口蹄疫抗體區別診斷方法之最佳化與確認

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摘要

口蹄疫 (FMD)、豬水疱病 (SVD)、水疱性口炎 (VS) 及水疱疹 (VE) 皆屬高度傳染性之動物水疱性疾病，這些疾病於臨床上常被疑惑而不易區別診斷。近幾年來，各種研發針對口蹄疫病毒 (FMDV) O/TW/1999 病毒株的 3ABC 非結構蛋白之抗原決定位的口蹄疫抗體檢測方法，包括 Chromatographic strip、Sandwich ELISA、Blocking ELISA、Sigleplex Luminex 及 Multiplex Luminex (xMAP)等 IVDs 方法。即利用重組蛋白產製於原核大腸桿菌 (*E.coli.*) 表現系統，經純化而高度保留口蹄疫病毒 (FMDV) O/TW/1999 病毒株的 3ABC 非結構蛋白之抗原決定位區域。以間接結合方式分別建立測試片、盤式及微珠等結合界面之檢測平台，以應用在血清樣品中的 FMDV 非結構蛋白 (NSP-3ABC) 評估與分析。試驗方法依據世界動物衛生組織 (OIE) 陸生動物手冊(2013) 1.1.5.章及澳大利亞之全國測試機構協會 (NATA) 2004 等確認指引，從各種試驗結果顯示 NSP 抗體檢測能力幾乎完全一致於 3ABC blocking ELISA 和 3B peptide indirect ELISA 等商品化試劑，且除了可檢測台灣 O 型口蹄疫病毒株之外，也可檢測其他六種血清型如 A、C、Asia 1、SAT 1、SAT 2、SAT 3 等牛源血清中的 NSP 抗體。這些研究顯示各種方法之敏感性及特異性皆高於 85 % 以上，可作為區別診斷及免疫狀況等評估之參考，且不會與口蹄疫病毒以外的水疱性疾病如豬水疱病病毒及水疱性口炎病毒等所誘發的抗體產生交叉反應，因此即具有高度的試驗特異性。

Optimisation and validation of antibody detection assays for differentiation of infected from vaccinated animals against Foot-and-mouth disease virus

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Abstract

Foot-and-mouth disease (FMD), swine vesicular disease (SVD), vesicular stomatitis (VS), and vesicular exanthema (VE) are highly contagious vesicular animal diseases, and they are not able to be differentiated clinically from each other. For the purpose of instant detecting FMD and differentiating it from the other vesicular diseases, many methods have been developed and evaluated in recent years. The in-house in-vitro diagnostic devices (IVDs) were developed by our institute, including the chromatographic strip, sandwich ELISA, blocking ELISA, singleplex Luminex, and multiplex Luminex (xMAP). The non-structural protein 3ABC gene of FMDV O/TW/1999 was cloned into expression vector, which was based on an *Escherichia coli* expression system. The expressed protein was employed to develop the complex interface measurement platforms for FMDV tests. Strip and plate and microsphere formats were developed and evaluated for their abilities in detecting serum antibodies against non-structural protein (NSP-3ABC) of the FMDV. The tests were based on the World Organization for Animal Health Terrestrial Manual 2013, chapter 1.1.5. and National Association of Testing Authorities Australia 2004. Almost perfect agreement between the results from in-house methods and those from the 3ABC blocking ELISA, and 3B peptide indirect ELISA kits were obtained. Moreover, antibodies to nonstructural proteins of the serotypes O, A, C, Asia 1, SAT 1, SAT 2 and SAT 3 were also detected in sera of infected cattle. These studies were showed that the sensitivity and specificity of the methods were higher than 85%, which can be as references for diagnosis and assessment of the immune status. Furthermore, the specificities of these assays were highlighted by the absence of cross-reactions generated by antibodies against the SVDV and VSV at different titers.