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[F1ANNEX 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

Textual Amendments

F1 Substituted by Commission Directive 2006/56/EC of 12 June 2006 amending the Annexes to Council Directive 93/85/EEC on the control of potato ring rot.

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Appendix 7

Validated reagents for FISH test

2. Fixative solution

[WARNING! THE FIXATIVE CONTAINS PARAFORMALDEHYDE WHICH IS TOXIC. WEAR GLOVES AND DO NOT INHALE. IT IS ADVISABLE TO WORK IN A FUME CUPBOARD]

- (i) Heat nine ml molecular grade water (e.g. Ultra pure water (UPW)) to about 60 °C and add 0,4 g paraformaldehyde. Paraformaldehyde dissolves after adding five drops of 1N NaOH and stirring with a magnetic stirrer.
- (ii) Adjust pH to 7,0 by addition of 1ml of 0,1 M phosphate buffer (PB; pH 7,0) and five drops of 1 N HCl. Check pH with indicator strips and adjust if necessary with HCl or NaOH.
 - [WARNING! DO NOT USE A PH METER IN SOLUTIONS WITH PARAFORMALEDHYDE]
- (iii) Filter the solution through a $0,22~\mu m$ membrane filter and keep dust-free at 4 °C until further use.
- (iv) Note:

Alternative fixative solution: 96 % ethanol.]