口蹄疫病毒非結構性蛋白抗體檢測方法之研發

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摘要

本研究主要針對口蹄疫病毒非結構蛋白(FMDV-NSP)免疫原區以PT-PCR利用特異性引子產出3ABC、3A、3B、2C及3D等五種FMDV-NSP特異性增幅產物，經核酸定序確認正確無誤，繼完成原核表現系統試量產、純化及透析後收集的FMDV-NSP可溶性重組蛋白。以西方墨漬法確認各重組蛋白的分子量皆與陽性抗血清反應呈正相關。然而，經試驗結果顯示分析敏感性最高達10^-2倍，以酵素免疫電泳轉漬法(EITB)法檢測FMDV感染陽性血清之診斷敏感性達94%及SPF、豬水疱病(SVD)抗血清等陰性血清之診斷特異性可達95%。另檢測免疫動物田間血清之分析特異性，結果顯示與商品化ELISA檢驗試劑比對符合率達93%。

Development of the method for detecting antibodies against non-structural proteins of foot-and-mouth disease virus

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Abstract

This study mainly targets the non-structural proteins of foot-and-mouth disease virus (FMDV-NSP) and uses PT-PCR to generate five FMDV-NSP specific amplification products, including 3ABC, 3A, 3B, 2C and 3D. The nucleic acid sequences were confirmed, and the soluble recombinant proteins of FMDV-NSP were collected after trial production, purification and dialysis of the prokaryotic expression system. The molecular weight of each recombinant protein was confirmed by Western blotting. However, the results show that the sensitivity of analysis is up to 10^{-2} folds. The diagnostic sensitivity of FMDV-positive sera detected by enzyme-linked immunoelectrotransfer blot (EITB) is 94%, SPF, swine vesicular disease (SVD) antiserum and other negative sera have a diagnostic specificity of 95%. In addition, the specificity of the sera of immunized animals was analyzed, and the results showed that the coincidence rate with commercial ELISA kits reached 93%.