Council Directive 93/85/EEC of 4 October 1993 on the control of potato ring rot

[F1 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

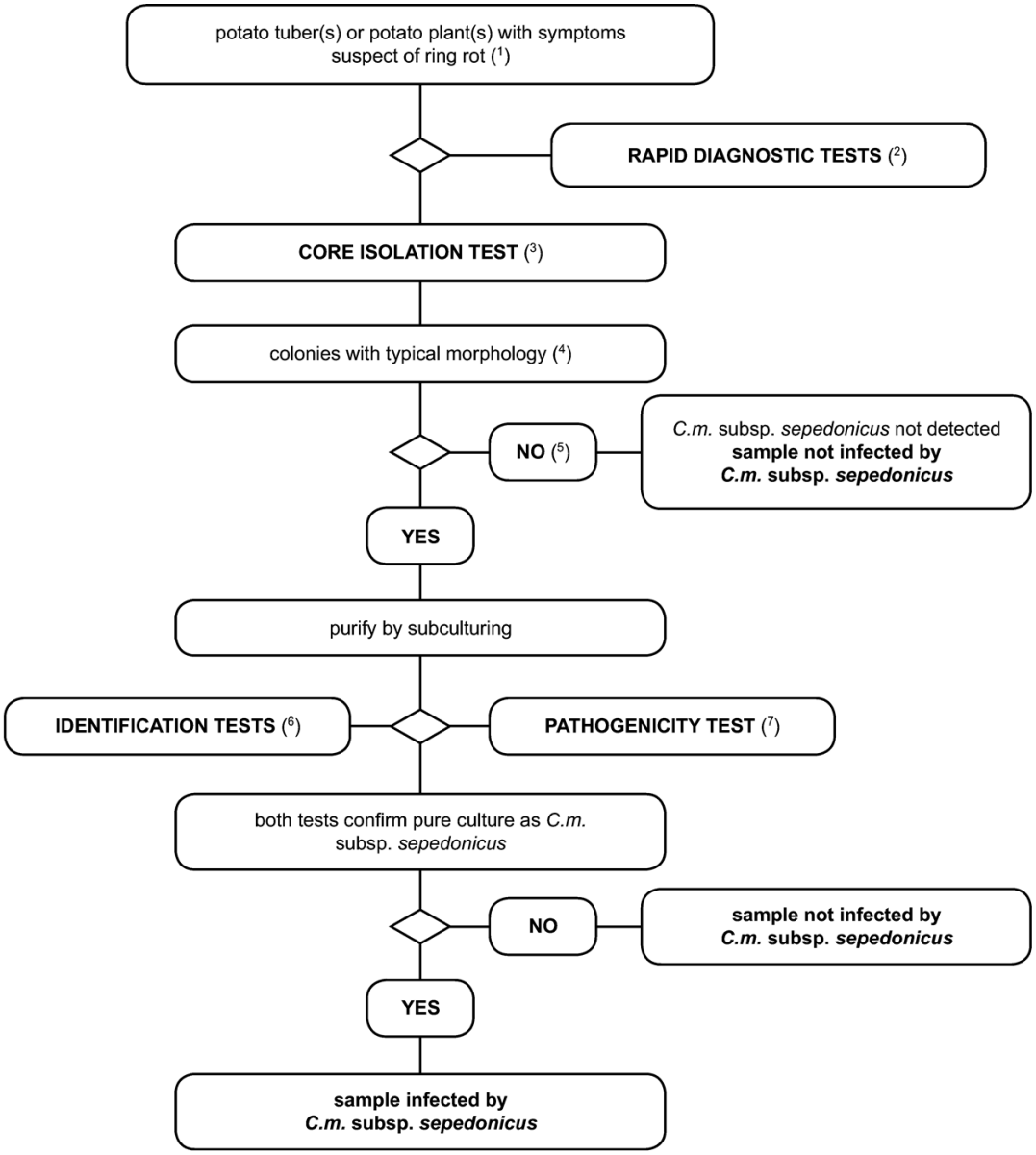
Textual Amendments

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.

1. FLOW CHART DIAGRAM PRESENTATION

1.1. Detection scheme for the diagnosis of Ring Rot in potato tubers and potato plants with symptoms of ring rot

The testing procedure is intended for potato tubers and plants with symptoms typical or suspect of ring rot. It involves a rapid screening test, isolation of the pathogen from infected vascular tissue on diagnostic media and, in case of a positive result, identification of the culture as *C. m.* subsp. *sepedonicus*.



(1) Description of symptoms is provided in section 2.

(2) Appropriate tests are:
— IF-test (section 4),
— PCR test (section 6),
— FISH test (section 5).

(3) Although isolation of the pathogen from plant material with typical symptoms by dilution plating is straightforward, culturing may fail from advanced stages of infection. Saprophytic bacteria which grow on diseased tissue may outgrow or inhibit the pathogen on the isolation medium. Therefore it is recommended to use both non selective and selective media, preferably MTNA (section 8) or Bioassay test (section 7).

(4) Description of typical colony morphology is provided in section 8.

(5) If the isolation test is negative, but disease symptoms are typical, then isolation must be repeated.

(6) Reliable identification of a pure culture of *C. m.* subsp. *sepedonicus* is achieved by using the tests listed in section 9.

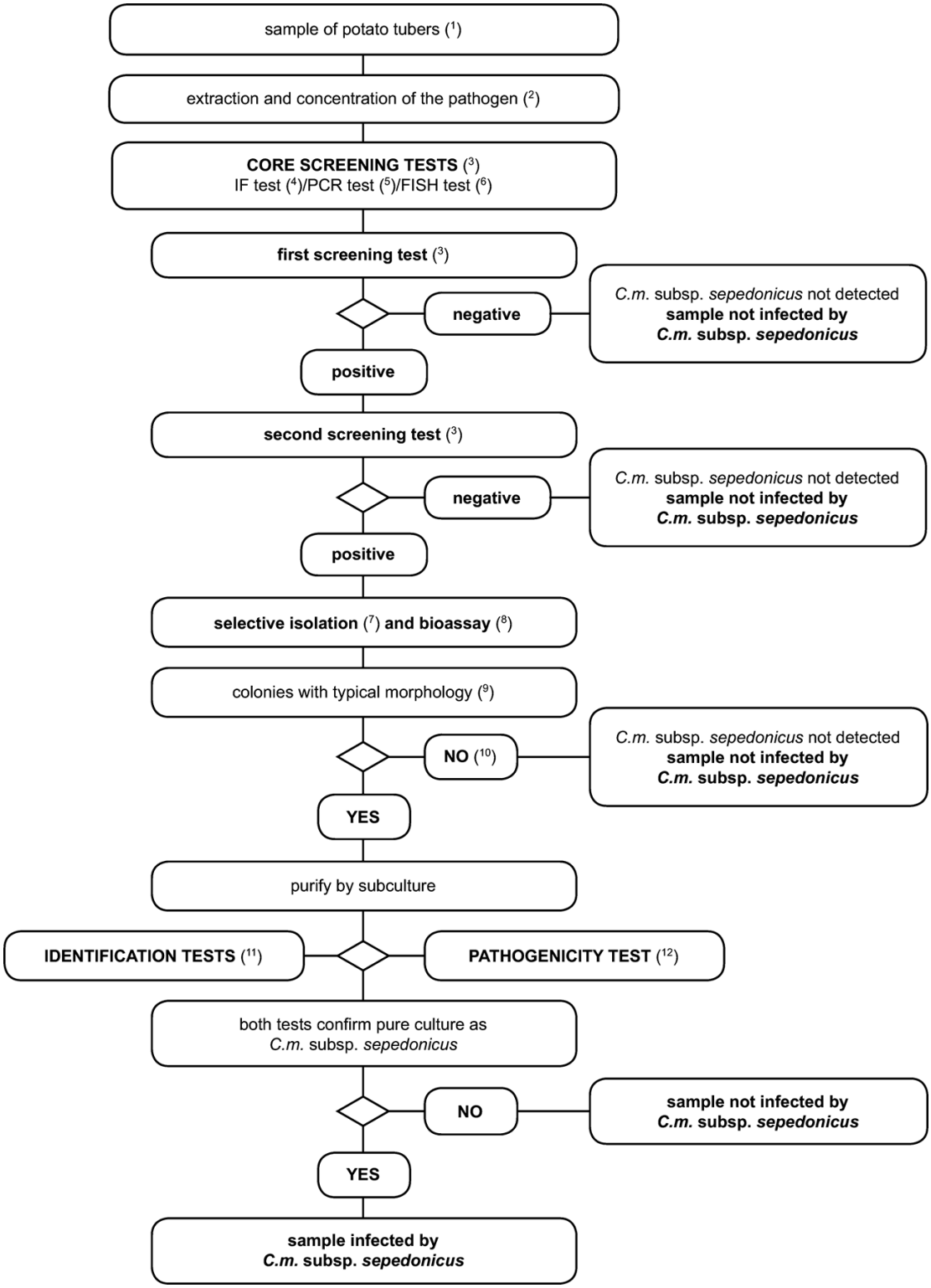
(7) The pathogenicity test is described in section 10.

1.2. Scheme for detection and identification of *Clavibacter michiganensis* ssp. *sepedonicus* in samples of asymptomatic potato tubers

Principle

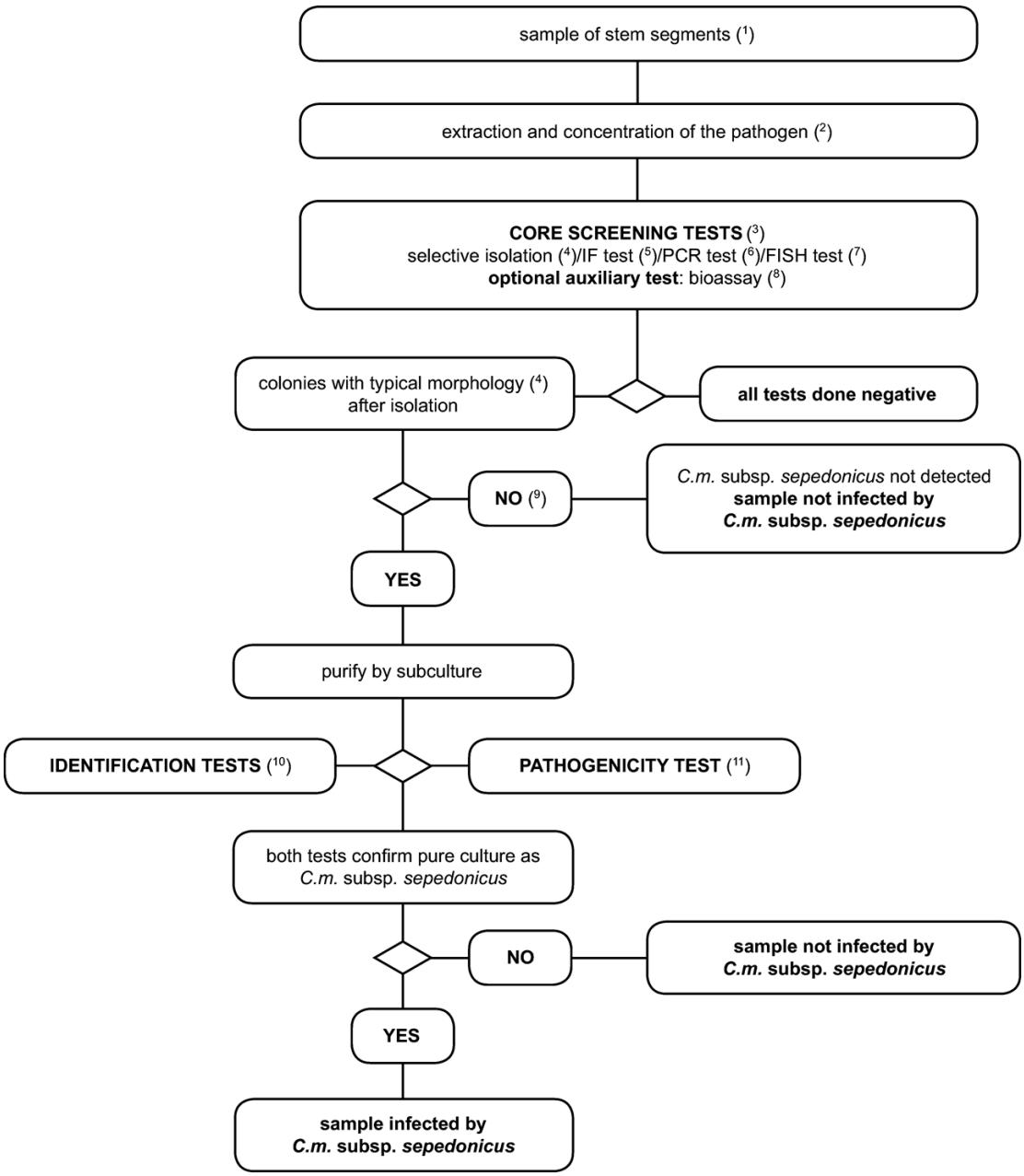
The testing procedure is intended for detection of latent infections in potato tubers. A positive result from at least two screening tests, based on different biological principles, must be complemented by the isolation of the pathogen; followed by, in case of isolation of typical colonies, confirmation of a pure culture as *C. m.* subsp. *sepedonicus*. A positive result from only one of the screening tests is not sufficient to consider the sample suspect.

Screening tests and isolation tests must permit a detection threshold of 10^3 to 10^4 cells/ml resuspended pellet, included as positive controls in each series of tests.



(1) The standard sample size is 200 tubers although the procedure can be used with smaller samples if 200 tubers are not available.
(2) Pathogen extraction and concentration methods are described in section 3.1.
(3) If at least two tests based on different biological principles are positive, isolation and confirmation have to be done. Perform at least one screening test. When this test is negative the sample is considered to be negative In case this test is positive a second or more screening tests based on different biological principles are required to verify the first positive result. If the second or other tests are negative the sample is considered negative. Further tests are not necessary.
(4) Immunofluorescence (IF) test.
Always use a polyclonal antibody for IF screening, additional monoclonal antibodies may provide more specifity (see section 4).
(5) PCR test.
Use appropriately validated PCR reagents and protocols (see section 6).
(6) Fish test.
Use validated reagents and protocols (see section 5).
(7) Selective isolation.
With the MTNA medium or NCP-88 medium and a 1/100 dilution of the resuspended pellet, this is in many cases a suitable method for direct isolation of *C. m.* subsp. *sepedonicus*. Typical colonies can be obtained 3 to10 days after planting. The pathogen then can be purified and identified. For full exploitation of its potential, the test requires careful preparation of heel end cores to avoid secondary bacteria associated with the potato tuber which are competitors with *C. m.* subsp. *sepedonicus* on the medium and may overgrow the pathogen. If the plate test fails isolation must be done from plants used for the bio assay (see section 8).
(8) The bioassay test is used for isolation of *C. m.* subsp. *sepedonicus* from potato extract pellets by selective enrichment in eggplants (*Solanum melongena*). The test requires optimal incubation conditions as specified in this method. Bacteria inhibitory to *C. m.* subsp. *sepedonicus* on the MTNA or NCP-88 medium will most likely not interfere in this test (see section 7).
(9) Typical colony morphology is described in section 8.
(10) Culturing or bioassays can fail due to competition or inhibition by saprophytic bacteria. If positive results are obtained in screening tests, but the isolation tests are negative, then repeat the isolation tests from the same pellet or by taking additional vascular tissue near the heel end from cut tubers of the same sample and, if necessary, test additional samples.
(11) Reliable identification of pure presumptive *C. m.* subsp. *sepedonicus* cultures is achieved using the tests described in section 9.
(12) The pathogenicity test is described in section 10.

1.3. Scheme for detection and identification of *Clavibacter michiganensis* ssp. *sepedonicus* in samples of asymptomatic potato plants**]**



(1) See section 3.2 for recommended sample sizes.

(2) Pathogen extraction and concentration methods are described in section 3.2.

(3) If at least two tests based on different biological principles are positive, isolation and confirmation have to be done.
Perform at least one screening test. When this test is negative the sample is considered to be negative. In case this test is positive a second or more screening tests based on different biological principles are required to verify the first positive result. If the second or other tests are negative the sample is considered negative. Further tests are not necessary.

(4) The selective isolation test and typical colony morphology are described in section 8.

(5) The IF test is described in section 4.

(6) PCR tests are described in section 6.

(7) The FISH test is described in section 5.

(8) The bioassay is described in section 7.

(9) Culturing or bioassays can fail due to competition or inhibition by saprophytic bacteria. If positive results are obtained in screening tests, but the isolation tests are negative, then repeat the isolation tests and, if necessary, test additional samples.

(10) Reliable identification of pure presumptive *C. m.* subsp. *sepedonicus* cultures is achieved using the tests described in section 9.

(11) The pathogenicity test is described in section 10.