Project Title: **Evaluation of a Fera antibody-based prototype LFD for on-site diagnosis of *Phytophthora***

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Cost: £15,000 (Defra Phytophthora Provision Funding)

Executive summary:

The Fera antibody-based prototype lateral flow device offers a good alternative to the Neogen antibody-based device currently in use. In the event of withdrawal of the Neogen antibody the Fera antibody could be used as a comparable alternative. However, in response to both laboratory staff observations and Inspectors comments further work to improve the intensity and speed of line development would enhance its performance and suitability for use.

Introduction

On-site testing for *Phytophthora ramorum* and *P. kernoviae* has been successfully achieved since 2005 with the use of a lateral flow device that detects all species of *Phytophthora* (approx 150 species). The technology is similar to that used in the home pregnancy testing kits, permitting diagnosis of *Phytophthora* species within 2- 10 minutes from any plant material (leaves, stems, bark). The kit has been evaluated on two separate occasions (Lane et al 2007; Kox et al., 2008) typically giving good comparison with either plating out or real-time PCR diagnoses (ca. 85% agreement, 10% false positives, 5% false negatives) and offering an excellent screening method at the point of inspection. As all three technologies are based on different techniques it is very difficult to decide which are' false positives/negatives' as the organism may or may have been present but it's detection is not permitted as either it is unviable so cannot be cultured or extraction is adversely affected due to inhibitors in decaying plant tissue.

The current lateral flow device in use is based on antibodies produced by the Neogen Corporation and manufactured by Forsite. Previous Defra-funded R&D (PH0412) developed a new *Phytophthora* genus-specific antibody that has been incorporated into a new prototype LFD. The advantages of this device are that the antibody would be cheaper to produce (LFD kits would be approximately £3.25 as opposed to the current £6 per test) and would ensure greater control and continuity of supply for these essential kits. Therefore a comparative trial of the Fera and Neogen antibody-based LFDs was commissioned with the PHSI after successful evaluation in the laboratory.

Aim

To establish if the Fera antibody-based Phytophthora lateral flow device is fit for purpose for field testing of plants for *P. ramorum* and *P. kernoviae*

Materials and methods

Six Plant Health and Seeds Inspectors (PHSI) experienced in the use of lateral flow devices were selected. The new kits were demonstrated and the trial explained at the PHSI Technical Conference.

A protocol was supplied to all participating inspectors; all steps in the testing process were identical for both devices. In summary, a small piece of potentially infected tissue was placed in the extraction bottle and shaken vigorously for one minute, two drops were then placed on each lateral flow device and the test kit results read after five minutes. All materials then returned to the laboratory for testing by isolation in order to identify the presence of any *Phytophthora* species.

Results

In total, 92 comparative trials of the LFDs were returned out of a total of 100 sent to six inspectors from around the country between mid January to mid March 2009. In total, 36 samples tested positive for *Phytophthora ramorum* (15), *P. kernoviae* (9) or other *Phytophthora* species (12); 56 tests gave negative results. The devices were tested from a representative range of hosts with a predominance of rhododendron as expected. There was a good level of agreement between the two devices (84/92; 91.3%): in 52 cases both LFDs gave the same positive result; in 32 cases both gave the same negative result. Where there was disagreement (8/92; 8.7%), in comparison with the laboratory test (isolation into culture) the Fera LFD gave 3 false negative results, whilst the Neogen LFD gave 5 false positive results. A summary of the results for these eight samples is as follows:

Neogen LFD result	**Fera LFD result**	**Lab result (isolation)**	**Number of samples**
Positive	Negative	Positive	3
Positive	Negative	Negative	5

When compared to the isolation results, on three occasions the Fera antibody-based device failed to detect *Phytophthora* ('false negative') whilst on the other five occasions the Neogen antibody-based device indicated a *Phytophthora* was present that could not be isolated ('false positive'). From a statutory perspective, LFD false positives have always been preferable to false negatives although neither is desirable!

Both devices performed well with similar levels of diagnostic sensitivity (Table 1). The diagnostic specificity of the prototype device was significantly better than the Neogen device as the former resulted in less 'false positives' (LFD positive but isolation negative); 18/92 as compared to 24/92 'false positives', respectively. In general, the number of false positives was higher than had been previously encountered in comparative trials (ca 10%). On a small number of occasions (7) samples from this trial were also tested by PCR. On each occasion, isolation agreed with the PCR result indicating that the discrepancy is primarily due to performance and interpretation of the lateral flow device. However, from a statutory point of view, as all LFD positives are submitted for laboratory testing then this discrepancy does not result in these pathogens being incorrectly diagnosed.

A number of inspectors also commentated on the performance of the two devices. In total, comments were provided on the performance of 17 devices with of the majority (12) in favour of the Neogen antibody-based device when compared to be Fera antibody-based device. In general, they preferred the current device due to the speed and intensity of line development.

Table 1 Comparison of Neogen and the Fera Prototype LFD devices with isolation (92 devices tested)

	Neogen (%)	**Prototype (%)**
Agreement	71.7	78.3
Diagnostic Specificity	56.4	69.9
Diagnostic Sensitivity	94.6	94.4
'False positives' (LFD +ve: Isolation –ve)	26.0	19.5
'False negatives' (LFD –ve: Isolation +ve)	2.2	2.2

Conclusions and recommendations

- The Fera antibody-based LFD performed well in comparison to the Neogen antibody-based LFD with good agreement (91.3%) between the two.
- Both devices had similar diagnostic sensitivity values (ca 95%) due to the small number of false negatives; whilst the Fera antibody-based LFD was less prone to false positives.
- The Neogen antibody-based LFD was preferred to the Fera antibody-based LFD by inspectors due to the speed and intensity of line development.
- The Fera antibody-based LFD offers a good alternative device that could be used now by inspectors. However, further work to improve line intensity and speed of development would assist in its uptake and address comments made by both inspectors and laboratory staff. Fera is working with Forsite to address these issues and investigating factors such as the influence of antibody purification and concentration on device performance.
- There are considerable cost savings from using a Fera antibody-based device (approx. 50% reduction).
- Consideration should be given to funding a larger-scale trial especially if the prototype could be improved further.

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