應用即時 RT-PCR 檢測牛流行熱

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摘要

參照澳洲AAHL實驗室之牛流行熱Taqman probe qRT-PCR方法，微調引子探針序列符合臺灣BEF病毒株，取1984年至2015年不同年代分離之6株毒株測試，可偵測極限為$10^{0\sim1}$ TCID50/mL病毒，較本實驗室常規BEF診斷用nested PCR敏感。因為2015年血液檢體屬臺灣新基因型病毒於細胞增殖第二代後，病毒RNA於探針位置發生點突變，而使qRT-PCR螢光反應變弱，終至無法檢出。故於G蛋白基因重新設計探針及引子，復以qRT-PCR試驗檢測3株BEF病毒株，極限皆為$10^{0}TCID_{50}$/mL與無法檢出變異株的探針引子相似，但螢光強度增為1.2倍以上且可檢出增殖的突變病毒株。取27個BEF陽性病毒血檢體，比較不同檢驗方法敏感性，qRT-PCR最優其檢出率為100%，nested PCR檢出率為96%，傳統RT-PCR檢出率為74%；陽性檢體Ct值介於14-23之間。BEF qRT-PCR為實驗室檢驗牛流行熱的一個方便且快速工具。

Detection of Bovine Ephemeral Fever Virus Using Real-Time RT-PCR

Lu-Jen Ting

Abstract

The method developed in this study was based on the protocol using theTaqMan quantitative reverse transcription polymerase chain reaction (qRT-PCR) for the detection of bovine ephemeral fever virus (BEFV) established at the Australian Animal Health Laboratory. The primers and probe sequences that we designed were slightly modified to be suitable for the detection of Taiwan BEFV strains. Our primer and probe set was tested on 6 strains isolated from 1984 to 2015, the detection limit was1 to 10 $TCID_{50}$/mL, more sensitive than then PCR protocol currently used in our laboratory. When applying the new method to detect Taiwan's new BEFV genotype, which was first discovered in 2015, we found that two point mutations within the region of probe annealing, generated during the second passage of the virus, may negatively affect the qRT-PCR reaction. Therefore, the probes and primers were redesigned, and three BEFV strains were tested. The detection limit was 100$TCID_{50}$/mL, similar to the previous probe and primer set. Yet the fluorescence intensity increased >1.2x. The mutant virus strain could also be detected. To evaluate the sensitivity between the new qRT-PCR method, the nested PCR protocol, and conventional RT-PCR, 27 BEFV-positive blood samples were tested. Our results demonstrated that the sensitivity of these methods were 100%, 96%, and 74%, respectively. The qRT-PCR method that we developed is therefore a sensitive tool for the detection of BEFVs currently extant in Taiwan.