NT PLANT QUARANTINE & BIOSECURITY GUIDANCE NOTE

NUMBER 3 – USE OF *Phytophthora*
LATERAL FLOW DEVICES (LFDs)

1.0 Background

How can they help us?
Symptoms of diseases caused by *Phytophthora* species can often be confused with other diseases, insect damage, over- or under-watering etc. In these situations, Lateral Flow Devices can be used to confirm if the symptoms are caused by a *Phytophthora* species. The devices have been used extensively by the Trust to test for *Phytophthora* diseases both in gardens and in plant sales areas. LFDs are also available for a range of other fungal, bacterial and viral diseases.

What are they?
LFDs use antibodies to detect antigens (proteins) using technology employed in home pregnancy testing kits. The antigens are produced by all species of *Phytophthora*, including *P. ramorum* and *P. kernoviae*.

How specific are they?
The kits are known to react to at least 13 species of *Phytophthora* including *P. ramorum, P. kernoviae, P. fragariae, P. cactorum and P. infestans* so are useful for general *Phytophthora* screening. They do not cross react with related organisms such as *Pythium* spp. or downy mildews.

How sensitive are they?
The kits are very sensitive and can detect just a few milligrams of infected rhododendron leaf tissue (equivalent to a few square millimetres permitting detection in leaf tissue which is less than 1% infected by *P. ramorum*.

What can and can't I test?
Leaves, stems, roots can be tested but the kits are not suitable for testing soil or water. They have been used successfully to test bleeding bark cankers.

Where can I buy them?
The kits are commercially available from Forsite Diagnostics Ltd (York, 01904 462660; website http://www.forsitediagnostics.com/).

2.0 Instructions for use – example *P. ramorum/kernoviae*

In order to prevent any cross contamination when breaking up the tissue prior to placing in the extraction bottle, either wear disposable gloves or wash hands with a proprietary alcohol gel between samples.

Leaves - Take approx. a 1-2 cm square of diseased tissue (Figure 1a). Fold up the piece of tissue and crush to help break down the sample (Figure 2a).

Stems **-** Shave-off about 5 small (ca. 1 cm long) slivers of the outer tissue from a necrotic area. Try to get tissue from the leading edge of infection (the boundary between brown and green) because the fungus is most active there. Crush if possible (Figures 1b and 2b). Clean knife before re-use.

Place the sample in the extraction bottle (Figure 3a) and shake vigorously for at 60 seconds (Figure 3b). Longer may be need for woody tissue.

With the pipette draw up the extraction solution (there should be a slight green to brown coloration if the extraction has been successful, Figure 4a).

Place 2-3 drops in the sample well (Figure 4b). If liquid does not start to flow across the membrane after 30 seconds (Figure 5a) add one or two further drops until fluid flows across the membrane. Do not use more than 4 drops as this will flood the membrane resulting in an invalid test.

Leave for 2-3 minutes.
Check to see if a blue line has appeared at 'C' (control), Figures 6a and b)
Check to see if a blue line has appeared at 'T' (test), Figures 6a and b)

You may check for lines up to 20 minutes but results after this time should not be considered.

In cold weather the devices may take longer to develop. If the kits are very cold (e.g. stored in a car overnight) it is advisable bring them back up to room temperature.

If the material appears dry or is very woody after the initial 60 seconds of shaking you can leave the sample in the bottle to soak to aid extraction for up to 1 hour before testing.

Interpretation of results

Control line is not blue - test invalid re-test with the same extract but a new LFD.
Control line is blue - test valid

Test line is blue within 10 minutes (Figure 6b - upper LFD [A]) - **Positive** for *Phytophthora* spp.

Test line is not blue within 20 minutes (Figure 6b - lower LFD [B]) - **Negative** for *Phytophthora* spp.

Health and Safety
Risk to user: The extraction buffer contains sodium azide, a detergent (Tween 20) and polyvinylpyrrolidine (PVP). The concentrations are such that any risks associated with the product are considered very low. However, ingestion of the buffer should be avoided, and care should be taken to avoid contact with skin or eyes. Should contact occur, rinse with copious amounts of water.

Storage
Store at ambient conditions. Do not place in the fridge or freezer. The kits have an expiry date on the foil packet. Once the foil packet has been opened, use the kits as quickly as possible (within several days). Where possible, seal opened foils with sticky tape until remaining LFD is required.

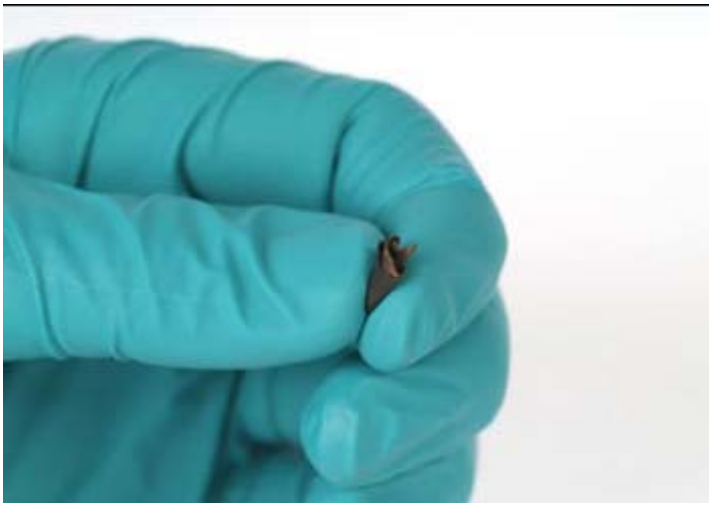
Sampling and Testing Procedure

Select a sample

1 a) Leaves

1 b) Stems

Break down sample prior to placing in the extraction bottle



2 a) Leaves - crush

2 b) Stems – slice and crush

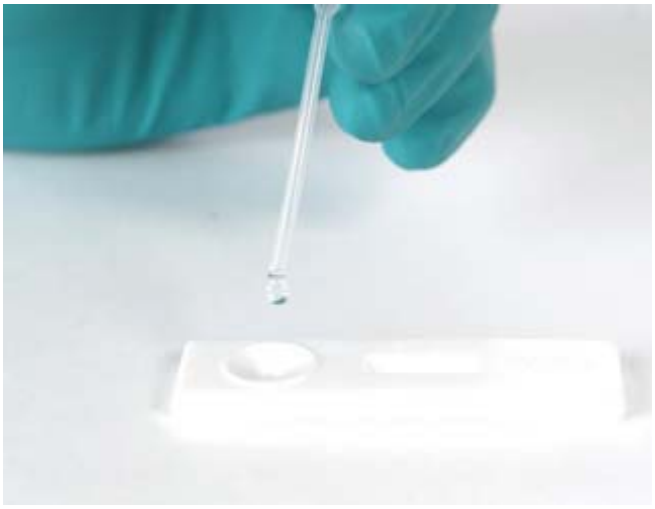
Place sample in extraction bottle and shake vigorously for 60 seconds

3 a) Place in bottle

3 b) Shake vigorously for 60 seconds

Draw up extraction fluid and pipette onto LFD

4 a) Check to see if the extraction liquid is discoloured

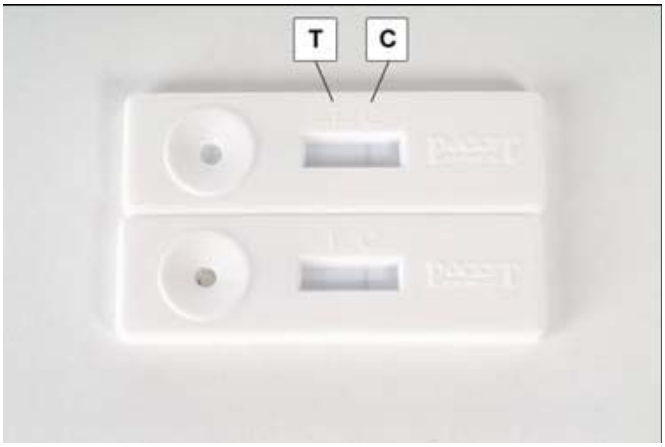


4 b) Pipette 2-3 drops onto sample well

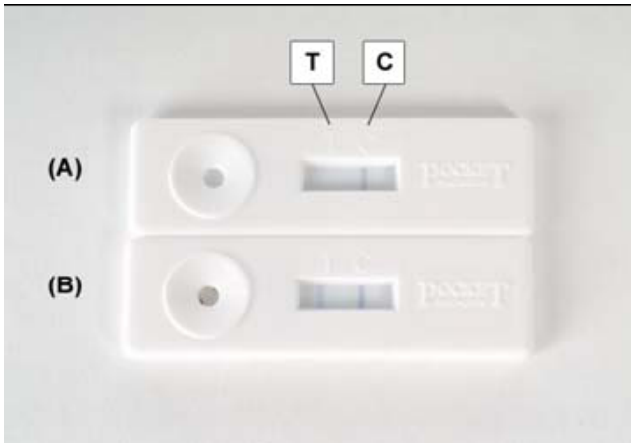
Check to see if the extraction fluid is running across the membrane

5a. Pale blue fluid runs across membrane

Read after 2-5 minutes



6 a) Test kit after 30 seconds - Feint control lines ('C') appearing; No test lines ('T') appearing



6 b) Test kit after 3 minutes: A) Test valid, sample negative; B) Test valid, sample positive

Conservation Directorate Guidance Note Information

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