

應用反轉錄環型恆溫核酸增幅法檢測新流感病毒

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摘要

2009年3月開始爆發新流感傳佈全球病導致超過18138人死亡，這需要發展一種容易、確實且簡單的方法來針對於新流感進行診斷。我們發展引子設計建立在基質蛋白基因片段的反轉錄環型恆溫核酸增幅法以達到快速的區別診斷新流感病毒，應用在臨床蒐集到的111個臨床鼻咽喉拭子檢體進行試驗，並以即時反轉錄聚合酶鏈反應及病毒分離做測試比較，以病毒分離做為參考標準。結果這方法可成功的檢出新流感病毒極限至約20個標的基因，比即時反轉錄聚合酶鏈反應較為敏感。此外，這方法能夠區別一般季節性的(H1N1&H3N2)流感病毒以及其他的呼吸道病毒如副流感病毒2型、副流感病毒3型、腺病毒、人類腸道致病性病毒7、柯沙奇病毒A10。基於病毒分離的實證，以基質蛋白基因特異的反轉錄環型恆溫核酸增幅法分析有100%的特異性及98.25%敏感性，而且大部分檢出的檢體(46/56)可在20分鐘內完成。這是一個確實且快速的分析系統，適合於如第一線的健康照護中心在只需要很少的設備的一般診斷實驗室進行，這將有助於臨床及公共衛生單位在疾病爆發時進行有效的處置。

Application of reverse transcription loop-mediated isothermal amplification for detection of pandemic (H1N1) 2009 virus

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Abstract

Since the initial outbreak in March 2009, the novel pandemic (H1N1) 2009 virus has affected individuals worldwide and caused over 18 138 deaths. There is an urgent need for the development of an easy, accurate and simple method for the diagnosis of this novel pandemic virus. Design Reverse transcription loop-mediated isothermal amplification assay (RT-LAMP) with primers targeting the M segment was established for the rapid differential diagnosis of pandemic (H1N1) 2009 virus. The performance of this assay was characterized using 111 clinic nasopharyngeal swabs, and the diagnosis accuracy was compared with real-time reverse transcription PCR (RRT-PCR) and virus isolation, the latter being the reference standard. This method successfully detected pandemic (H1N1) 2009 virus with a detection limit of approximately 20 copies of the target RNA per reaction, which is a comparably sensitivity to the RRT-PCR assay. Furthermore, this assay was able to discriminate pandemic (H1N1) 2009 virus from seasonal influenza viruses, such as H1N1 and H3N2, and other respiratory viruses (parainfluenza type 2 and 3, adenovirus, echovirus 7, and coxsackievirus A10). Based on validation by virus isolation, the specificity and sensitivity of this M-specific RT-LAMP assay were 100% and 98A25%, respectively. Moreover, the RT-LAMP amplification of most positive samples (46 out of 56) was achieved in < 20 min. This is an accurate and fast analysis system suitable for general diagnostic laboratories with only limited equipment, e.g. first-line health care centre. This assay will help clinicians and public health officials to react effectively during an outbreak.