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Multiplex LAMP detection of *Phytophthora ramorum*, *P. kernoviae* and *P. lateralis* with plant universal primer set as an internal control

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Purpose: Recently, several *Phytophthora* species cause destructive disease in forest trees and nursery plants. *P. ramorum* is a causal agent of sudden oak death, most commonly observed on Camellia, Magnolia, Pieris and Quercus spp. *P. kernoviae* causes similar symptoms to *P. ramorum*, bleeding stem cankers, foliar blight, and shoot dieback on Fagaceae and other host plants. Unlike others, *P. lateralis* is especially aggressive to *Chamaecyparis lawsoniana*. Previously, we have designed species-specific LAMP primer sets for rapid detection of these three species in import quarantine inspection. In this study, we try to establish multiplex LAMP with plant universal primer set to determine consequences of false-negative and evaluate the reaction.

Methods and Results: We performed fluorescence LAMP assay to distinguish two amplified products from mycelial and plant DNA by peak temperature of anneal derivative curve. By using mycelial and plant DNA, we identified appropriate reaction conditions for these three combinations as follows; reaction temperature is 65°C, concentrations of the species-specific primer is same as before and plant universal primer was 0.08 times lower than before. By the reaction conditions, we were able to detect mycelial DNA when it existed in DNA sample, and if it was absent, plant DNA was detected. We confirmed the multiplex LAMP detection was applicable to inoculated plant samples of Rhododendron, Pieris and Camellia.

Conclusions: This method can be used for rapid and accurate detection of these three *Phytophthora* species.