應用恆溫環形核酸增幅法（LAMP）快速診斷山羊痘

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摘要

應用恆溫環形核酸增幅法（LAMP），成功建立快速診斷山羊痘之技術。LAMP 試驗可檢測 60 copy 以上的羊痘 DNA，但傳染性化膿性皮膚炎（orf）之 DNA 不會有產物被增幅。取羊痘種毒(力價為 $10^{6.3}$ $TCID_{50}$/mL)萃取病毒 DNA，DNA 連續 10 倍稀釋後分別進行 LAMP 試驗、PCR 及巢式 PCR 等三種檢測方法，比較其敏感性，。結果顯示 LAMP 試驗的敏感性可達到 $10^{2.3}$ $TCID_{50}$/mL 稀釋階，優於傳統 PCR（$10^{3.3}$ $TCID_{50}$/mL），但不及巢式 PCR（$10^{1.3}$ $TCID_{50}$/mL）法敏感。分別採集 212 頭罹患羊痘病羊，皮膚痘瘡、鼻拭和抗凝血液以 LAMP 試驗篩檢，結果痘瘡之檢出率為 100%、鼻拭次之檢出率為 88.21%、血液之檢出率最低為 36.79%。另取 120 個臨床檢體評估 LAMP 試驗和 PCR 兩者一致性為 92%， Kappa 值為 0.81，故兩種實驗完全吻合;LAMP 試驗和巢式 PCR 兩者一致性為 85%，Kappa 值為 0.69 兩種實驗為高度的吻合。

Development of loop-mediated isothermal amplification for detection of goatpox virus

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Abstract

A loop-mediated isothermal amplification (LAMP) assay using six primers targeting a highly conserved region of RNA polymerase gene has been developed to diagnose goatpox virus. The sensitivity of the LAMP assay, which was determined to be sixty copy of the standard plasmid, furthermore, no cross-reactivity was founded with the other tested viruses. Goatpox seed virus DNA were tested using PCR, nested PCR and LAMP assay, was 10 fold higher than PCR and 10 fold less than nested PCR. A total of 212 goatpox infected goats, the papule, nasal swab, blood were collected from each one, the samples were tested using PCR, nested PCR and LAMP assay and the positive rates were 100%, 82.21% and 36.79%, respectively. The LAMP assay allows easy, rapid, accurate and sensitive detection of infection with goatpox virus and is especially applicable in a resource-limited situation. LAMP assay revealed 92% concordance with PCR, κ values was 0.81, two tests were almost perfect. LAMP assay revealed 85% concordance with nested PCR, κ values was 0.69, two tests were substantial.