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## [<sup>F1</sup>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

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#### **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 5

### Media for isolation and culture of *C. m. subsp. sepedonicus*

#### (a) General growth media

##### Nutrient agar (NA)

Nutrient Agar (Difco)	23,0 g
Distilled water	1,0 L

Dissolve ingredients and sterilise by autoclaving at 121 °C for 15 min.

##### Nutrient dextrose agar (NDA)

Difco bacto nutrient agar containing 1 % D(+) glucose (monohydrate). Sterilize by autoclaving at 115 °C for 20 minutes.

##### Yeast peptone glucose agar (YPGA)

Yeast Extract (Difco)	5,0 g
Bacto-Peptone (Difco)	5,0 g
D(+) Glucose (monohydrate)	10,0 g
Bacto-Agar (Difco)	15,0 g
Distilled water	1,0 L

Dissolve ingredients and sterilise by autoclaving at 121 °C for 15 min.

##### Yeast extract mineral salts medium (YGM)

Bacto-Yeast-Extract (Difco)	2,0 g
D(+) Glucose (monohydrate)	2,5 g
K <sub>2</sub> HPO <sub>4</sub>	0,25 g
KH <sub>2</sub> PO <sub>4</sub>	0,25 g
MgSO <sub>4</sub> .7H <sub>2</sub> O	0,1 g
MnSO <sub>4</sub> .H <sub>2</sub> O	0,015 g
NaCl	0,05 g
FeSO <sub>4</sub> .7H <sub>2</sub> O	0,005 g
Bacto-Agar (Difco)	18 g
Distilled water	1,0 L

Dissolve ingredients and sterilize 0,5 litre volumes of medium by autoclaving at 115 °C for 20 minutes.

#### (b) Validated selective growth media

##### MTNA medium

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Unless otherwise stated all media components are from BDH.

Yeast extract (Difco)	2,0 g
Mannitol	2,5 g
K <sub>2</sub> HPO <sub>4</sub>	0,25 g
KH <sub>2</sub> PO <sub>4</sub>	0,25 g
NaCl	0,05 g
MgSO <sub>4</sub> .7H <sub>2</sub> O	0,1 g
MnSO <sub>4</sub> .H <sub>2</sub> O	0,015 g
FeSO <sub>4</sub> .7H <sub>2</sub> O	0,005 g
Agar (Oxoid no. 1)	16,0 g
Distilled water	1,0 L

Dissolve ingredients, adjust pH to 7,2. After autoclaving (at 121 °C for 15 minutes) and cooling down to 50 °C, add the antibiotics: trimethoprim 0,06 g, nalidixic acid 0,002 g, amphotericin B 0,01 g.

Stock antibiotic solutions: trimethoprim (Sigma) and nalidixic acid (Sigma) (both at 5 mg/ml), in 96 % methanol, amphotericin B (Sigma) (1 mg/ml) in dimethyl sulfoxide. Stock solutions are filter-sterilized.

Note:

Durability of basal medium is three months. After antibiotics are added durability is one month when stored refrigerated.

NCP-88 medium

Nutrient agar (Difco)	23 g
Yeast extract (Difco)	2 g
D-mannitol	5 g
K <sub>2</sub> HPO <sub>4</sub>	2 g
KH <sub>2</sub> PO <sub>4</sub>	0,5 g
MgSO <sub>4</sub> .7H <sub>2</sub> O	0,25 g
Distilled water	1,0 L

Dissolve ingredients, adjust pH to 7,2. After autoclaving and cooling down to 50 °C, add the following antibiotics: Polymyxin B sulphate (Sigma) 0,003 g, nalidixic acid (Sigma) 0,008 g, Cycloheximide (Sigma) 0,2 g.

Dissolve antibiotics in stock solutions as follows: nalidixic acid in 0,01 M NaOH, cycloheximide in 50 % ethanol, polymyxin B sulphate in distilled water. Stock solutions are filter-sterilized.

Note:

Durability of basal medium is three months. After antibiotics are added durability is one month when stored refrigerated.]