropriate labelling and shipping.

Our contact details appear at the end of this article, and we can send additional information or answer any questions.

Unfortunately, not all grapevine viruses have had antisera developed for use in ELISA testing. What's more, if a pathogen is present in a vine at extremely low levels, the test may come back with a "false negative" result. Despite its limitations, however, ELISA remains a valuable component of any vine-health regime.

Tests can be done for one or more viruses or for composites (to a maximum of five vines per test).

#### WHAT IS PCR?

Where ELISA looks for indirect evidence that a virus or bacteria is present by searching for an antibody reaction, the Polymerase Chain Reaction looks directly for a virus's unique DNA or RNA (the genetic component of grapevine viruses).

In wide use for little more than a decade, PCR has had an unprecedented impact on modern science and medicine.

PCR has been called a "molecular photocopier", because it targets genetic

material and copies it until quantities are large enough for detection, verification and analysis.

It does this by exploiting the natural function of the enzymes known as "polymerases". These enzymes are present in all living things and it's their job to copy, and correct, genetic code.

Each grapevine pathogen — be it a virus, bacteria or fungus — has its own "genome" (a unique molecular "sequence" or "code"). Recently, plant pathologists have isolated and identified the sequences that mark many pathogens, gradually adding to the list of grapevine diseases for which a PCR test has been developed.

It is PCR's ability to amplify a pathogen's genome that makes the technique so precise — by some estimates, between 100-1000 times more sensitive than ELISA.

Thus, even when a virus is present in a vine in extremely low amounts, it can often be detected using PCR, even when the corresponding ELISA test would come up negative. Other grapevine viruses, such as those causing rupestris stem pitting, simply cannot be detected using ELISA, making PCR the only option for diagnosis.

PCR involves an automated process, using purpose-built equipment, materials and trained personnel, which

make PCR more expensive than ELISA.

At Linnaeus, we routinely test for 15 of the major grapevine viruses using PCR, versus the two viruses we regularly screen for using ELISA tests (see the chart on facing page for a description of the samples and timing appropriate to each technique).

Again, tests can be done for one or more viruses or for composites (to a maximum of five vines per test, as with ELISA).

#### USING THE APPROPRIATE TEST

Both ELISA and PCR have their place in viticultural diagnostics. Each technique is accurate when used correctly; by using a combination of methods, testing programmes can be designed to offer a high degree of reliability, yet keeping costs as low as possible.

For example, though ELISA is much less costly when large volumes of testing material and routine viruses are involved, it's actually cheaper to use PCR testing to cover a broader range of viruses for only a few samples.

ELISA tests are typically sold in minimum batches, usually 500 tests, so buying an ELISA kit for a "one-off" of, say, a non-routine virus in a handful of samples will actually be more expensive than PCR.

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When plant materials are wanted for propagation purposes as "mother vines", PCR is unquestionably the preferred diagnostic technique. More viruses can be screened — all year round — and the tests are the most sensitive available.

As in all laboratory testing, the number of samples submitted bears directly on the confidence you can place in the results: the more the better.

As a result of the significant volumes of plant material processed at Linnaeus, we have developed systematic protocols for sampling and testing to ensure the highest degree of accuracy.

The laboratory can advise clients on sampling techniques, ranging from individual vines to bay and/or composite testing, based on our protocol for viticultural developments.

It's also worth emphasising no diagnostic test is perfect. A vine can be infected by more than one disease and infections are unlikely to be uniformly distributed throughout a vineyard, or even a single vine.

If your sample was selected from a part of the vine that does not yet contain the virus, for example, the result will come back as "not detected".

If you suspect a vine is diseased but test results are negative, don't stop there — test again. Remember, the test confirming that a vine is in perfect health has not yet been invented, nor is it likely to be. PCR and ELISA tests are simply designed to determine whether a particular pathogen is present in a particular vine or group of vines, a job they do extremely well.

#### BIOGRAPHICAL NOTE:

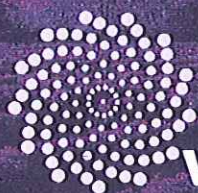
Technical Director Dr. Roderick Bonfiglioli, Laboratory Manager Fran Edwards and Laboratory Assistant Sue McGregor are the key staff at Linnaeus Laboratory, the only viticultural virus-testing laboratory to have been accredited by IANZ (International Accreditation New Zealand). For more information, contact Fran Edwards (telephone: 06-867-5279; mobile: 027-298-8458; or email: fran@riversun.co.nz).

## Guidelines for sampling and disease testing grapevines<sup>1</sup>

DISEASE	WHEN TO TEST	TISSUE TO SAMPLE	ELISA	PCR
Fanleaf, yellow vein (and other nepoviruses, including Arabis mosaic)	Spring is best; fall and winter are okay	Active shoot tips in spring; canes for cambium scrapings in fall and winter	Reliable in spring	Reliable in spring, less reliable in fall and winter
Leafroll (GLRaV)	Late summer, fall and winter	Leaf in late summer; canes for cambium scrapings in fall and winter	GLRaVs 1, 2, 3, 5 and 7 only Reliable in late summer, fall and winter	GLRaVs 1, 2, 3, 4, 5, 6, 7 and 9 Reliable in late summer, fall and winter
Rupestris stem pitting	Year round	Petioles, leaves or cambial scrapings	Not available	Reliable
Vitivirus	Spring, fall and winter	Petioles, leaves or cambial scrapings	GVA only Most reliable in spring	GVA, GVB, GVD Reliable
Fleck	Spring, fall and winter	Shoot tips and young leaves in spring; canes for cambium scrapings in fall and winter	Reliable in spring	Reliable in spring, fall and winter
Pierce's disease	Late summer and fall	Symptomatic leaves and shoots	Reliable in late summer and fall	Reliable in late summer and fall

<sup>1</sup>All tests are available from Linnaeus. This chart has been adapted from: "Laboratory testing for grapevine diseases," by Ed Weber, Deborah Golino and Adb Rowhani, in *Practical Winery & Vineyard Magazine*, Jan/Feb 2002 (with the authors' permission)

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