

Rapid lateral flow test for detection of *Dickeya* sp, causative agent of "agressive rot" in Hyacinth bulbs

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Background

The bulbous plant Hyacinth has to cope with some major challenges in order to maintain its quality and the growers/retailers have to keep the market stable. Infection by *Dickeya* creates a lot of damage in the sector annually. *Dickeya* bacteria cause bulbs to rot completely. In the case of Hyacinth rot two types of bacteria may be involved; *Dickeya* ("agressive rot") or the far less pathogenic *Pectobacterium* ("white rot"), which in terms of control and extent of damage, differ substantially from each other. Visually, distinction between *Dickeya* and *Pectobacterium*, is often impossible (figure 1). At the moment a labour intensive and expensive laboratory PCR test is required for a good distinction between the two.

To support farmers and inspection services in early and correct diagnosis of diseased bulbs, we developed a rapid method that is based on the well-known Lateral Flow ImmunoAssay (LFIA) principle.

Objectives

- To develop a simple and rapid method to diagnose infected Hyacinth bulbs whether they are affected by *Dickeya* ("agressive rot") or by *Pectobacterium* ("white rot").
- Comparison of performance characteristics of the newly developed rapid lateral flow test with results obtained with Polymerase Chain Reaction (PCR) as the "gold standard".

Results

Rapid Lateral Flow ImmunoAssay

A specific polyclonal antiserum directed against *Dickeya* bacterial cells was used both as capture ligand on the nitrocellulose membrane and as detection ligand immobilised on the surface of carbon nanoparticles (figure 2). If only the control line shows up, the assay is valid and there is no *Dickeya*, if both lines develop, the assay is valid and there is a *Dickeya* infection.

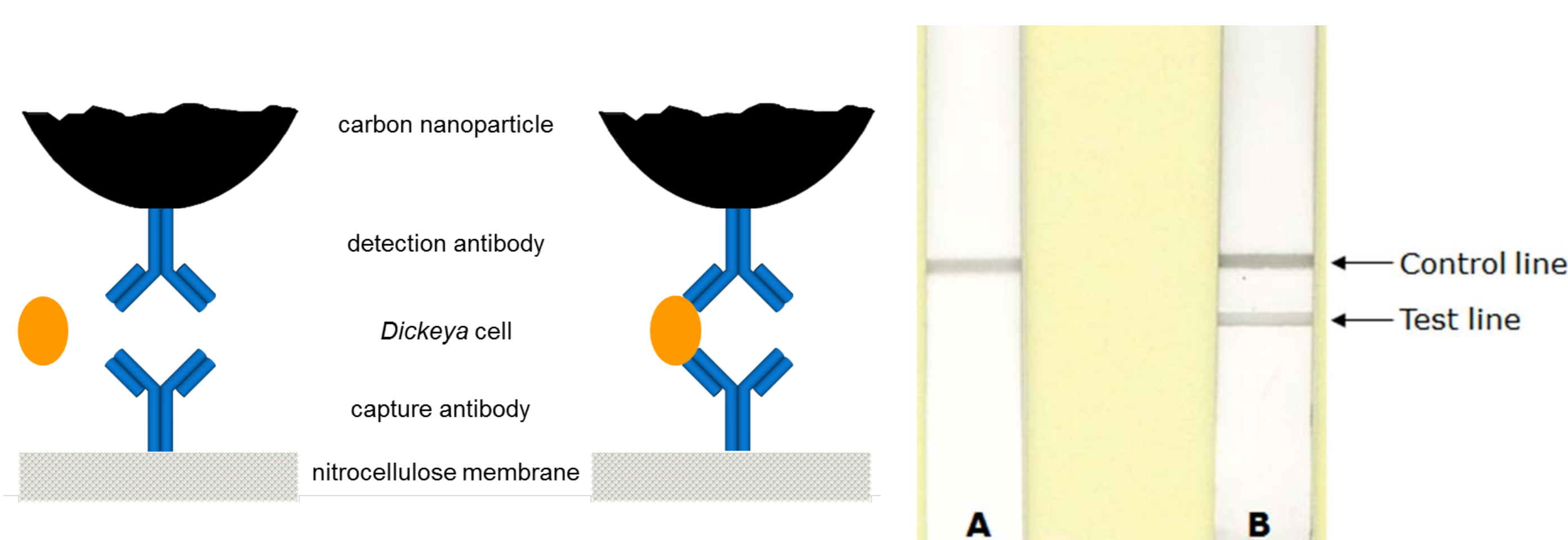


Figure 2. LFIA test format. Left: a schematic view of the Dickeya-LFIA. Right: LFIA results of Hyacinth bulb extracts; A – healthy bulb, B – bulb infected with Dickeya.



Figure 1. Hyacinth bulb infected by *Dickeya* (top) and by *Pectobacterium* (bottom).

Sample preparation

Sample extracts were prepared from healthy and infected bulb tissue material using disposable micro-potters. Samples (1 µl) were 1:100 diluted in assay buffer and following extraction directly applied to the LFIA. (figure 3). Several bulb sample extracts were tested with the *Dickeya* LFIA. The same samples were also tested with a *Dickeya* specific PCR and a *Pectobacterium* specific PCR (table 1).



Figure 3. Disposable micro-potter with battery-driven hand-held rotor for homogenisation of bulb tissue in extraction buffer.

Table 1. Hyacinth bulb samples tested with *Dickeya* LFIA and with *Dickeya* and *Pectobacterium* specific PCR.

Nr.	Sample (code)	<i>Dickeya</i> LFIA	<i>Dickeya</i> specific PCR (ADE1/ADE2)	<i>Pectobacterium</i> specific PCR (Y1/Y2)
1	Miss Saigon	-	-	+
4	Miss Saigon	-	-	+/-
6	Pink Pearl	-	-	+
20	C. Dream	+	+	-
23	Louvre	+	+	-
33	Top Hit, (TH2)	+	+	-
34	Louvre, (NEW)	+	+	-
35	Monster 44746	+	+	-
3	Miss Saigon	-	-	-
7	Pink Pearl	-	-	-
40	WP, 22-07	-	-	-
41	PP, 22-07	-	-	-
42	Louvre	+	+	-
43	Top Hit	+	+	-

Figure 4 shows the results of infected bulb samples tested by PCR-gelelectroforesis (top left) and by LFIA (top right and bottom).

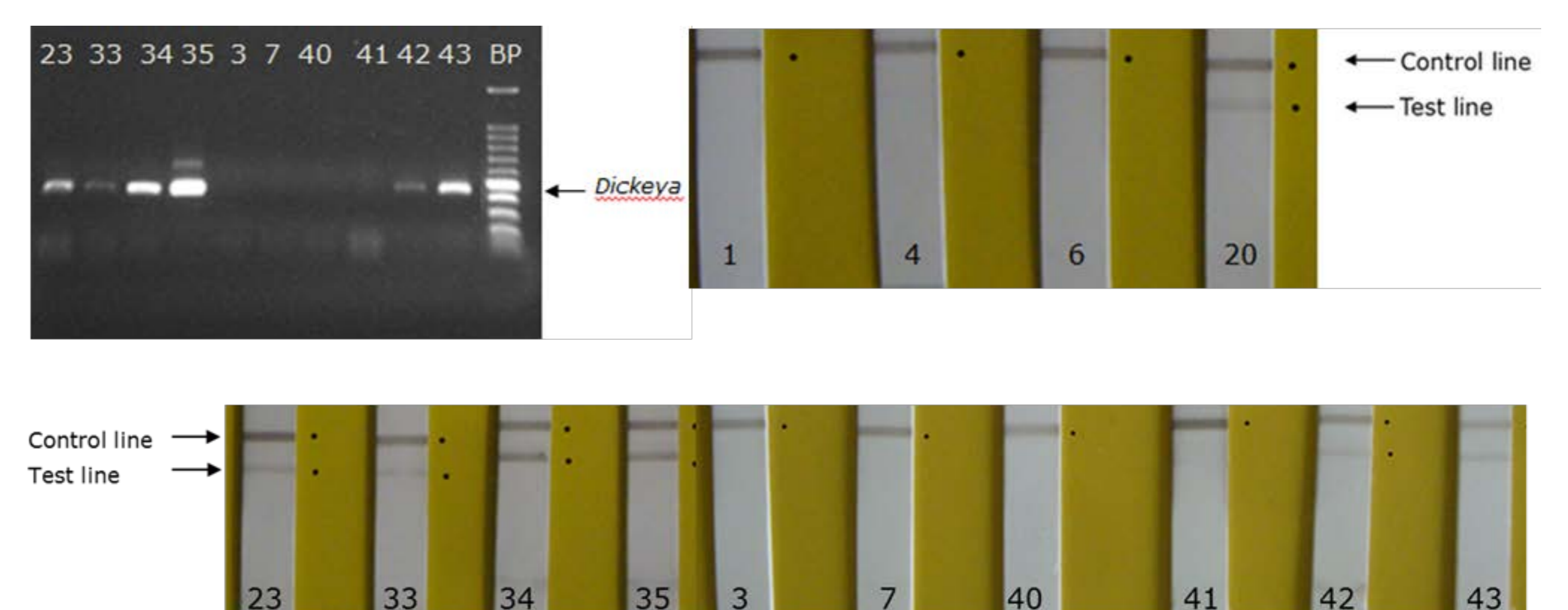


Figure 4. PCR and LFIA results obtained with healthy or infected (agressive rot, white rot) Hyacinth bulb samples. Top left; PCR results on agarose gel. Top right and bottom; LFIA test strip results. For numbers see Table 1.

Conclusions

- We demonstrate specific detection of *Dickeya* infection (agressive rot) in Hyacinth bulb tissue using a simple, cheap and rapid (result within one hour) Lateral Flow ImmunoAssay (LFIA).
- Preliminary validation experiments showed very good correlation between the *Dickeya* and *Pectobacterium* specific PCR results and the results obtained with the *Dickeya* LFIA.

Wageningen UR Applied Plant research offers the new test for interested Hyacinth growers.