Validation report

qPCR HLVd sets

Article No.: 899600 (qPCR HLVd set 96) / 899200 (qPCR HLVd set 192)

General information:

Target Pathogen	HLVd (hop latent viroid)
Genus	*Cocadviroid*
Method	Real-Time RT-qPCR, TaqMan

Technical information:

Fluorophores	FAM: HLVd | ROX: COX
Cycling program	➢ 50°C for 20 min (Reverse Transcription) ➢ 95°C for 5 min (RT inactivation) ➢ 40 Cycles: o 95°C for 15 sec (Denaturation) o 60°C for 30 sec (Annealing / Extension)
Controls	Internal positive control (IPC): cytochrome oxidase (COX) Negative control (NC): plant RNA from leaf Positive control (PC): HLVd-infected plant extracts

Host matrix:

Tested plant material	Leaf, roots, stem mechanical transmittable
Tested species infected	*Humulus lupulus* (Hop) *Cannabis sativa* (Hemp)
Sampling	Right sampling is essential since HLVd is not evenly distributed on the leaves. It is recommended to use multiple leaves per plant and mixed extract from all selected leaves.

Specificity:

Analytical Specificity	100%
Number of tested samples from target organism (True Positives)	>100
Diagnostic Specificity	100%
Number of tested samples non-target organism (True Negatives)	>100
Detected isolates / geographic regions (Inclusivity)	HLVd HT #8,10,12,101,104,105 (Switzerland, Hemp) HLVd SO2 #33 (Switzerland, Hemp) HLVd HQ x CT #13,14,15 (Switzerland, Hemp) HLVd CR #21,22 (Switzerland, Hemp) HLVd V+ #28 (Switzerland, Hemp) HLVd leaves (Slowenia, Hop)
Cross reaction with (Exclusivity)	None known
No cross reaction tested with (Exclusivity)	AMV (Alfalfa mosaic virus) ArMV (Arabis mosaic virus) BYDV (Barley yellow dwarf virus) CMV (Cucumber mosaic virus) ErLV (Erysimum latent virus) GLRaV-4 (Grapevine leafroll-associated virus 4) GLRaV-6 (Grapevine leafroll-associated virus 6)

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	PLRV (Potato leafroll virus) RBDV (Raspberry bushy dwarf virus) SLRSV (Strawberry latent ringspot virus) TMV (Tobacco mosaic virus) TRSV (Tobacco ringspot virus)
No matrix effect observed with (Selectivity)	*Humulus lupulus* (Hop) *Cannabis sativa* (Hemp)

Sensitivity:

Analytical Sensitivity / LoD	10^{-1} to 10^{-4}
Sensitivity on host matrix	Hop leaves: 10^{-3} to 10^{-4} Hemp leaves: 10^{-3} to 10^{-4} Hemp roots: 10^{-1} to 10^{-2} Hemp stem: 10^{-3} to 10^{-4}
Other sensitivity characteristics	Infected leaves from hop and hemp plants were mixed with leaves of healthy plants: Infected plant: Ct 23.95 Mixed sample 3 plants: Ct 24.02 Mixed sample 5 plants: Ct 24.33 Mixed sample 10 plants: Ct 25.95 Mixed sample 25 plants: Ct 27.03

Validation:

Internal validation	01.11.2022 – 30.03.2023
External validation	-
Reproducibility	100% (BIOREBA)
Repeatability	100% (BIOREBA)
Validation information	One validation was done at BIOREBA, by retesting of already PCR-tested (unknown external laboratory) HLVd-infected and healthy samples.

Validation release Date:
April, 12th, 2023

QC manager:

Version: 2 – 23.01.2024 – Information about tested plant material (root, stem) and specificity added.

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