

[^{F1}ANNEX 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.](#)

Status: EU Directives are being published on this site to aid cross referencing from UK legislation. After IP completion day (31 December 2020 11pm) no further amendments will be applied to this version.

Appendix 3

A. Commercially available standardised control material

(a) Bacterial isolates

The following bacterial isolates are recommended for use as standard reference material either as positive controls (Table 1) or during optimisation of tests to avoid cross-reactions (Table 2). All strains are commercially available from:

1. National Collection of Plant Pathogenic Bacteria (NCPBP), Central Science Laboratory, York, UK
2. Culture Collection of the Plant Protection Service (PD), Wageningen, the Netherlands.
3. Collection Française de Bactéries Phytopathogènes (CFBP), INRA Station Phytobactériologie, Angers, France.

Table 1

SMT REFERENCE PANEL OF ISOLATES OF *R. SOLANACEARUM*

NCPPB code	SMT#	Other codes	Country of origin	Biovar
NCPPB 4153	6	CFBP 4582, Pr 3020, EURS11	Egypt	2
NCPPB 4154	10	CFBP 4585, 550, EURS21	Turkey	2
NCPPB 3857	12	CFBP 4587, Pr 1140, EURS26	England	2
NCPPB 1584	23	CFBP 4598, EURS49	Cyprus	2
NCPPB 2505	24	CFBP 4599, EURS50	Sweden	2
NCPPB 4155	26	CFBP 4601, 502, EURS55	Belgium	2
NCPPB 4156 ^a	71 ^a	PD 2762, CFBP 3857	Netherlands	2
NCPPB 4157	66	LNPV 15.59	France	2
NCPPB 4158	39	CFBP 4608, Port 448, EURS80	Portugal	2
NCPPB 4160	69	IVIA-1632-2	Spain	2
NCPPB 4161	76	B3B	Germany	2
NCPPB 325	41	CFBP 2047, KEL60-1, R842	USA	1

^a Use as standard reference strain of *R. solanacearum* biovar 2 (race 3).

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NCPPB 3967	42	CFBP 4610, R285, GONg7	Costa Rica	1
NCPPB 4028	43	CFBP 4611, R303/571, CIP310, SEQ205	Colombia	2
NCPPB 3985	44	CFBP 4612, R578, CIP312	Peru	2T
NCPPB 3989	45	CFBP 4613, R568, CIP226	Brazil	2T
NCPPB 3996	46	CFBP 3928, R276/355, CIP72, SEQ225	Peru	3
NCPPB 3997	47	CFBP 4614, R280/363, CIP49, HAY0131a	Australia	3
NCPPB 4029	48	CFBP 4615, R297/349, CIP121, CMib2861	Sri Lanka	4
NCPPB 4005	49	CFBP 4616, R470	Philippines	4
NCPPB 4011	50	CFBP 4617, R288, HEmps2	China	5

a Use as standard reference strain of *R. solanacearum* biovar 2 (race 3).

Note: Authenticity of the above strains can be guaranteed only if obtained from an authentic culture collection.

Table 2:

SMT REFERENCE PANEL OF SEROLOGICALLY- OR GENETICALLY-RELATED BACTERIA FOR USE IN OPTIMISATION OF DETECTION TESTS

NCPPB code	SMT#	Other code	Identification
NCPPB 4162	51	CFBP 1954	<i>Bacillus polymyxa</i> ^a
NCPPB 4163	52	CFBP 1538	<i>Pseudomonas marginalis</i> pv. <i>marginalis</i> ^a
NCPPB 4164	—	CFBP 2227	<i>Burkholderia cepacia</i> ^b

a Potential cross-reacting strain in serological tests (IF and/or ELISA) with polyclonal antisera.

b Strain from which PCR product can be amplified in some laboratories of a similar size to that expected using specific primers OLI-1 and Y-2 (see Appendix 6).

c Likely to cross-react in most tests but known to occur only on banana in Indonesia.

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NCPPB 4165	—	CFBP 2459	<i>Ralstonia pickettii</i> ^b
NCPPB 4166	58	CFBP 3567 CSL Pr1150	<i>Ralstonia pickettii</i> ^a
NCPPB 4167	60	CFBP 4618 PD 2778	<i>Ralstonia</i> sp. ^a
NCPPB 1127	53	CFBP 3575	<i>Burkholderia andropogonis</i> ^a
NCPPB 353	54	CFBP 3572	<i>Burkholderia caryophylli</i> ^a
NCPPB 945	55	CFBP 3569	<i>Burkholderia cepacia</i> ^a
NCPPB 3708	56	CFBP 3574	<i>Burkholderia glumae</i> ^a
NCPPB 3590	57	CFBP 3573	<i>Burkholderia plantarii</i> ^a
NCPPB 3726	59	CFBP 3568	Banana Blood Disease Bacterium ^{abc}
NCPPB 4168	61	CFBP 4619 IPO S339	<i>Enterobacter</i> sp. ^a
NCPPB 4169	62	IPO 1695	<i>Enterobacter</i> sp. ^a
NCPPB 4170	63	CFBP 4621 IPO S306	<i>Ochrobactrum anthropi</i> ^{ab}
NCPPB 4171	64	CFBP 4622 IPO 1693	<i>Curtobacterium</i> sp. ^{ab}
NCPPB 4172	65	IPO 1696a	<i>Pseudomonas</i> sp. ^a
NCPPB 4173	—	PD 2318	<i>Aureobacterium</i> sp. ^b
NCPPB 4174	81	IVIA 1844.06	<i>Flavobacterium</i> sp. ^{ab}

a Potential cross-reacting strain in serological tests (IF and/or ELISA) with polyclonal antisera.

b Strain from which PCR product can be amplified in some laboratories of a similar size to that expected using specific primers OLI-1 and Y-2 (see Appendix 6).

c Likely to cross-react in most tests but known to occur only on banana in Indonesia.

(b) Commercially available standardised control material

The following standard control material is available from the NCPPB culture collection.

Freeze dried pellet of potato extract from 200 healthy potato tubers as negative control for all tests.

Freeze dried pellet of potato extract from 200 healthy potato tubers containing 10^3 to 10^4 and 10^4 to 10^6 cells *R. solanacearum* biovar 2 (strain NCPPB 4156 = PD 2762 = CFBP 3857) as positive controls for serological and PCR tests. Since cell viability is affected during freeze-drying, these are not suitable as standard controls for isolation or bioassay tests.

Formalin-fixed suspensions of *R. solanacearum* biovar 2 (strain NCPPB 4156 = PD 2762 = CFBP 3857) at 10^6 cells per ml as positive controls for serological tests.]