USE OF MORE LARVAE IN THE "AFB TEST" KIT - A SYSTEM TO DIAGNOSE AMERICAN FOUL BROOD

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Continuing the study on the reliability and practicality of the AFB Test kit from Vita Europe Ltd., during 2004, the Institute of Agrarian Entomology of Milan and the National Apiculture Institute worked together in a new phase of testing with a view to gaining further knowledge about some aspects of application, particularly the possibility of using more larvae in the same test.

The tests performed during the preceding year showed that, at low spore concentration levels (20,000,000 per ml extractant solution contained in the phial used to homogenise the larva), the intensity of the stroke of the T line (which shows the result of the test) was either very low or non-existent. Below this limit, there is a risk of not recording the infection when analysing a single larva containing spores of *Paenibacillus larvae* subsp. *larvae*.

However, increasing the number of larvae that are probably infected, and testing them at the same time with the same ELISA kit, increases the number of spores in the solution and thus the legibility of the T line. At a practical level, therefore, the engineer or beekeeper, when dealing with "dubious" larvae in view of the absence of clear symptoms of American foul brood (i.e. at a sub-clinical level), could examine more larvae from the same colony using the same test.

TEST METHOD AND RESULTS

The test was performed using the following scheme.

Group I
Healthy larvae taken prior to operculation were examined, following confirmation by microscopic examination that there were no spores present. The test was first performed on one larva and then on homogenised preparations, each with more larvae, from two to eight. In this case, in the total absence of infection, all that was monitored was the intensity of the C stroke (Control) that appears in the window of the kit device. Each homogenate of one or more larvae was tested at the same time on five devices (five repetitions for eight cases).

Group II
Prepupae were examined with evident symptoms and with a viscous liquid consistency, and dark or light brown in colour. In this case, too, several solutions were prepared involving an increasing number of larvae. Each solution was tested on five devices and the intensity of both the C (Control) and T (Test) strokes was monitored.

RESULTS AND DISCUSSION

As can easily be seen from the tables, the line for the control (C) **in Group I** (Table 1), which involved the introduction of up to six larvae into the assay solution, remained relatively stable; beyond six larvae, the line starts to become poorly legible. Then, using infected larvae (**Group II**), the C line remains strongly visible up to a maximum of three larvae used simultaneously (Table 2), whereas it can become invisible beyond five larvae, which frustrates the purpose of the test.

Table 1

Visibility of control line C - Group I					
	Repeat				
No. of larvae per phial	1	2	3	4	5
1	+++	+++	++	++++	+++
2	++++	++++	++++	++++	+++
3	++	+++	++	++	+++
4	++++	++++	++++	++++	++
5	++	+++	++++	+++	+++
6	+++	++++	+++	++	+++
7	+++	+	+++	++	+
8	+	+	+	+	+

Table 2

Visibility of control line C - Group II					
	Repeat				
No. of larvae per phial	1	2	3	4	5
1	++	++	+++	+++	+++
2	+	+++	+	+	++
3	++++	+++	+++	+++	+++
4	+	++	+	+	+
5	++	+	+	+	+
6	+	+	+	-	-
7	+	-	+	+	+

In the same group, the T line remained strong for up to five larvae, but its strength reduced with a higher number of larvae, though remaining visible (Table 3).

These observations enable us to conclude that increasing the recording capacity of the test permits the test to be applied to a larger larva pool. However, it is recommended not to exceed three or four larvae at a time, otherwise there is a risk of compromising the reliability of the test.

Indeed, with a higher number of larvae, the C line, which indicates that the test is working properly, might not form, whereas the intensity of the T line, which indicates the specific reaction, could be markedly reduced with the attendant risk of not being recorded, even in the presence of infected larvae (i.e. false negatives).

We therefore believe that the AFB Test kit by Vita Europe Ltd. is a valid tool for the diagnostic confirmation of American foul brood in the field or the laboratory; however, like any tool, it must be used with the appropriate attention based on a knowledge of its characteristics and principles of operation.

Table 3

Visibility of test line T - Group II					
	Repeat				
No. of larvae per phial	1	2	3	4	5
1	++++	++++	++++	++++	++++
2	++++	++++	++++	++++	++++
3	++++	++++	++++	++++	++++
4	+++	+++	+++	+++	+++
5	++	+++	+++	+++	++
6	++	++	+	++	+
7	+	+	++	+	+