2026

Development and Validation of a real-time PCR kit for the detection of the *Ralstonia Solanacearum* Species Complex (RSSC)

Beatrice Manser, Michele Frapolli, Marco Kaiser

BIOREBA AG, Switzerland

Bacterial wilt in Switzerland

In 2023, an outbreak of *R. pseudosolanacearum* in ginger was detected at twelve Swiss farms

Ralstonia pseudosolanacearum wütet bereits in sieben Kantonen

In der Schweiz wird das Bakterium Ralstonia pseudosolanacearum bekämpft. Es kann grosse Schäden an mehr als 200 Pflanzenarten anrichten.

von Jonas Bucher

530 40 141

3/4

Ende Juli sei der Eidgenössische Pflanzenschutzdienst EPSD informiert worden, dass aus Deutschland gelieferte Ingwer-Pflanzen möglicherweise mit Ralstonia pseudosolanacearum befallen seien. (Symbolbild)
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Real-time PCR for RSSC

Ralstonia solanacearum species complex (RSSC)

Bacterial wilt caused by *R. solanacearum* is one of the most destructive plant diseases worldwide

R. solanacearum is a soil-borne bacterium infecting over 200 plant species from more than 50 families.

It enters through wounds, root tips, or lateral roots, colonizes the root cortex, and moves into the xylem, where it rapidly multiplies. This systemic spread causes wilting and ultimately plant death.

CIP

EPPO

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Ralstonia solanacearum species complex (RSSC)

Bacterial wilt caused by *R. solanacearum* is one of the most destructive plant diseases worldwide

Historically, the species complex was described as several phenotypically diverse strains from five pathogenic races and five biovars.

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2014: Reclassification by Safni et al. into three distinct species

- *Ralstonia solanacearum* (Phylotype II)
- *Ralstonia pseudosolanacearum* (Phylotypes I and III)
- *Ralstonia syzygii* (Phylotype IV)
    - *R. syzygii* subsp. *syzygii*
    - *R. syzygii* subsp. *celebesensis*
    - *R. syzygii* subsp. *indonesiensis*

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Real-time PCR for RSSC

Design of real-time PCR for RSSC detection

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Development of a method for ISO17025-accredited testing service laboratory, as well as a commercial real-time PCR-set

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RSSC primer and probes:

- Primer-sequences from Körner et al., 2017

  Modification of an existing primer set to reduce false-positive results with *Advenella kashmirensis*

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  Modification to avoid false-positive results with non-target DNA in potato tubers

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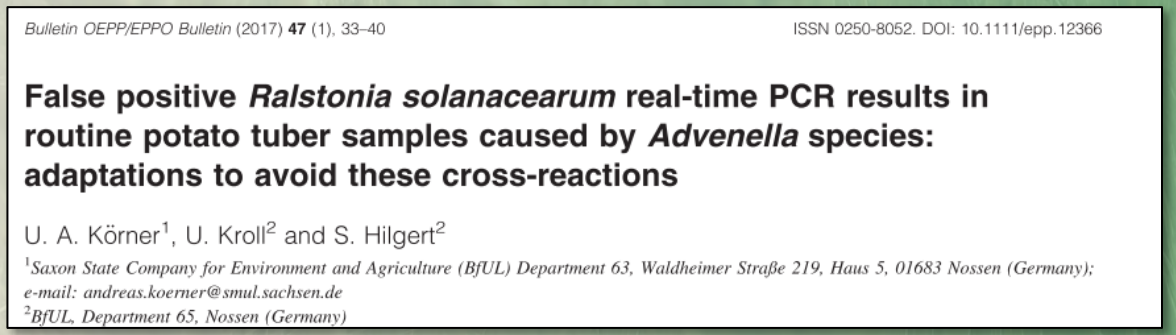


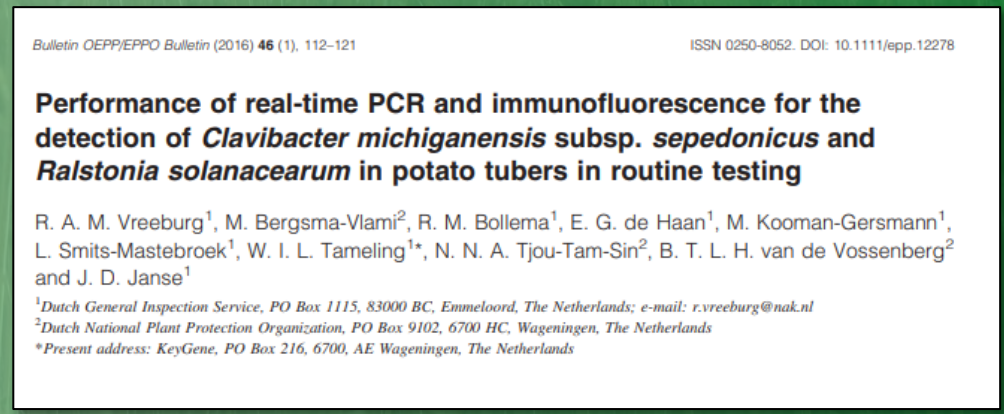
Bulletin OEPP/EPPO Bulletin (2017) **47** (1), 33–40 ISSN 0250-8052. DOI: 10.1111/epp.12366

False positive *Ralstonia solanacearum* real-time PCR results in routine potato tuber samples caused by *Advenella* species: adaptations to avoid these cross-reactions

U. A. Körner[1], U. Kroll[2] and S. Hilgert[2]

[1]*Saxon State Company for Environment and Agriculture (BfUL) Department 63, Waldheimer Straße 219, Haus 5, 01683 Nossen (Germany); e-mail: andreas.koerner@smul.sachsen.de*

[2]*BfUL, Department 65, Nossen (Germany)*



Bulletin OEPP/EPPO Bulletin (2016) **46** (1), 112–121 ISSN 0250-8052. DOI: 10.1111/epp.12278

Performance of real-time PCR and immunofluorescence for the detection of *Clavibacter michiganensis* subsp. *sepedonicus* and *Ralstonia solanacearum* in potato tubers in routine testing

R. A. M. Vreeburg[1], M. Bergsma-Vlami[2], R. M. Bollema[1], E. G. de Haan[1], M. Kooman-Gersmann[1], L. Smits-Mastebroek[1], W. I. L. Tameling[1]*, N. N. A. Tjou-Tam-Sin[2], B. T. L. H. van de Vossenberg[2] and J. D. Janse[1]

[1]*Dutch General Inspection Service, PO Box 1115, 83000 BC, Emmeloord, The Netherlands; e-mail: r.vreeburg@nak.nl*

[2]*Dutch National Plant Protection Organization, PO Box 9102, 6700 HC, Wageningen, The Netherlands*

**Present address: KeyGene, PO Box 216, 6700, AE Wageningen, The Netherlands*

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Primer and probes for internal control (IC):

IC serves as an extraction and amplification control.

The amplified gene should be present in all RSSC host plants. Candidates = COX, 18S, NAD, Actin

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Primer and probes for internal control (IC):

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The amplified gene should be present in all RSSC host plants. Candidates = COX, 18S, NAD, Actin

➢ **18S** could be amplified in all tested host plants and was selected as IC

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Optimization of RSSC real-time duplex PCR

Dilution series to assess primer efficiency and analytical sensitivity

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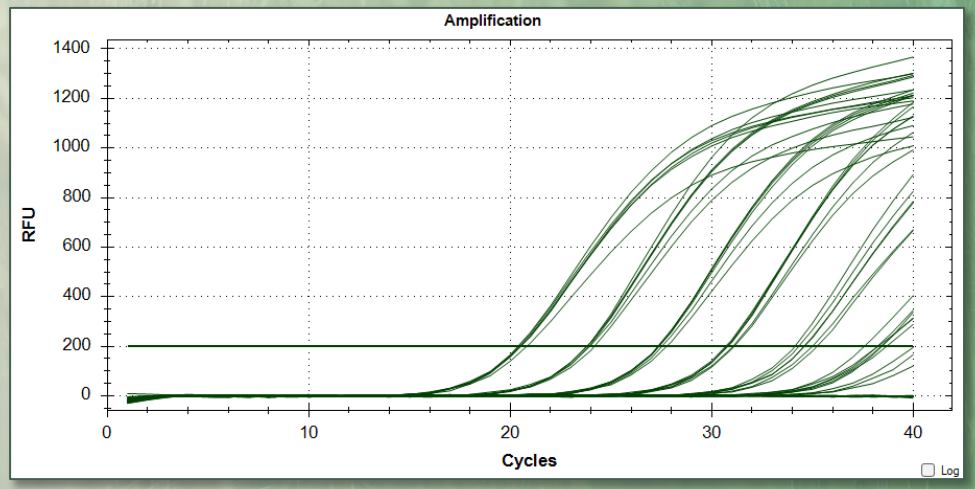
Dilution series to assess primer efficiency and analytical sensitivity

1. Definition of RFU-threshold



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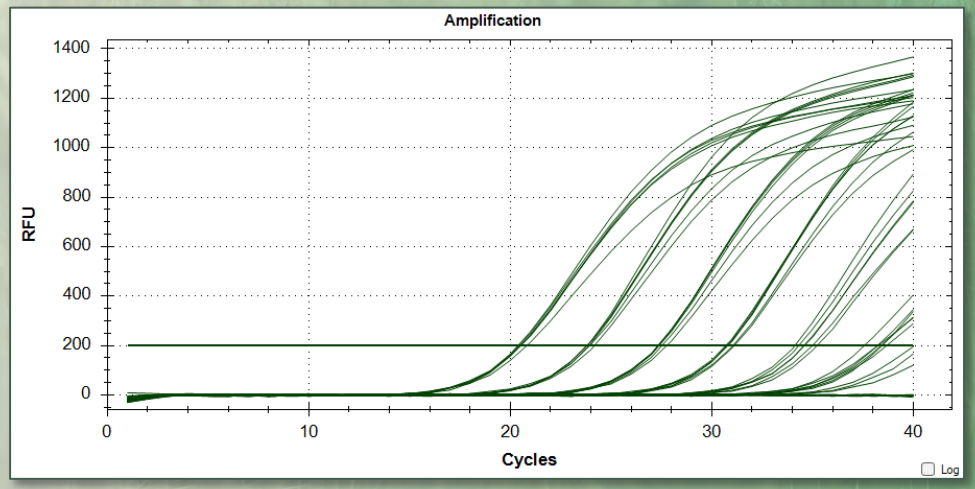

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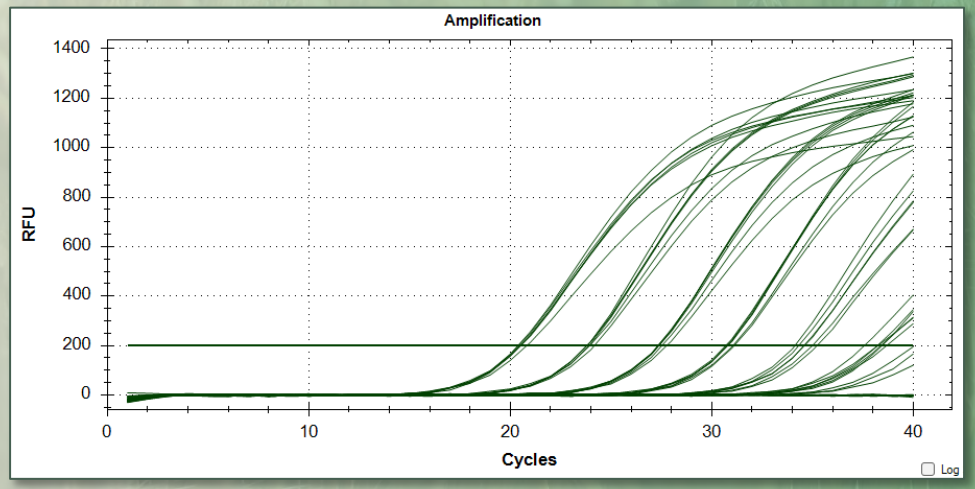

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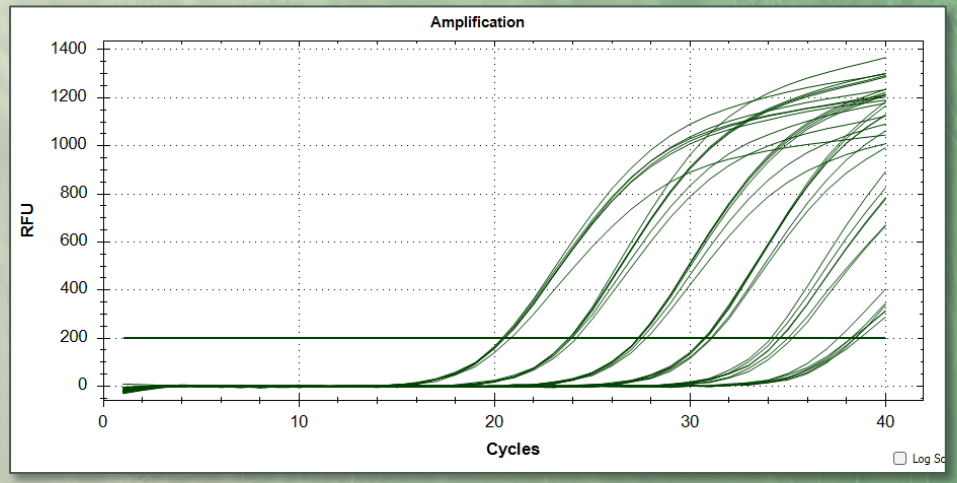

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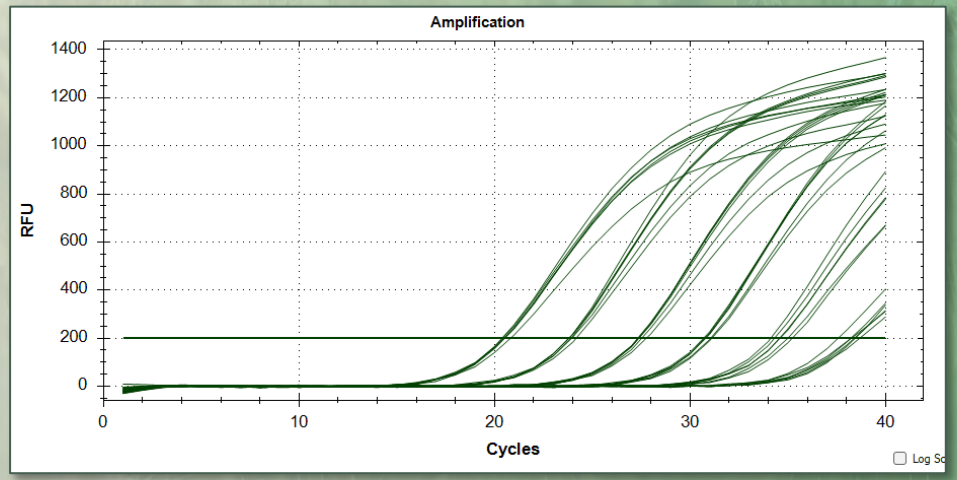

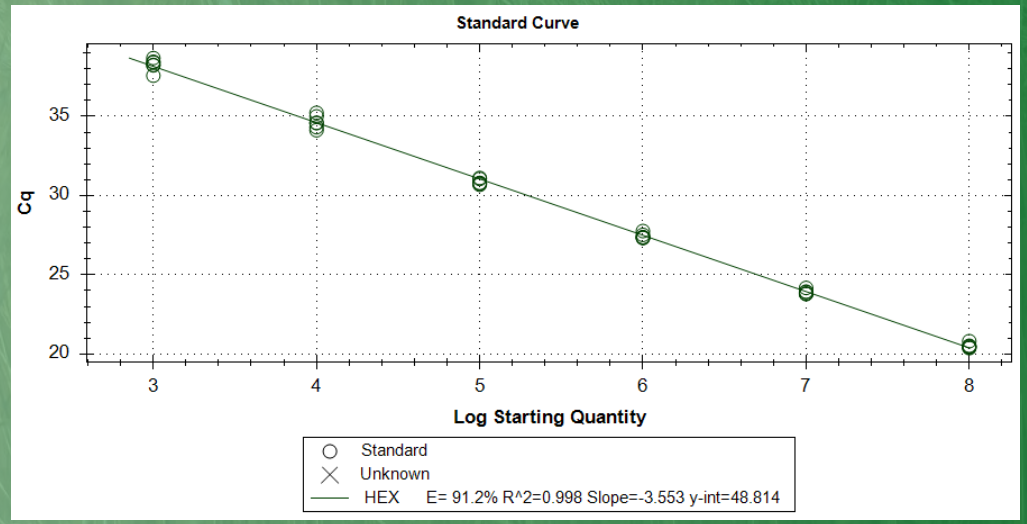

Primer efficiency = 91.2% -> OK

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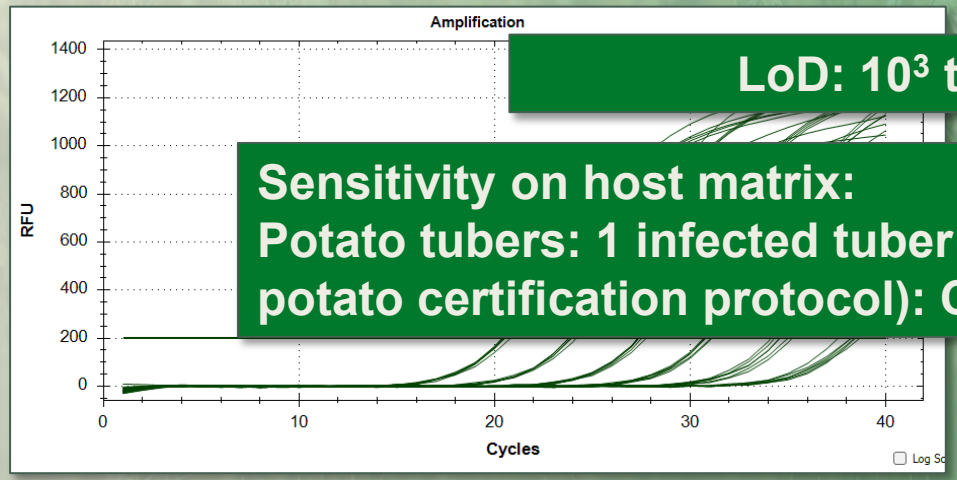
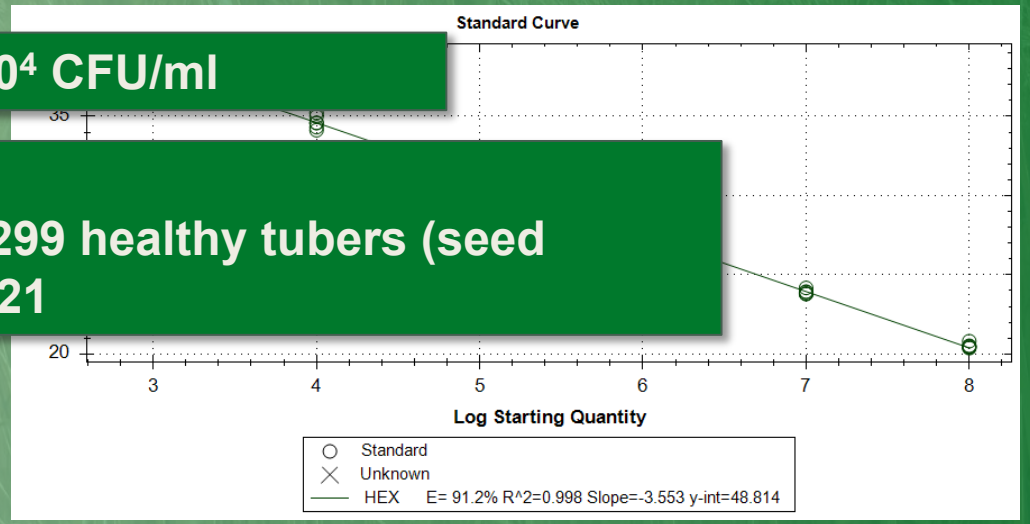


LoD: 10^3 to 10^4 CFU/ml

Sensitivity on host matrix:
Potato tubers: 1 infected tuber in 299 healthy tubers (seed potato certification protocol): Ct ~21



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Validation of real-time PCR for RSSC detection

Specificity testing - Inclusivity

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Isolate	Phylotype	Host plant	Origin	Recognized
R. pseudosolanacearum	unknown	Ginger	Switzerland	+
R. pseudosolanacearum NCPPB 3181	III	Sodom Apple	Gambia	+
R. pseudosolanacearum NCPPB 1018	III	Potato	Angola	+
R. pseudosolanacearum	I	Rose	Netherlandes	+
R. pseudosolanacearum	I	Tomato	Indonesia	+
R. pseudosolanacearum	I	Curcuma	Peru	+
R. solanacearum	II	Potato	Germany	+
R. solanacearum	II	Potato	Switzerland	+
R. syzygii subsp. *syzygii*	IV	Clove	Indonesia	+
R. syzygii subsp. *indonesiensis*	IV	Tomato	Indonesia	+
R. syzygii subsp. *celebensis*	IV	Banana	Indonesia	+

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No cross-reaction with related bacteria, pathogens on the same host, or host matrix

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Pathogen	Recognized
Candidatus Arsenophonus phytopathogenicus	-
Candidatus Phytoplasma solani	-
Clavibacter michiganesis subsp. sepedonicus	-
Pectobacterium wasabiae	-
Pectobacterium atrosepticum	-
Pectobacterium parmentieri	-
Pectobacterium carotovorum subsp. Brasiliense	-
Pectobacterium carotovorum subsp. Carotovorum	-
Dickeya solani	-
Pseudomonas syringae spp.	-

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Pectobacterium atrosepticum	-
Pectobacterium parmentieri	-
Pectobacterium carotovorum subsp. Brasiliense	-
Pectobacterium carotovorum subsp. Carotovorum	-
Dickeya solani	-
Pseudomonas syringae spp.	-

Host plant	Recognized
Potao	-
Tomato	-
Ginger	-
Eggplant	-
Rose	-
Bell pepper	-
Chilli	-
Watermelon	-
Banana	-
Cucumber	-
Tobacco	-

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Production of a qPCR Rs set

For the use in ISO 17025-accredited testing service laboratory and for commercial sale

Real-time PCR for RSSC

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Rs Lyo qPCR Master Mix (2x)	Lyophilized MM including DNA-Polymerase and Primer/Probe Mix
Reconstitution Buffer for MM	For rehydration of Lyo qPCR MM
Rs qPCR positive control	With a defined Ct-value
qPCR negative control	- Control for Rs, but positive for IC
Nuclease free water	Dilution of templates or No-Template Control

Production of a qPCR Rs set

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- Lyophilized Master Mix allowing room-temperature transport
- Integration of DNA-Polymerase and Primer/Probe mix into a single tube
- Lot-to-lot consistency
- Certificate of Analysis available with Ct-values from QC

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External validation and participation in interlaboratory comparison (ILC)

- External Validation of the Rs qPCR set through a collaborator in Belgium
- ILC carried out within the scope of EUPHRESCO 203-F-431 ExoRal

Test panel

- 4 potato tuber extracts (*Solanum tuberosum*)
- 2 ginger rhizome extracts (*Zingiber officinale*)
- 2 rose plant extracts (*Rosa* sp.)
- 2 concentrated water samples

Euphresco

Real-time PCR for RSSC detection

The method is being used in our testing service laboratory to test store-bought ginger rhizomes from Peru and China, used for propagation in Switzerland

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THANK YOU

Feel free to contact me at: ***manser@bioreba.ch***

In collaboration with:

Agroscope

Euphresco