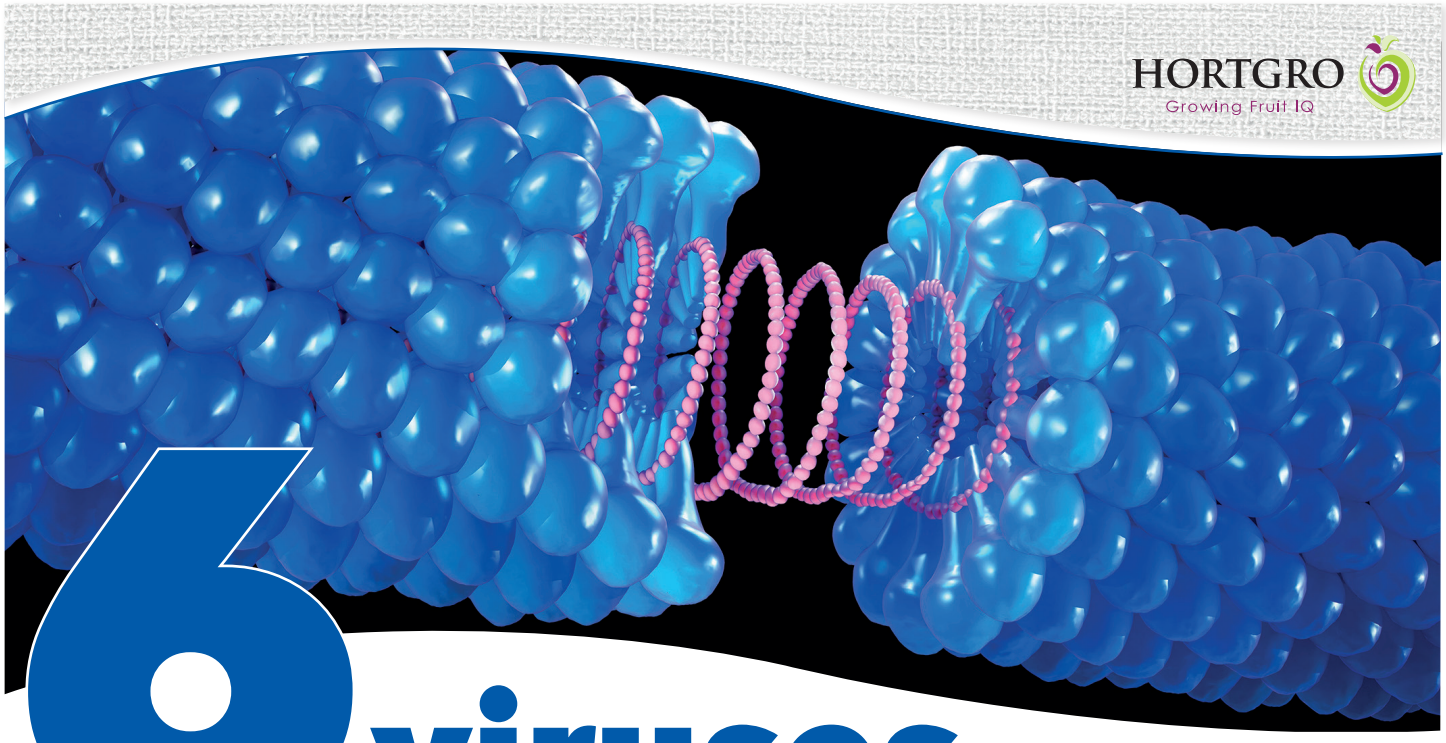
WHAT IS A VIRUS?

HORTGRO
Growing Fruit IQ

WHY IS IT IMPORTANT? | HOW DO YOU TEST FOR IT?



6 viruses and a viroid

A graphic representation of a virus similar to apple chlorotic leaf spot virus. The blue structure is its protein coat, and the pink structure is its genetic material.

The essentials explained for pome- and stone-fruit growers seeking to prevent losses.

The first thing to realise about viruses and viroids is that signs of infection in fruit trees show up most clearly in financial statements. South African stone-fruit growers were recently reminded of this with the emergence of plum marbling. Plum marbling is caused by a viroid that renders fruit unmarketable due to its impact on external appearance and internal quality.

Viral infections are associated with reduced growth and yields in both pome- and stone-fruit trees. Researchers in New Zealand and Germany found growth reductions of about 40% in apple trees infected with apple mosaic virus compared to non-infected trees. A South African study showed that yields in apple trees infected with apple mosaic virus were reduced by a third. Similar – and worse – outcomes have been reported for some viral infections of stone fruit.

So what exactly are viruses and viroids? And what can growers do to protect their orchards?

Viruses and viroids defined

A basic virus particle consists of a protein coat enclosing a set of genes. These genes are instructions for making the virus. Viruses hijack the cellular machinery of their hosts and turn this machinery to producing more viruses. Sometimes the host cell is damaged or destroyed in the process and signs of disease develop.

A viroid is similar but different. It is just a short loop of genetic material that is copied inside the host cell. Viroids are much simpler than viruses. They do not contain any protein-coding genes and have no protein coats. Scientists are still figuring out how viroids cause disease.

Most viruses and viroids are pathogens. The word pathogen literally means disease-causing. The name of a pathogen is often different to that of the disease it causes. For example, the coronavirus that causes the disease COVID-19 is called SARS-CoV-2. The trichovirus that causes apple chlorotic leaf spot, plum pseudopox, and other diseases, is called apple chlorotic leaf spot virus.

Virus particles are very small and measured in nanometres – millionths of a millimetre. Apple chlorotic leaf spot virus is about 700 nanometres long and 12 nanometres wide. You could fit nearly 1 500 of them on the head of a pin if you laid them end-to-end – and had a lot of time.

Bacteria and fungi are other examples of pathogens. Bacterial cells are roughly 10 to 100 times bigger than viral particles, and fungi are even larger. Bacteria and fungi have their own cellular machinery and are therefore far more complex than viruses.

The big six

The Deciduous Fruit Plant Certification Scheme includes testing for six viruses: apple chlorotic leaf spot virus, apple mosaic virus, apple stem pitting virus, apple stem grooving virus, prune dwarf virus, and Prunus necrotic ringspot virus. Plum viroid 1 – the cause of plum marbling – is a recent addition to the Scheme.

These pathogens are part of the Scheme because they have an economic impact. Certain other viruses and viroids occur in SA but are not part of the Scheme because there is no evidence that they cause financial losses. The Scheme also does not cover dangerous pests and diseases that do not occur in SA. These are prevented from entering through testing and quarantine of imported plant material.

The big six viruses are somewhat related, which explains why they have a lot in common. Apple mosaic virus, prune dwarf virus, and Prunus necrotic ringspot virus are all in the same genus. The other three are in three different genera but share a family. All

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Table 1. Summary of the big six viruses and the viroid for which plant material is tested under the Deciduous Fruit Plant Improvement Scheme

		Apple chlorotic leaf spot virus	Apple mosaic virus	Apple stem grooving virus	Apple stem pitting virus	Prune dwarf virus	Prunus necrotic ringspot virus	Plum viroid 1
	Also known as	Plum pseudopox virus			Pear stony pit virus			
	Abbreviation	ACLSV	ApMV	ASGV	ASPV	PDV	PNRSV	PlVd-1
	Family	*Betaflexiviridae*	*Bromoviridae*	*Betaflexiviridae*	*Betaflexiviridae*	*Bromoviridae*	*Bromoviridae*	*Pospiviroidae*
	Genus	*Trichovirus*	*Ilarvirus*	*Capillovirus*	*Foveavirus*	*Ilarvirus*	*Ilarvirus*	*Apscaviroid*
	Fruit type most affected	Pome and stone fruit	Pome and stone fruit. Hazelnuts.	Pome and stone fruit. Citrus.	Pome fruit	Stone fruit	Stone fruit	Stone fruit
SIGNS OF DISEASE	**Abnormal leaves**	Yes	Yes	Yes	Yes	Yes	Yes	No
	Abnormal fruit	Mostly in stone fruit	No	Yes	Yes	Yes	Yes	Yes
	Graft union failure	Yes	No	Yes	Yes	Yes	Yes	No
	Reduced growth and yields	Yes	Yes. Also reduced life span.	Yes	Yes	Yes. Also reduced life span.	Yes. Also reduced life span.	No. But can reduce fruit size.
	Other	Dieback and cankers	–	–	Dieback	Bark splitting	Dieback and cankers	–
TRANSMISSION	**Plant material**	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	Root grafting	Yes	Yes	Yes	Yes	Yes	Yes	Unknown
	Pollen	No	Inconclusive	Yes	No	Yes	Yes	Unknown
	Seed	No	No	Yes	No	Yes	Yes	No

the members of the two families to which the big six belong are plant pathogens.

Plum viroid 1 is not a virus, so it is not related to the big six. But it shares a viroid genus with pathogens of especially citrus and vines.

Which plants are at risk?

All the big six viruses primarily infect woody plants in the rose family. Apple chlorotic leaf spot virus and apple mosaic virus are of economic importance in both pome and stone fruit. Apple stem grooving virus and apple stem pitting virus mostly affect pome fruit, whereas prune dwarf virus and Prunus necrotic ringspot virus mostly affect stone fruit.

All these viruses can also infect other plants, including herbaceous species, but usually only under experimental conditions.

Plum viroid 1 has so far been found in Japanese plums and apricots.

The big six viruses all have a worldwide distribution and tend to occur wherever their host trees are commercially farmed. Plum viroid 1 has only been reported from SA.

How do trees become infected?

Propagation of infected plant material is the most important means of spread of the big six viruses. It has allowed these pathogens to infect pome- and stone-fruit trees on nearly every continent. Plum viroid 1 is also transmitted through infected plant material.

The big six viruses and plum viroid 1 are so good at spreading through propagation material because infected trees may appear healthy. Just as you cannot know whether someone is infected with SARS-CoV-2 or HIV by looking at them, you cannot know whether trees are infected with viruses or viroids by looking at them. Testing is the only way to identify infection.

None of the big six viruses is transmitted by insect vectors. In other words, insects that feed on infected trees will not spread viruses by feeding on uninfected trees. However, prune dwarf virus and Prunus necrotic ringspot virus occur in pollen, and insects can shuttle infected pollen between trees.

Western flower thrips and honeybees have been reported to carry infected pollen. One researcher noted that strains of Prunus necrotic ringspot virus were regularly spread between Washington State and California by honeybees used for pollination. This resulted in the introduction of new strains of the virus to Washington State.

Prune dwarf virus and Prunus necrotic ringspot virus can also be transmitted in seed. Seedling rootstocks from untested sources therefore pose a risk – growers should be wary of uncertified rootstocks grown from seed obtained from processing facilities such as canneries.

Although vegetative propagation and sexual reproduction account for most infections, the big six viruses can also be transmitted through natural root grafting. The roots of many woody

Propagation of infected plant material is the most important means of spread.

Right Distortion and discoloration caused by apple chlorotic leaf spot virus. Other viruses can cause similar changes. **Below** Leaf discoloration in a cherry tree. Several viruses cause similar signs.

> plants can form natural grafts when in contact. Apple mosaic virus is one example of a virus that has been shown to spread between apple trees through root grafts.

Researchers studying apple mosaic virus in roses discovered that 10% of uninfected bushes became infected within one year of planting near virus-infected bushes. Nearly half became infected within two years. Root grafting was thought to be the means of transmission.

What happens to infected trees?

This is where it gets complicated. The past few years have shown us how infection with SARS-CoV-2 can cause sickness and death in some individuals while others never so much as cough. Viral infections in trees tend to be the same – some trees show signs of disease while others appear normal. Why?

The outcome of most infections depends as much on the tree as on the pathogen. Trees may be less impacted due to their genetic makeup or their overall health. Even cultivars within the same fruit type can vary in their response to infection. For example, Malling and Malling-Merton rootstocks are tolerant to apple stem grooving virus, which causes graft union failure with many other rootstocks. Plum viroid 1 causes different fruit abnormalities in different plum cultivars.

Trees that enjoy ideal conditions are less likely to show symptoms of infection than trees that are under stress. This may be why diseases like plum marbling are more noticeable in some years than others.

Environmental conditions may also interact directly with pathogens. For example, apple mosaic virus is sensitive to high temperatures, so signs of disease are reported to fade in stone fruit after early summer when the weather heats up.

To further confuse the issue, trees are frequently simultaneously infected with two or more viruses. And there is one way in which viral infections in trees are less like COVID-19 and more like AIDS – infection is lifelong. Once infected, trees may have good years in which they show no signs of disease, but they still retain viruses in their tissue.

So what happens in the bad years? Viral infections typically cause distortion and discoloration of leaves. Leaves often have white or yellow spots or streaks. A tree may have few or many affected leaves. Tree growth and fruit yields can be significantly reduced.

Some viruses and viroids affect fruit quality. For example, apple chlorotic leaf spot virus can cause fruit deformities in plums and apricots. Apple stem pitting virus is responsible for pear stony pit, while Prunus necrotic ringspot virus gives rise to small, discoloured fruit. And of course, plum viroid 1 is behind plum marbling.

Apple chlorotic leaf spot virus, apple stem grooving virus, and apple stem pitting virus are also associated with graft union failure and decline of top-worked trees.

The bottom line

A few things should be obvious from the above. Firstly, the impact of viral infections in fruit trees can be anything from negligible to devastating. Secondly, signs of infection vary wildly for any given virus and overlap a lot for the different viruses.

Even worse, trees suffering from viral infections can show the same signs as trees suffering from other problems, such as nutritional deficiencies. For all of these reasons, it is not possible to diagnose a viral or viroid infection in a pome- or stone-fruit tree based on visual examination – testing is indispensable.

The only way that growers can manage the risk of these viral and viroid infections in their orchards is by only planting trees that have been propagated from material that has tested negative. There are no antivirals or vaccines for pome- and stone-fruit trees. <

Compiled by Anna Mouton

VIRAL economics

The financial repercussions of diseases go further than you might think.

The cost of disease is easy to see when infection equals death or disability. But viruses and viroids of fruit trees seldom kill their hosts and may cause few obvious signs of disease. So why are they so bad for the bottom line? There are five main reasons.

1. Direct losses

Viral infections can result in direct losses when trees die, or fruit is affected. For example, apple chlorotic leaf spot virus, apple stem grooving virus, apple stem pitting virus, prune dwarf virus, and Prunus necrotic ringspot virus can all cause graft union failure in susceptible cultivars.

Prunus necrotic ringspot and prune dwarf viruses can also decrease bud take after grafting. Infection of either scion or rootstock with prune dwarf virus has been reported to reduce bud take by up to 50% in almonds. A German study found that viral infections could reduce bud take in apple nurseries by as much as two-thirds.

Pear stony pit caused by apple stem pitting virus is an example of fruit damage related to viral infection. Affected pears are deformed and gritty. Up to 94% of the fruit on an infected tree can be damaged in a bad year.

In SA, plum viroid 1 causes sporadic losses in certain plum cultivars due to marbling. Infected trees look fine but abnormal appearance and quality render some fruit unmarketable. The occurrence of marbling seems to be influenced by environmental stress – a pattern also seen with many viral infections.

Researchers reported yield reductions of 40 - 50% in Golden Delicious and Red Delicious infected with apple mosaic virus.

2. Reduced growth and yields

Failure to thrive is arguably a more significant – and underrated – outcome of viral infections in deciduous fruit trees than direct losses. One reason is that poor tree performance due to infection can be hard to disentangle from other causes such as drought or replant disease. Another is that the consequences of infection are cultivar dependent.

For example, researchers reported yield reductions of 40 - 50% in Golden Delicious and Red Delicious infected with apple mosaic virus, while yield reductions in McIntosh were less than 10%.

Infections with multiple viruses further muddy the waters. Whereas peach trees infected with Prunus necrotic ringspot virus had yield reductions of 18%, those infected with both Prunus necrotic ringspot

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and prune dwarf virus had reductions of 30%. Other authors have noted up to 60% lower yields in peaches simultaneously infected with both these viruses.

Many studies have documented poor growth associated with viral infections. German apple trees infected with apple mosaic virus grew 40% less than uninfected trees over five years. A New Zealand trial found that Freyberg apple trees infected with apple mosaic virus produced 42% less wood over 12 years than uninfected trees. Golden Delicious is a parent of Freyberg.

New Zealanders also showed that Jonathan apples were smaller when trees showed moderate to severe signs of mosaic compared to fruit from trees with mild or no signs.

Viral infections have similar potential to stunt growth in nursery trees. For example, experimental infections of nursery trees with apple stem grooving virus reduced height by an average of 23% and stem diameter by an average of 14%, compared to uninfected controls. Golden Delicious was the worst affected cultivar in the trial, with a 64% reduction in height and a 43% reduction in stem diameter.

3. Reduced survival

Lack of performance may shorten the lifespan of a tree when the grower loses patience with it. But the viral infections themselves can also reduce tree survival. For example, up to 80% of trees died in one study on four peach cultivars infected with both Prunus necrotic ringspot and prune dwarf virus.

Another example of reduced survival is top-working disease – the decline of apple trees within a few years of grafting. Apple chlorotic leaf spot, stem grooving and stem pitting viruses were reported to cause top-working disease in apple trees in Japan and India. It tends to affect only some rootstocks – trees grafted on Malling and Malling-Merton rootstocks do not develop the disease.

4. Management costs

Infected trees can often appear healthy provided they are not stressed in any way. This suggests that the impact of viruses can be mitigated through careful management. But ensuring perfect fertilisation and irrigation at all times comes at a cost – and might be impossible during times of drought.

Furthermore, infected trees may not be able to carry the same crop load as uninfected trees. Meanwhile, they are a potential source of infection for healthy trees. So the best strategy is to keep viruses away by only planting trees that have tested free.

In SA, plant material should be tested free of apple chlorotic leaf spot virus, apple mosaic virus, apple stem grooving virus, apple stem pitting virus, prune dwarf virus, Prunus necrotic ringspot virus, and plum viroid 1. These are the viruses and viroid included in the Deciduous Fruit Plant Improvement Scheme.

Testing and certification of plant material is an unavoidable cost in a virus-ridden world. There is no doubt, however, that this form of prevention is far more economical than trying to manage chronically diseased trees.

5. Quarantine

Viruses affect the movement of plant material. Even though several viruses of pome- and stone-fruit trees already occur in SA, there are many others that we have so far escaped. Plum pox virus is a good example.

Plum pox has been described as one of the most destructive diseases of stone fruit. Unlike the viruses listed above, plum pox virus is transmitted not only by grafting but also by aphids. One study published in 2006 estimated the global cost of plum pox management over 30 years at more than €10 billion.

Plum pox renders fruit unmarketable. Because plum pox virus is not yet ubiquitous, it is a quarantine disease in certain countries, thereby affecting trade.

Diseases such as plum pox are one reason for the strict regulation of plant-material importation. Although our deciduous-fruit industry clearly has to be protected from invasive pests and diseases, this does constitute yet another cost of viruses.

Many of the figures for reduced tree performance due to viral infections are decades old, such as a South African publication from 1949 stating that apple yields were reduced by a third in trees infected with apple mosaic virus.

More recent reports are scarce because testing and certification of plant material have become standard practice in most countries – sparing many growers losses due to viral infections. But the risk is that we begin to focus so much on the price of control that we forget the much higher price of disease.

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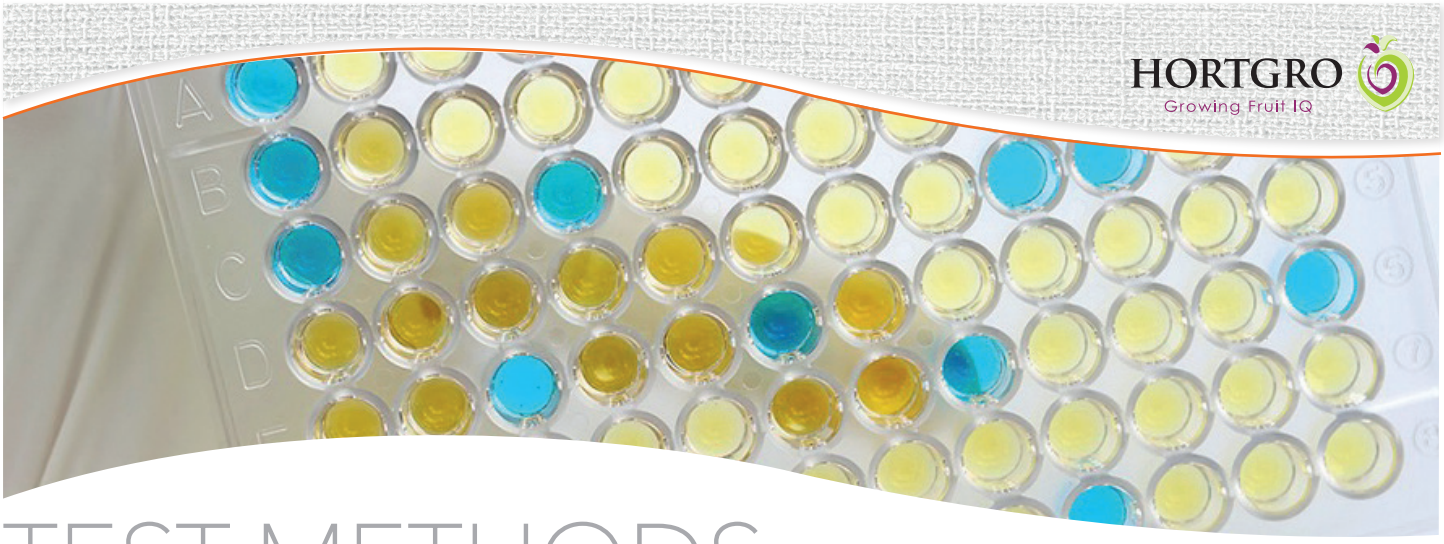


Positive and negative results in the ELISA test are indicated by a colour change.

TEST METHODS demystified

Do you think ELISA is the girl next door and RT-PCR is a new stone-fruit rootstock? Then you need to read this article.

Viruses and viroids have the potential to inflict serious financial damage on pome- and stone-fruit growers. The best way to mitigate this risk is by establishing orchards using healthy trees – but trees can be infected without showing signs of disease. So what do growers need to know about testing their trees?

Five things to keep in mind about testing

1. The sample is not the whole

Good sampling protocols use science and statistics to provide a high probability of disease detection but there is still a chance that a test of an infected individual or population returns a negative result. This is because we seldom test an entire organism or population. We typically sample parts of plants and those only from a subset of the plant population.

Successful disease control will reduce infections, and all else being equal, increase the sample size needed to detect infection in the population.

2. No test is perfect

The performance of a test can be judged by its sensitivity and specificity. Sensitivity is the ability of the test to detect very low levels of the target pathogen. Specificity is the ability of the test to differentiate its target from similar pathogens. The perfect test would be 100% sensitive and 100% specific – but there are no perfect tests in the real world.

The trade-off between sensitivity and specificity is shown in figure 1. As a rule of thumb, the greater the sensitivity of a test, the greater the risk of a false positive, and the greater the specificity, the greater the risk of a false negative.

3. Pathogens can change

Pathogens such as viruses mutate over time and new strains replace older ones. A test that worked for an older strain will not always perform as well on a new strain. Like the antivirus software on your computer, tests for plant pathogens need regular updating.

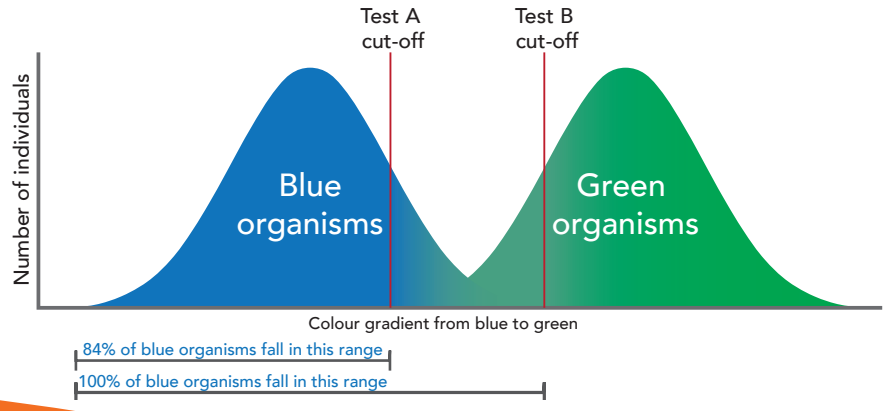
FIGURE 1: The trade-off between sensitivity and specificity

Two tests – A and B – can identify a hypothetical blue organism by detecting its colour

Test A is 100% specific. It is very strict about what counts as blue and will never call a green organism blue. But it also throws out the 16% of blue organisms that are turquoise.

Test B is 100% sensitive. It detects any degree of blueness, so it never misses a blue organism. However, it will classify about 16% of green organisms as blue because they are turquoise.

The figures for real tests and real organisms will depend on the technology and how much the organisms overlap, but the fundamental trade-off still applies.



ELISA in 4 steps

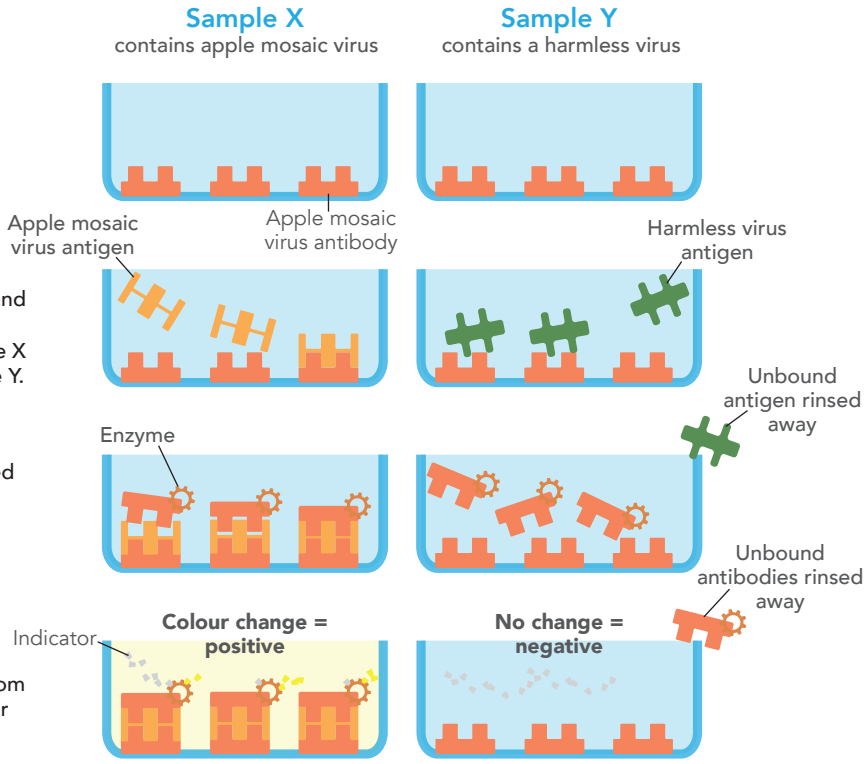
FIGURE 2

1. Attach apple mosaic virus – ApMV – **antibodies** to two containers.

2. Add **antigens** extracted from the samples to different containers. Sample X contains ApMV and sample Y contains a harmless virus. ApMV antibodies bind to the ApMV antigens in sample X but not to the harmless virus antigens in sample Y.

3. Remove unbound antigens. Add enzyme-labelled antibodies. **Enzymes** catalyse – speed up – chemical reactions. Labelled antibodies bind to the ApMV antigen in sample X.

4. Remove unbound labelled antibodies. Add the **indicator**. The enzyme changes the indicator from a colourless to a coloured compound so a colour change indicates a positive result for ApMV.



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4. You only find what you look for

Except for high-throughput sequencing, all the tests discussed below will only detect the pathogen they were designed to detect. For example, trees that tested free of the six viruses covered by the Deciduous Fruit Plant Certification Scheme were recently found to be infected with the viroid that causes plum marbling. The trees had not previously been tested for plum viroid 1 because no one knew it existed.

Plum viroid 1 is now included in the Scheme, but it is unlikely that this will be the last time that a new pathogen emerges in fruit trees.

5. Things can go wrong

Like any undertaking, testing is subject to human error. The best way to mitigate this risk is to use reputable laboratories that have preferably been accredited by SANAS – the South African National Accreditation System.

What do tests detect?

The tests discussed below are used in SA to detect viruses and viroids. There are six viruses and one viroid covered by the Deciduous Fruit Plant Certification Scheme. They are included in the Scheme because they occur in SA and are economically important.

Many other significant viruses and viroids do not occur in SA – testing imported plant material helps to keep them out.

Viruses all consist of genetic material enclosed in a protein coat. Tests for these viruses are designed to detect either their genetic material or their proteins. Viroids do not have a protein coat, so tests for viroids only target genetic material.

Why does this matter? Because it explains a key difference between ELISA and PCR. ELISA tests are based on viral proteins while PCR tests sniff out viral or viroid genetic material. LAMP assays and high-throughput sequencing likewise target genetic material.

None of these technologies is used exclusively for finding viroids and viruses in fruit trees. They are applied daily throughout the world to test a wide range of biological samples for both diagnostic and research purposes.

ELISA

ELISA is short for enzyme-linked immunosorbent assay, a laboratory workhorse that has been around for more than 50 years. ELISA testing is available for apple chlorotic leaf spot virus, apple mosaic virus, apple stem grooving virus, prune dwarf virus, and Prunus necrotic ringspot virus.

ELISA relies on the ability of antibodies to bind to antigens. Antibodies are proteins produced by the immune system. These proteins recognise and help inactivate foreign material such as viruses or bacteria in the body.

Antigens are defined in relation to antibodies – an antigen is something to which an antibody binds. Certain immune cells also bind antigens.

The key to understanding the ELISA is knowing that antibodies are specific. An antibody will only bind to a specific antigen in the same way as a key will only open a specific lock. Scientists can manufacture antibodies to specific viral proteins and then incorporate those antibodies in an ELISA to detect the virus in a sample.

There are umpteen variations of the basic ELISA, but the fundamentals are the same for

all of them. Figure 2 shows how a double antibody sandwich ELISA works. This is a method commonly used in SA to test for plant viruses.

PCR

The invention of the polymerase chain reaction – PCR – nearly 40 years ago was a seismic event in biology. PCR is behind everything from forensics in television shows like CSI to schemes to revive the extinct woolly mammoth.

Closer to home, PCR testing is used for apple chlorotic leaf spot virus, apple mosaic virus, apple stem grooving virus, apple stem pitting virus, prune dwarf virus, Prunus necrotic ringspot virus, and plum viroid 1.

PCR works because DNA usually consists of two complementary strands. Either

FIGURE 3

RT-PCR IN 4 STEPS

What is?

- PCR = Polymerase chain reaction
- RT-PCR = Reverse transcription PCR
- Real-time PCR = PCR monitored in real-time
- qPCR = Quantitative PCR

RNA vs DNA: the bases Both consist of chains of molecules called bases. RNA usually has one strand. The bases in RNA are

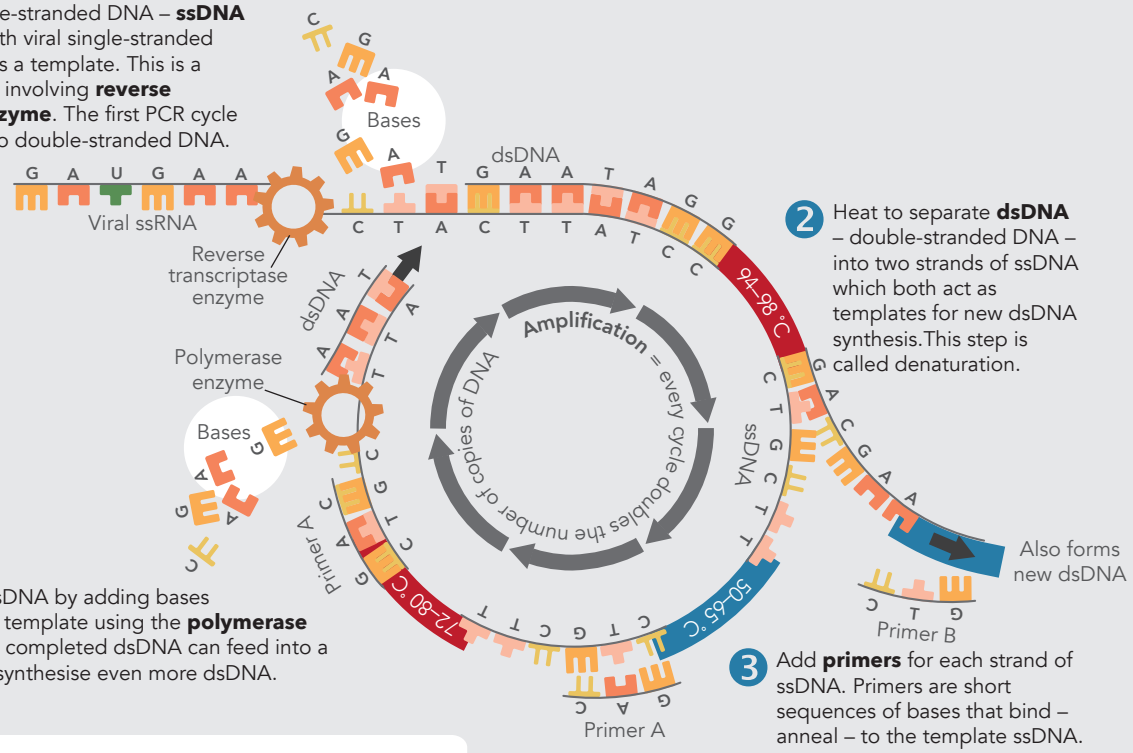
- Adenine
- Uracil
- Guanine
- Cytosine.

C U G C U A

DNA is double-stranded. It also has the bases A, G, and C, but instead of U it has Thymine.

G A C G A T
C T G C T A

The two strands in DNA are held together by bonds between opposite pairs of bases. A always bonds to T. G always bonds to C. This means that **either strand of DNA can be used to reconstruct the double-stranded version**.



From amplification to result If the primer matches the sample DNA, each cycle of the PCR process will generate two new copies of dsDNA. A typical PCR reaction of 30 cycles would produce approximately 1 billion copies of dsDNA per template. This is called **amplification**.

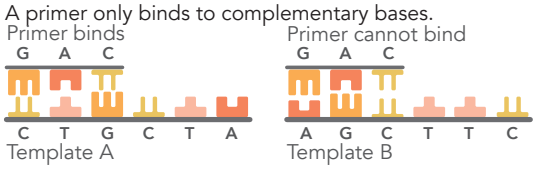
Amplification can be measured in several ways. In conventional PCR the sizes of the amplified DNA strands are compared to a control in a process called gel electrophoresis, carried out after the PCR process.

In real-time PCR, amplification is measured during the PCR process – hence the term real-time PCR.

Note Although the basics of PCR are the same, the details of any given PCR test will vary depending on many factors, for example the sample type, primer set, and polymerase enzyme.

The primacy of primers New dsDNA is synthesised from the template ssDNA by building a matching second strand of DNA with the help of polymerase enzyme. **Primers** provide the starting point from where the enzyme can add bases – polymerase cannot begin work without a primer.

A primer only binds to complementary bases.



If we know the DNA or RNA sequence of the virus – or other target – we want to find, **we can design primers** that will only bind to that DNA sequence **so that only target DNA is amplified during the PCR**.

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strand can serve as a template to recreate the other strand. The PCR process splits double-stranded DNA and then recreates two copies of the original DNA from the two strands. Repeat the cycle once and two copies become four copies. Repeat the cycle 30 times and you end up with 1 billion copies – this is called amplification.

The reason PCR is so useful is that one can control which DNA is amplified. For example, if you want to test a plant sample for apple mosaic virus, you can design a PCR to only target that virus and not any other viruses or DNA from the plant itself.

Dealing with targets that have RNA instead of DNA – such as many viruses – requires first converting RNA to DNA. Reverse-transcription PCR – RT-PCR for short – refers to PCR that includes this step. See figure 3 for more details.

LAMP

The principles of DNA amplification are the same for LAMP – loop-mediated isothermal amplification – as for PCR, except that a different strategy for amplification is employed. At present, LAMP testing is only available for plum viroid 1 in SA.

Coloured pH indicators or the incorporation of fluorescent dyes can be used to detect LAMP-amplified DNA. The pH-dependent colour change can be seen by the eye, while the dye-based tests generally require instruments, some of which are basic and others that are sophisticated – and pricey.

One advantage of LAMP over PCR is that sample preparation for LAMP is simpler, which reduces cost.

High-throughput sequencing

Also known as next-generation sequencing, high-throughput sequencing – HTS – is a method for determining the sequence of the bases in a DNA molecule. DNA has four bases – think of it as an alphabet with only four letters – that spell out the instructions for making an organism. The instructions for building a human are obviously different from those for building a pear tree and this is reflected in their genome sequences.

Genome sequencing has many practical uses. For example, potential pathogens can be smoked out by sequencing samples from diseased and healthy individuals and seeing what genetic material is present in the former but not in the latter. This is how plum viroid 1 was identified.

High-throughput sequencing differs from most other tests in being non-specific. Whereas PCR will only detect what it was designed to detect, HTS can potentially detect previously unknown things – like plum viroid 1.

The drawback of HTS is that interpreting the results can be challenging and requires expert analyses of reference sequence information that may not be available. High-throughput sequencing is also an expensive and time-consuming process. This makes it unsuitable for testing large numbers of samples.

Table 1 Brief comparison of different tests used for plant viruses

	ELISA	PCR	LAMP	HTS
Full name	Enzyme-linked immunosorbent assay	Polymerase chain reaction	Loop-mediated isothermal amplification	High-throughput sequencing
Detects	Viral proteins	Viral and viroid genetic material	Viral and viroid genetic material	Viral and viroid genetic material
Main use	Testing	Testing and research	Testing	Research. Less often testing.
Good for large sample numbers?	Yes	Real-time PCR: Yes. Conventional PCR: Less so.	Yes	No
Sensitivity	Moderate	Very good	Very good	Very good
Specificity	Good	Good	Can be excellent	Not applicable
Advantages	Easy sample preparation and simple robust test method	Relatively robust test method	Minimal sample preparation	Can detect previously unknown pathogens
Disadvantages		Costly sample preparation. High equipment costs.	Prone to false positives due to contamination	Slow turnaround time. Very high equipment costs
Notes on test variations		Reverse transcription step needed for RNA targets*.	Reverse transcription step needed for RNA targets*. Conventional LAMP is technically challenging. Real-time LAMP is more reliable but has high equipment costs.	
COST	$$	$$$	$$	$$$$

*Applies to all viruses and viroids covered by the Deciduous Fruit Plant Improvement Scheme