CORRIGENDUM: VALIDACIJA METODE DUPLEX RT-QPCR ZA DOLOČANJE VIROIDA RAZPOKANOSTI SKORJE AGRUMOV (CBCVD) NA HMELJU Z UPORABO MRNA1192 INTERNE KONTROLE

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Corrigendum odpravlja napako v članku Guček in Radišek (2023), objavljenem v Hmeljarskem biltenu letnik 30, str. 26-39.

V članku na strani 34 v besedilu in preglednici 8 je navedena mejna vrednost (Cq cut-off) za CBCVd pri uporabi SensiFast kompleta reagentov kot 31,2. Na podlagi rezultatov analiz, izvedenih v Diagnostičnem laboratoriju za varstvo rastlin (DL) v letih 2024 in 2025, smo ugotovili, da je bila je mejna vrednost za CBCVd postavljena previsoko. Izračun je temeljil na rezultatih gBlocks (umetno sintetiziran CBCVd), kar ne ustreza realnim vzorcem hmelja okuženega s CBCVd.

Leta 2025 smo izvedli dodatno validacijo metode duplex RT-qPCR za hkratno detekcijo CBCVd in notranje kontrole mRNA1192. V ta namen smo testirali serijo redčitev (od 10^0 do 10^{-7}) večjega števila CBCVd pozitivnih in negativnih vzorcev hmelja ter izračunali nove Cq cut-off vrednosti po metodi Mehle in sod. (2013). Izračune smo izvedli ločeno za CBCVd in mRNA1192 ter za vsak komplet reagentov (SensiFast in AgPath). Mejno vrednost (Cq cut-off) smo določili na podlagi zadnjih pozitivnih vrednosti v seriji redčitev. Izračunali smo povprečje teh vrednosti, ga zaokrožili navzgor za pol enote in prišteli 0,5. Na tej osnovi smo določili vrednosti: CBCVd, SensiFast (27 vzorcev)= 28,0; CBCVd, AgPath (12 vzorcev)= 34,0; mRNA1192, SensiFast (25 vzorcev)= 31,0; mRNA1192, AgPath (12 vzorcev)= 35,5 (preglednica 1).

Preglednica 1: Mejne vrednosti za CBCVd in mRNA1192 za komplet reagentov SensiFast in AgPath

Komplet reagentov*	Število vzorcev testiranih na CBCVd	Cq cut-off CBCVd	Število vzorcev testiranih na mRNA1192	Cq cut-off mRNA1192
SensiFast	27	28,0	25	31,0
AgPath	12	34,0	12	35,5

* SensiFAST Probe No-ROX Kit (Bioline) + MultiScribe Reverse Transcriptase (TFS)

AgPath-IDTM One Step RT-PCR, Thermo Fisher Scientific

Za dodatno zanesljivost rezultatov smo uvedli interval nezaupanja – vse vzorce z rezultati ±1 Cq glede na določene Cq cut-off vrednosti ponovno analiziramo.

Poleg tega smo med validacijo analizirali vpliv različnih redčitev vzorcev. Ugotovili smo, da je zaradi prisotnosti nespecifičnih signalov najbolj ustrezna 100-kratna redčitev vzorca, ki jo zdaj rutinsko uporabljamo v kombinaciji z novimi Cq cut-off vrednostmi.

Poudarjamo, da so te mejne vrednosti specifične za našo kombinacijo reagentov, instrumentov in pogojev dela. Vsak laboratorij mora zato na podlagi lastne validacije določiti svoje ustrezne Cq cut-off vrednosti.

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CORRIGENDUM: VALIDATION OF THE RT-qPCR METHOD FOR THE DETECTION OF CITRUS BARKCRACKING VIROID (CBCVd) ON HOPS INCLUDING mRNA1192 AS INTERNAL CONTROL

Corrigendum corrects an error in the article by Guček and Radišek (2023), published in Hop Bulletin, Volume 30, pages 26–39.

On page 34 of the article, in both text and Table 8, the Cq cut-off value for CBCVd when using SensiFast kit is stated as 31.2. Based on the results of analyses conducted at the Plant Health Diagnostic Laboratory (DL) in 2024 and 2025, we determined that the Cq cut-off value for CBCVd had been set too high. The original calculation was based on gBlocks (artificially synthesized CBCVd), which does not fully reflect the performance of real hop samples infected with CBCVd.

In 2025, we performed an additional validation of the duplex RT-qPCR method for the simultaneous detection of CBCVd and the internal control mRNA1192. For this purpose, we tested a dilution series (from 10^{0} to 10^{-7}) using a larger number of CBCVd-positive and -negative hop samples and calculated new Cq cut-off values following the method described by Mehle et al. (2013). Calculations were carried out separately for CBCVd and mRNA1192, as well as for each kit (SensiFast and AgPath). The cut-off value was determined based on the last consistently positive values in the dilution series. The average of these values was calculated, rounded up to the nearest half Cq, and then 0.5 was added. Based on this method, the following cut-off values were determined: CBCVd, SensiFast (27 samples): 28.0; CBCVd, AgPath (12 samples): 34.0; mRNA1192, SensiFast (25 samples): 31.0; mRNA1192, AgPath (12 samples): 35.5 (Table 1).

Table 1: The Cq cut-off value for CBCVd and mRNA1192 for SensiFast in AgPath kits.

Kit*	Number of samples tested for CBCVd	Cq cut-off CBCVd	Number of samples tested for mRNA1192	Cq cut-off mRNA1192
SensiFast	27	28.0	25	31.0
AgPath	12	34.0	12	35.5

* SensiFAST Probe No-ROX Kit (Bioline) + MultiScribe Reverse Transcriptase (TFS)

AgPath-IDTM One Step RT-PCR, Thermo Fisher Scientific

To further ensure the reliability of results, we established a confidence interval around the cut-off – all samples with results within ±1 Cq of the Cq cut-off are reanalyzed. Additionally, during validation we examined the effect of different sample dilutions and determined that a 100-fold dilution was optimal due to the occurrence of nonspecific signals. This dilution is now used routinely in combination with the newly established Cq cut-off values.

We emphasize that these cut-off values are specific to our particular combination of reagents, instruments, and working conditions. Therefore, each laboratory must establish its own Cq cut-off values based on its own validation data.

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