ANNEX I

TEST SCHEME FOR DIAGNOSIS, DETECTION AND IDENTIFICATION OF THE RING ROT BACTERIUM, *CLAVIBACTER MICHIGANENSIS* (Smith) Davis *et al.* ssp. *SEPEDONICUS* (Spieckermann et Kotthoff) Davis *et al.*

SCOPE OF THE TEST SCHEME

6. PCR TEST

Principles

When the PCR test is used as the principal screening test and found to be positive, the IF test must be performed as a second compulsory screening test. When the PCR test is used as the second screening test and found to be positive, further testing according to the flow scheme is required to complete the diagnosis.

Full exploitation of this method as principal screening test is only recommended when specialised expertise has been acquired.

Note:

Preliminary testing with this method should permit reproducible detection of 10^3 to 10^4 cells of *C. m.* subsp. *sepedonicus* per ml added to sample extracts which previously tested negative. Optimisation experiments may be required to achieve maximum levels of sensitivity and specificity in all laboratories.

Use validated PCR reagents and protocols. Preferably select a method with an internal control.

Use appropriate precautions to avoid contamination of sample with target DNA. The PCR test should be performed by experienced technicians, in dedicated molecular biology laboratories, in order to minimise the possibility of contamination with target DNA.

Negative controls (for DNA extraction and PCR procedures) should always be handled as final samples in the procedure, to make evident whether any carry over of DNA has occurred.

The following negative controls should be included in the PCR test:

— sample extract that previously tested negative for *C. m.* subsp. *Sepedonicus*,

— buffer controls used for extracting the bacterium and the DNA from the sample,

— PCR-reaction mix.

The following positive controls should be included:

— aliquots of resuspended pellets to which *C. m.* subsp. *sepedonicus* has been added (preparation see Appendix 2),

— a suspension of 10^6 cells per ml of *C. m.* subsp. *sepedonicus* in water from a virulent isolate (e.g. NCPPB 2140 or NCPPB 4053),

— if possible also use DNA extracted from positive control samples in the PCR test.

To avoid potential contamination prepare positive controls in a separate environment from samples to be tested.

Sample extracts should be as free as possible from soil. It could therefore in certain cases advisible to prepare extractions from washed potatoes if PCR protocols are to be used.