Performance Evaluation of DNA Amplification Reagent Kits for the Molecular Detection of Lumpy Skin Disease Virus Using Real-time PCR

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Abstract

Background: Lumpy skin disease (LSD) is an economically important viral disease affecting cattle and buffaloes and is caused by the Lumpy skin disease virus (LSDV), a member of the genus *Capripoxvirus* within the family *Poxviridae*. The disease has a substantial impact on animal health and livestock production. Laboratory diagnosis using real-time polymerase chain reaction (real-time PCR) is widely accepted as a standard diagnostic method because of its high sensitivity, specificity, and ability to provide rapid and reliable results. In addition, this technique plays an important role in disease surveillance, animal movement control, and the certification of disease-free farms. However, the accuracy and reliability of molecular detection may be affected by several factors, particularly the selection of an appropriate and high-quality DNA amplification reagent kit (PCR Master Mix) for routine laboratory use.

Objectives: This study aimed to evaluate and compare the analytical performance of three commercial DNA amplification reagent kits (Kit F, Kit L, and Kit Q), currently used in the virology laboratory of the Western Veterinary Research and Development Center for the molecular detection of LSDV by real-time PCR.

Methods: The analytical performance of the three reagent kits was assessed using plasmid samples serially diluted to concentrations ranging from 10^6 to 10 copies/5 µL. Each dilution level was tested in triplicate. The reagent kits were evaluated based on their ability to detect LSDV genetic material across the dilution series. Cycle threshold (Ct) values obtained from each kit were statistically compared using analysis of variance (ANOVA).

Results: All three reagent kits were able to reliably detect LSDV genetic material at concentrations as low as 10^2 copies/5 µL. At the lower concentration of 10 copies/5 µL, Kit Q successfully detected all replicates (3/3), whereas Kit F detected only one (1/3), and Kit L failed to detect any replicates (0/3). Statistical analysis using ANOVA revealed no statistically significant differences in Ct values among the three reagent kits across all detectable dilution levels ($p > 0.05$).

Conclusion: The findings of this study indicate that all three reagent kits are suitable for the molecular detection of LSDV by real-time PCR. However, Kit Q demonstrated superior analytical sensitivity for the detection of low-copy-number samples, while Kit F and Kit L offered practical advantages in terms of lower cost and shorter assay time. Therefore, the selection of an appropriate reagent kit should take into account not only analytical performance but also cost-effectiveness, operational efficiency, and procurement feasibility, in accordance with the specific requirements and operational context of the laboratory.

Keywords: Lumpy skin disease, Lumpy skin disease virus, DNA amplification reagent kit, Real-time PCR

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