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• 中文關鍵字	登革熱；血清型；快速診斷；預防醫學
• 英文關鍵字	Dengue fever；Serotype；Rapid diagnosis；Preventive medicine
• 中文摘要	登革病毒屬黃質病毒科,依其抗原性之不同,可分爲四種血清型,主要流行於熱帶及亞熱帶地區;台灣亦屬登革熱之流行地區,四種血清型的登革病毒均曾出現本土株,並有本土化跡象,甚或有出血性登革熱病例,故登革病毒的檢測與防範,帡爲流行病學與預防醫學之重要課題。 本計畫之目的爲簡化檢體 RT-PCR 之前處理,並建立登革病毒快速診斷定型之流程。利用 96 孔微量滴定盤,配合化學呈色法及登革病毒特異型探針(Type-specific probe)雜交技術(Hybridization)偵檢 PCR 產物,以提高靈敏度與特異性(Specificity);而利用酵素免疫分光光度計(ELISA reader)讀取 96 孔微量滴定盤檢體之化學呈色吸光值,有利於操作的自動化、標準化,且方便、省時,並具低成本特性;PCR-ELISA 系統之建立,除適用於處理大量檢體外,尙具定量(Quantitation)功能,或能提供患者體內病毒量與疾病嚴重度之關聯性。 本研究室利用 TouchDown PCR 方法以提高 RT-PCR 產物之特異性與產量,亦即先以高溫(66.degree.C)進行 Polymerization 反應,以提高反應引子之結合專一性,藉以提升產物特異性,但爲兼顧產量,故將 Annealling 溫度以每 cycle TouchDovon -0.5.degree.C 至 58.degree.C 爲止。此外,反轉錄之反應溫度亦從 42.degree.C 提升至 53.degree.C,並縮短反應時間至 30min。此外,以 Streptavidin 或 DNA 塗佈之 96 孔微量滴定盤,配合化學呈色法及登革病毒特異型探針(Type-specific probe)雜交技術,本研究室目前可偵檢靈敏度爲 1fg,並可降低交叉反應,以提高特異性。另外,並具有下列優點: (1)利於自動化、標準化,故方便、省時,並降低成本; (2)適時處理大量檢體,並具定量化的功能。 台灣地區爲登革病毒之流行區域,登革熱已名列台灣十大重要傳染病之一,故發展一簡單、快速又可靠之診斷方法,以利登革熱疫情之預防與掌握,實爲流行病學與預防醫學的重要課題。本計畫之成果,近程可提供防疫單位實際診斷操作應用的參考,兼具學術性與實用性。此外,因流程簡化省時、成本降低,故可節省公帑;更因其易於自動化與標準化,故本研究成果在遠程上,有助於登革病毒 RT-PCR

	檢驗試劑組之開發。
• 英文摘要	Dengue virus, a flaviviridae, can be distinguished to four serotypes due to their epitopes. Taiwan is an endemic area. Local strains and local cases were discovered in recent years. Therefore, the prevention and diagnosis of dengue virus are an important course in the policy-making for public health. Our laboratory was supported by the Department of Health (DOH88-TD-1002) to establish a system of rapid diagnosis and serotyping for dengue viral infection. Results showed that the standardized RT-PCR/ELISA method was performed very well and had the advantages of low cost, short diagnosis course (<8hr) and low labor intensive with automatic potential. The best RT-PCR condition was established by a TouchDown PCR method with high starting annealing temperature (66.degree.C) abd touchdown by -0.5.degree.C/cycle for 15 cycles. After this procedure, another 15 cycles were proceeded with 58.degree.C annealing temperature. Such PCR protocol can obtain products with high specific quality and a lot of quantity. Moreover, by streptavidin or DNA coated plate coupled hybridization and colorimetric detection method, as low as 1fg dengue viral RNA with high sensitivity and specificity. Based on this protocol, the diagnosis can be completed within 8hr. However, it needs further investigation to evaluate the potential of RT-PCR/ELISA to replace the conventional nested RT-PCR method. Furthermore, our ambition is to combine the nucleic acid hybridization and ELISA-like colorimetric detection technique to develop a high sensitivity and specificity dengue viral diagnosis and serotyping kit which with automatic, standardized, convenient and low cost characteristics. On the other hand, such kind of method can also be applied to diagnosis the infection of entrovirus or hantavirus.