Council Directive 98/57/EC of 20 July 1998 on the control of Ralstonia solanacearum (Smith) Yabuuchi et al.

[F1ANNEX II

TEST SCHEME FOR DIAGNOSIS, DETECTION AND IDENTIFICATION OF *RALSTONIA SOLANACEARUM* (SMITH) YABUUCHI *ET AL.*

Textual Amendments

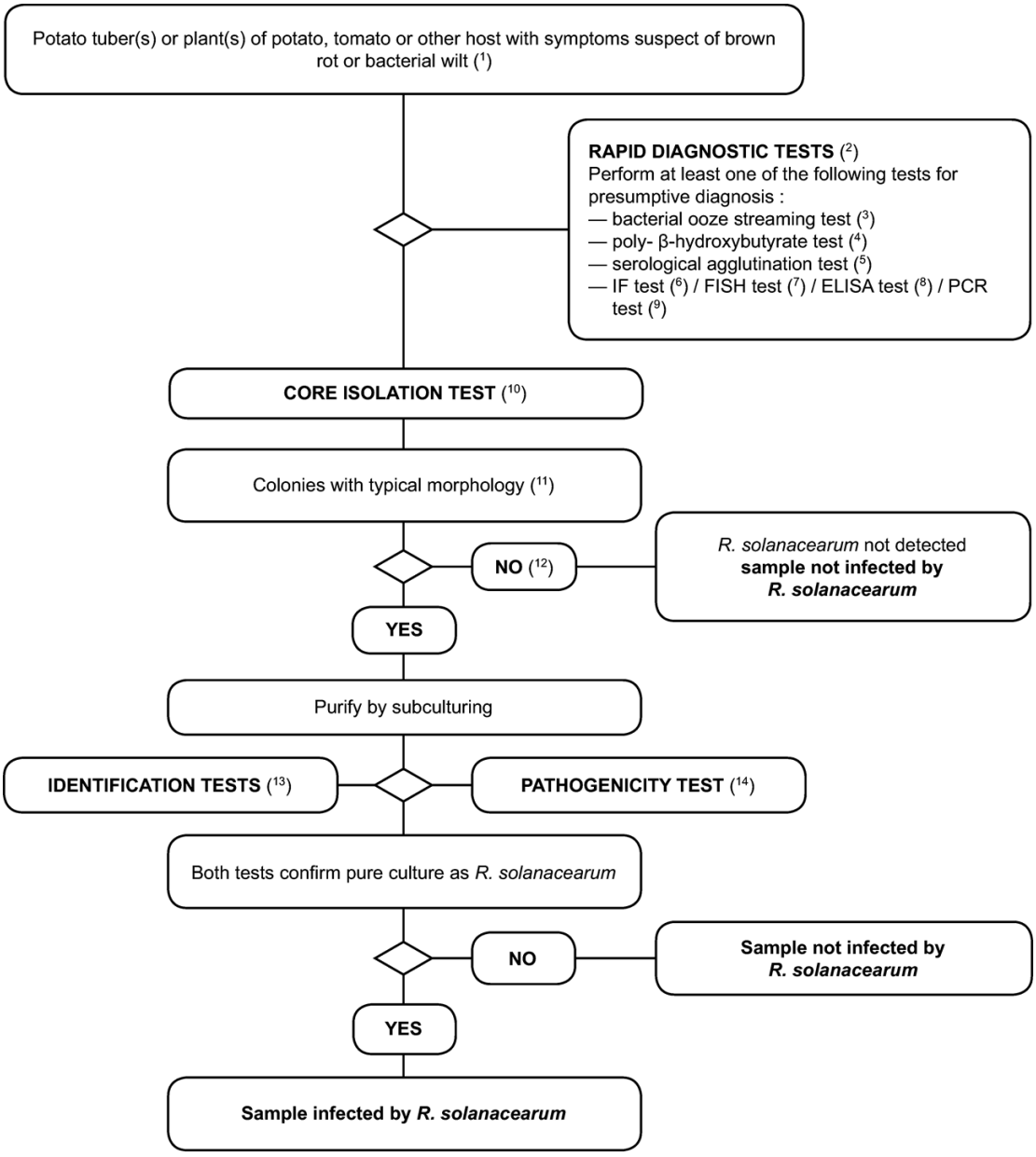
F1 Substituted by Commission Directive 2006/63/CE of 14 July 2006 amending Annexes II to VII to Council Directive 98/57/EC on the control of Ralstonia solanacearum (Smith) Yabuuchi et al..

SECTION I

APPLICATION OF THE TEST SCHEME

1. Detection scheme for the diagnosis of brown rot and bacterial wilt (*Ralstonia solanacearum*) in potato tubers and potato, tomato or other host plants with symptoms of brown rot or bacterial wilt.

The testing procedure is intended for potato tubers and plants with symptoms typical or suspect of brown rot or vascular wilt. It involves a rapid screening test, isolation of the pathogen from infected vascular tissue on (selective) medium and, in case of a positive result, identification of the culture as *Ralstonia solanacearum*.



(1) For description of symptoms see Section II.1.

(2) Rapid diagnostic tests facilitate presumptive diagnosis but are not essential. A negative result does not always guarantee absence of the pathogen.

(3) Streaming test for bacterial ooze from vascular stem tissue is described in Section VI.A.1.

(4) Test for poly-ß-hydroxybutyrate granules in bacterial cells is described in Section VI.A.2.

(5) Serological agglutination tests on bacterial ooze or extracts from symptomatic tissue are described in Section VI.A.3.

(6) IF test on bacterial ooze suspended in water or symptomatic tissue extracts is described in Section VI.A.5.

(7) FISH test on bacterial ooze suspended in water or symptomatic tissue extracts is described in Section VI.A.7.

(8) ELISA test on bacterial ooze suspended in water or symptomatic tissue extracts is described in Section VI.A.8.

(9) PCR test on bacterial ooze suspended in water or symptomatic tissue extracts is described in Section VI.A.6.

(10) The pathogen is usually easily isolated from symptomatic plant material by dilution plating (Section II.3).

(11) Typical colony morphology is described in Section II.3.d.

(12) Culturing may fail from advanced stages of infection due to competition or overgrowth by saprophytic bacteria. If disease symptoms are typical, but the isolation test is negative, then the isolation must be repeated, preferably using a selective plate test.

(13) Reliable identification of pure cultures of presumptive *R. solanacearum* isolates is achieved using the tests described in Section VI.B. Sub-specific characterisation is optional but recommended for each new case.

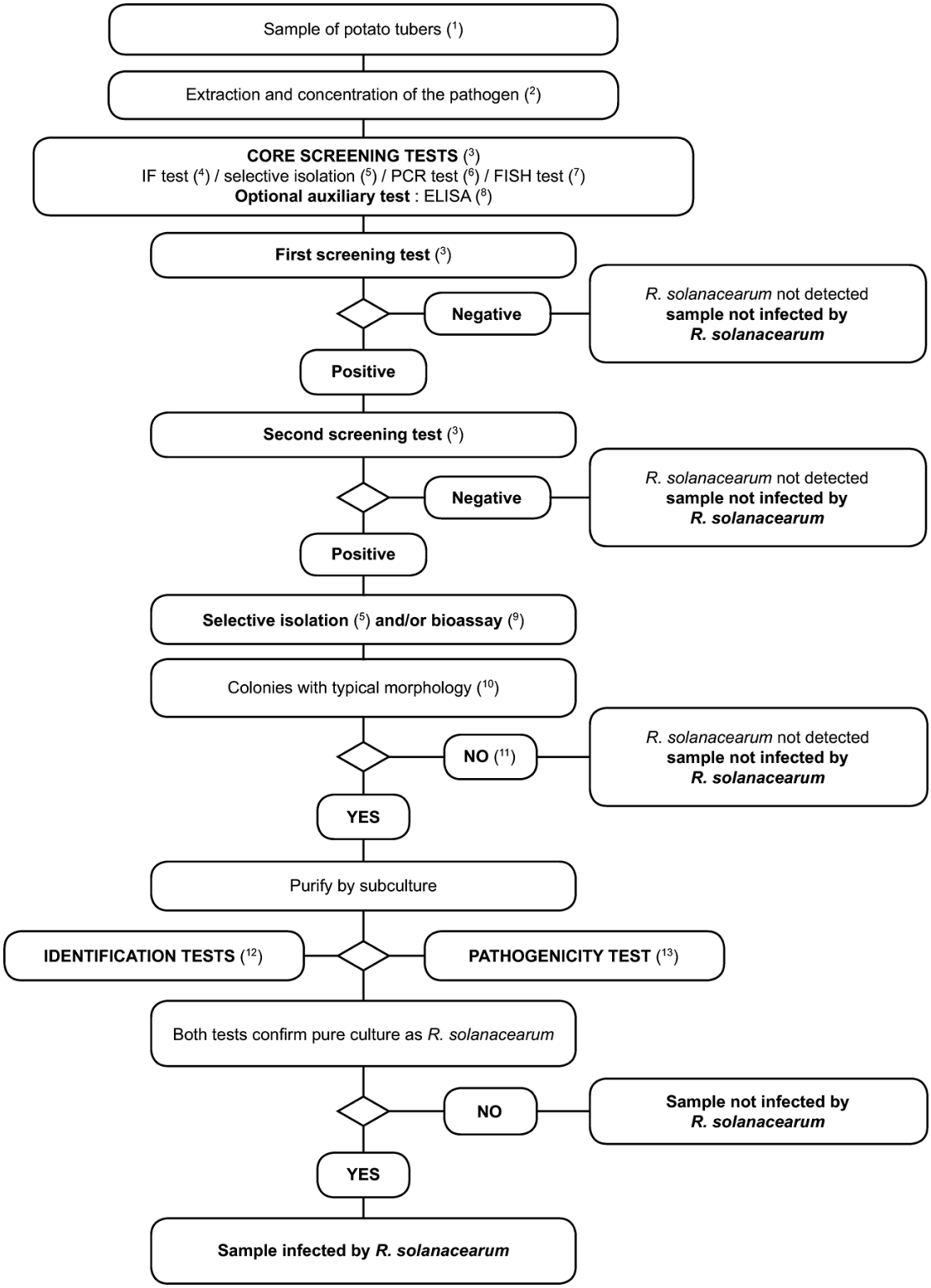
(14) The pathogenicity test is described in Section VI.C.

2. Scheme for detection and identification of *Ralstonia solanacearum* 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 ([3]), 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 *R. solanacearum* 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 detection of 10^3 to 10^4 cells/ml of 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 III.1.1.

(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) The IF test is described in Section VI.A.5.

(5) The selective isolation test is described in Section VI.A.4.

(6) PCR tests are described in Section VI.A.6.

(7) The FISH test is described in Section VI.A.7.

(8) ELISA tests are described in Section VI.A.8.

(9) The bioassay is described in Section VI.A.9.

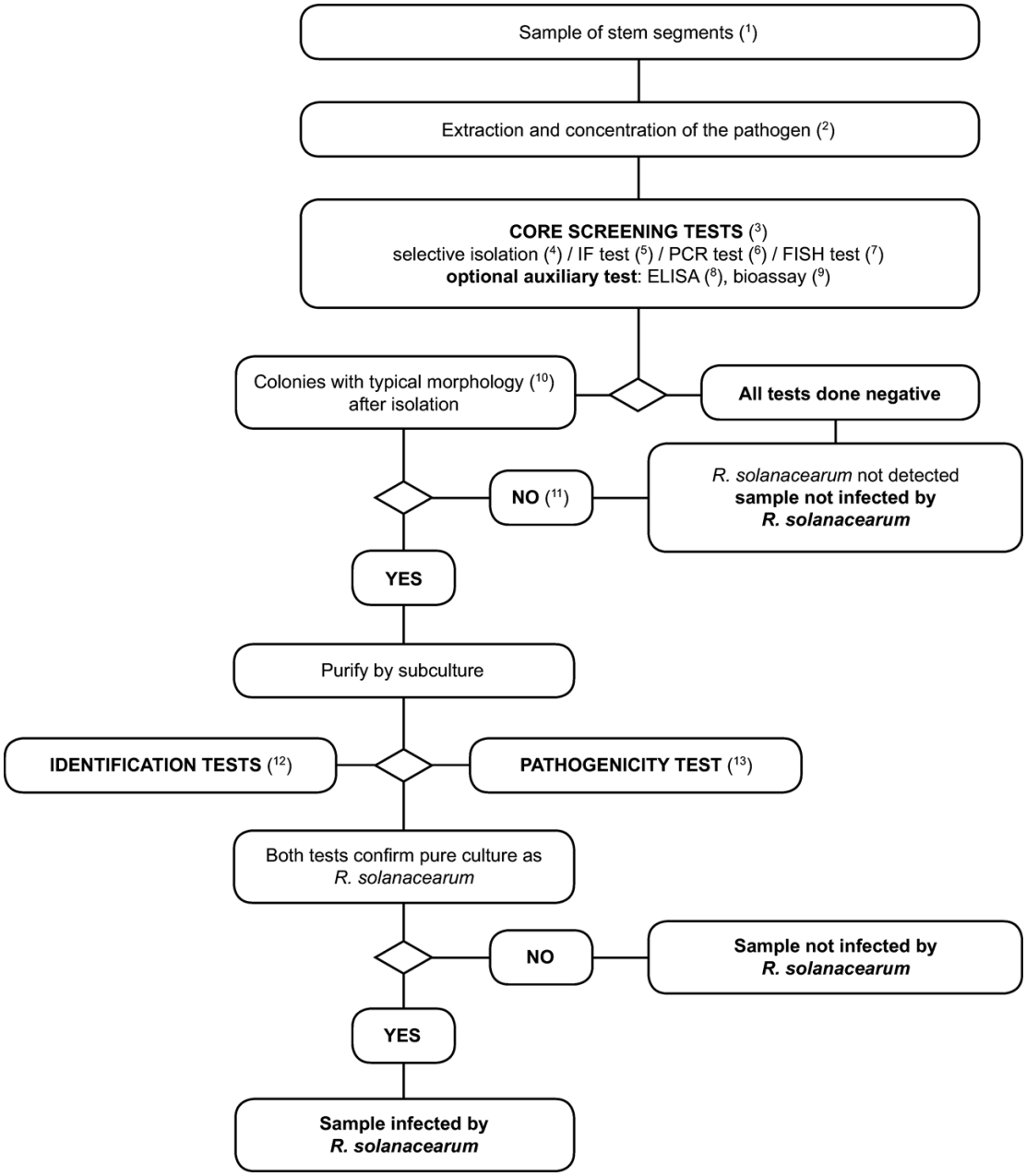
(10) Typical colony morphology is described in Section II.3.d.

(11) Culturing or bioassays can fail due to competition or inhibition by saprophytic bacteria. If clear 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.

(12) Reliable identification of pure cultures of presumptive *R. solanacearum* isolates is achieved using the tests described in Section VI.B.

(13) The pathogenicity test is described in Section VI.C.

3. Scheme for detection and identification of *Ralstonia solanacearum* in samples of asymptomatic potato, tomato or other host plants**]**



(1) See Section III.2.1. for recommended sample sizes.

(2) Pathogen extraction and concentration methods are described in Section III.2.1.

(3) If at least two tests based on different biological priciples 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 is described in Section VI.A.4.

(5) The IF test is described in Section VI.A.5.

(6) PCR tests are described in Section VI.A.6.

(7) The FISH test is described in Section VI.A.7.

(8) ELISA tests are described in Section VI.A.8.

(9) The bioassay is described in Section VI.A.9.

(10) Typical colony morphology is described in Section II.3.d.

(11) 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.

(12) Reliable identification of pure presumptive *R. solanacearum* cultures is achieved using the tests described in Section VI.B.

(13) The pathogenicity test is described in Section VI.C.